THE EFFECT OF GRAPEFRUIT JUICE, A P-GLYCOPROTEIN INHIBITOR, ON ORGANIC ACID AND CONJUGATES URINARY PROFILE IN HEALTHY HUMAN SUBJECTS.

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The Lord is my strength and my shield,
my heart trusted in Him, and I am helped;
therefore my heart greatly rejoices,
and with my song will I praise Him

Psalms 28: 7

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Abbreviations

ABC	ATP-binding cassette
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
AUC	Area under the curve
α	Alpha
BSA	N, O-bis-(trimethylsilyl) acetamide
BSTFA	N, O-bis-(trimethylsilyl) trifluoracetamide
β	Beta
CFTR	Cystic fibrosis transmembrane conductance regulator
CNS	Central nervous system
CoA	Coenzyme A
CO_2	Carbon dioxide
CsA	Cyclosporin A
CYP 450	Cytochrome P450
CYP 3A4	The most abundant P450 present in the liver and the small bowel
DNA	Deoxyribonucleic acid
FAD	Flavin adenine dinucleotide
FADH	The reduced form of flavin adenine dinucleotide
GC-MS	Gas chromatography-mass spectrometry
GTP	Guanosine triphosphate
γ	Gamma
H	A hydrogen atom
H^+	Proton (electron-deficient hydrogen atom) / hydrogen cation

H_2O	A water molecule
HDL	High density lipoproteins
HPA	p-Hydroxyphenylacetate
MSD	Membrane-spanning domain
NAD	Nicotinamide adenine dinucleotide
NADH	The reduced form of nicotinamide adenine dinucleotide
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NBF	Nucleotide-binding fold
O_2	Molecular oxygen
P_i	Inorganic phosphate
P-gp	P-glycoprotein
SEM	Standard error of the mean
TM	Transmembrane
TMS	Trimethylsilane

Glossary

Amphipathic compounds: Compounds that have both a hydrophilic and a hydrophobic part.

Autosomally inherited: A genetic trait that is encoded on a chromosome other than the sex chromosomes and, therefore, is inherited equally in males and females.

Atherosclerosis: The deposition of yellowish plaques (atheromas) containing cholesterol, lipoid material and lipophages on the intima (the most inner part) of large and medium-sized arteries.

Beta oxidation: A process of fatty-acid breakdown producing acetyl CoA.

Citric–acid cycle: The cyclic metabolic mechanism by which the complex oxidation of the acetyl moiety of acetyl-coenzyme A is accomplished.

Chromatography: A method for separating mixtures of different molecules into pure or partly pure fractions. The molecules in the mixture bind to a stationary phase – paper, thin layer, or particles packed into a column – and are differentially eluted by a mobile phase.

Cystic fibrosis: A common genetic disease characterised by viscous mucus secretions obstructing the exocrine glands.

Cytotoxin: A toxin or antibody that is toxic to the cells of a certain organ for example, nephrotoxin is toxic to the cells of the kidney.

Encephalopathy: Any degenerative disease of the brain.

Erythrocyte: A mature red blood cell. It is the major cellular element of the circulating blood and transports oxygen as its principal function.

Eukaryote: An organism whose cells have a true nucleus i.e. one bounded by a nuclear membrane. Eukaryotic cells also contain many membrane-bound compartments (organelles) in which cellular functions are performed.

Genes: A gene determines an inherited trait. Genes are encoded in the sequence of the cellular DNA.

Glycolysis: The process of converting glucose or glucose phosphate to pyruvate or lactate and producing ATP. This can occur in the absence of oxygen.

Haemodynamics: The study of the movement of the blood and the forces concerned therein.

Hydrophobic: A tendency to repel water molecules, a quality possessed by nonpolar radicals or molecules that are more soluble in organic solvents than in water.

Hypolipidaemia: Abnormally low concentration of any or all of the lipids in the plasma.

Isoenzyme: Isoenzymes are different enzymes that carry out the same chemical reaction. They are different proteins usually with different kinetic properties.

Jejunoileostomy: The formation of an anastomosis between the proximal jejunum and the terminal ileum.

Lipids: Very hydrophobic molecules that can be released from cells with hydrophobic solvents.

Lipophilic: A tendency to attract or absorb fat.

Metabolic pathway: A series of enzymatic reactions in which the product of one reaction is the substrate for the next reaction in the pathway.

Multidrug resistance: A decrease in sensitivity of cells and tissues to a wide variety of structurally and chemically unrelated compounds.

Neuroendocrine: The interaction between the nervous and endocrine system; hormones elaborated in the nervous system and ultimately secreted by endocrine gland.

Neurotoxin: A toxin that is poisonous to or destroys nerve tissue.

Neutropenia: (Agranulocytosis) A decrease in the number of neutrophilic leukocytes in the blood characterised by lesions of the throat and other mucous membranes of the gastrointestinal tract and of the skin.

Nontropical sprue: A chronic form of malabsorption syndrome precipitated by the ingestion of gluten-containing foods; pathologically, the proximal intestinal mucosa loses its villous structure surface epithelial cells exhibit degenerative changes and their absorptive function is severely impaired.

Nucleotide: A component of nucleic acids and other biological molecules. A nucleotide is a sugar, such as ribose, connected to an organic base, such as adenine, and to one or more phosphate groups.

Obstetric cholestases: Interruption in the flow of bile through any part of the biliary system, from liver to duodenum due to pregnancy.

Oxidative phosphorylation: The formation of ATP from ADP plus inorganic phosphate during the transfer of hydrogen or electrons down the electron-transport system.

Pleitropic transporter: A protein that transports a multiple, different and apparently unrelated compounds.

Prokaryote: Cellular organisms (such as bacteria) lacking a true nucleus and nuclear membrane. Their nuclear material consists of a single double-stranded DNA molecule not associated with basic proteins.

Scleroderma: Chronic hardening and thickening of the skin, which may be a finding in several different diseases, occuring in a localised or focal form and as a systemic disease.

Substrate: A compound that is converted into product by an enzyme-catalysed reaction.

Tangier disease: A disease characterised by deficient efflux of lipids from peripheral cells and a very low level of high density lipoproteins.

Toxin: A poison, usually one produced by or occuring in a plant or microorganism.

Transamination: The movement of nitrogen from one carbon skeleton to another carbon skeleton, usually by the action of amino transferases.

Transmembrane: Stretching from one end of the membrane straight across to the other end of the membrane.

Xanthoma: A benign fatty fibrous yellowish plaque, nodule, or tumour that develops in the subcutaneous layer of skin, often around tendons. The lesion is characterized by the intracellular accumulation of cholesterol and cholesterol esters.

Xenobiotics: Organic substances that are foreign to the body, such as drugs or organic poisons.

Abstract

P-glycoprotein (p-gp), a member of the superfamily ATP-binding cassette (ABC) is known to be present in the absorptive enterocytes of the gastro-intestinal tract and many other tissues in the body where it acts mainly as a defence mechanism against exogenous assault. Defects in p-gp is speculated to result in the development of diseases as mutations in genes are causes of numerous diseases in the metabolic mosaic that underlies health. Due to the importance of p-gp, particularly in the intestines, mutation of the gene encoding this protein may lead to the presence of unusual compounds, xenobiotics in the body and the urine. It is thought that defective p-gp in the intestine might also lead to the absorption of some metabolites of bacterial origin and residue of digestion which normally would have been effluxed back into the gut by the p-gp.

To investigate if defective p-gp may be involved in the manifestation of unusual compounds and organic acids in the urine, inhibition of intestinal p-gp was proposed. Grapefruit juice (GJ), a natural beverage commonly taken by the majority of the populace has been reported to inhibit p-gp activity in the intestine (Spahn-Langguth & Langguth, 2001). Grapefruit juice was administered to healthy subjects in this study and the sugars and organic acids content of the urine sample after administration was analysed and compared with the controls (urine samples taken from the same set of subjects before grapefruit juice administration). These were determined by thin layer chromatography and gas chromatography-mass spectrophotometry respectively.

The thin layer chromatography revealed that there was no difference between the concentrations of sugars in the control and samples after the administration of grapefruit juice. This might indicate that the inhibition of p-gp or mutation of the gene encoding p-gp does not result in the presence of sugars in the urine. The analysis of organic acids by gas chromatography-mass spectrophotometry method showed a remarkable difference between the organic acids present in the controls and urine samples after the administration of grapefruit juice as well as their concentrations. The organic acids solely from microbial origin were statistically analysed and the

results gave statistically significant increase in these organic acids in the adults. There was no statistically significant increase in the children.

In conclusion, this study confirmed that grapefruit juice inhibits p-gp in the intestine and this resulted in the presence of unusual organic acids from microbial origin in the urine of the adults. The presence of some of these organic acids have been indicated in some metabolic disorders and are also known to give rise to toxic effects on brain, liver, muscle and other tissues. There is the need to do more study on p-gp expression in children so that its functional roles and effect of the mutation of the gene encoding this protein can be known.

Chapter 1

Introduction

Many known transporter proteins in both pro- and eukaryotes belong to the ATP-binding cassette (ABC) superfamily, which comprises transporters for amino acids, sugars, ions, peptides, proteins, lipids and various organic and inorganic compounds. While some ABC transporters translocate single substances across membranes with high specificity, others transport a wide variety of lipophilic compounds (Dean, 2003). ABC transporters are responsible for physiological and pathophysiological processes alike and contribute to the development of a number of diseases (Efferth, 2001). In the human, many ABC transporters are associated with genetic diseases including cystic fibrosis, Tangier diseases, obstetric cholestases and drug resistance of cancers (Higgins, 2001).

For example, two ATP-binding cassette (ABC) proteins, ABCG5 and ABCG8 have recently been associated with the accumulation of dietary cholesterol in the sterol storage disease, sitosterolemia (Albrecht *et al.*, 2002). Increased levels of phytosterols (plant sterol) such as sitosterol and campesterol are found in blood, plasma, erythrocytes, and especially in xanthomas and arteries of affected subjects. Increased intestinal absorption of phytosterols as well as decreased biliary and faecal excretion of cholesterol and phytosterols contribute to the abnormal lipid composition of blood and tissues from these patients. Also, mutation of ABC1 protein, a member of the large ATP-binding cassette family of proteins, has been identified in the original Tangier disease and this establishes that defects in the ABC1 gene are a cause for the disease (Remaley *et al.*, 1999).

Many transporters mediate efflux of hydrophobic molecules from cells. The p-glycoprotein (p-gp), also designated as ABCB1, transports a wide variety of hydrophobic compounds, including steroids, out of cells (Higgins & Gottesman, 1992).

P-glycoprotein (p-gp) is a plasma membrane glycoprotein of about 170 kDa and belongs to the superfamily of ATP-binding cassette (ABC) transporters in pro- and eukaryotes (Borst & Schinkel, 1997). One important physiological role of p-gp appears to be the protection against toxins, achieved by exporting xenobiotics from the body into the bile, urine or gut. There is accumulating evidence from studies in animal models, and preliminary studies in humans, that p-gp has a significant role in limiting substrate penetration into the CNS and is an important determinant of pharmacological effects and toxicity within the CNS (Schinkel, 1999).

The identification of p-gp in normal tissue (and moreover at the cellular level) allows speculation of its role in normal physiologic functions. These findings suggest that p-gp, the pleitropic transporter protein, may be utilised for different purposes by different types of cells. Its tissue-specific functions may include excretion of toxic substances (providing a major and general route of detoxification), maintenance of homeostatic levels of steroid hormones in the adrenal gland cells (through an intracellular transporter function), and the maintenance of blood-brain, blood-testis, and placental barriers. The importance of p-gp due to its functional role and its expression in several normal human tissues associated with secretory or barrier functions and in some bone marrow and peripheral blood cells, led to the postulation that a defect in a p-gp gene would certainly initiate the development of some genetic disorders (Thiebaut *et al.*, 1987).

The products of carbohydrate digestion utilised in the body are monosaccharides - the major ones being D-glucose, D-galactose and D-fructose. The presence of other monosaccharides may therefore be indicative of some form of physiological defect that may result in the presence of unusual organic acids and carbohydrates in the urine as products of their metabolism. P-gp is known to act as a detoxifier, thus recognising molecules that do not belong in the membrane and removing them.

The sugar moieties of p-gp show significant changes in their structures and alterations of these carbohydrate chains have been observed in diseases. Knowledge of the biological functions of p-gp is instrumental in the development of better diagnostic paradigms (Brockhausen & Kuhns, 1997).

The consumption of grapefruit juice has been reported to increase the bioavailability of certain p-gp substrates in recent years (Bailey *et al.*, 1998). This indicates that constituents of grapefruit juice may inhibit p-gp, thus increasing its substrates bioavailability. The inhibition was also found to be localised to the intestinal p-gp (Bistrup *et al.*, 2001). Three glasses of grapefruit juice taken by adults per day were found to have an inhibitory effect on gut p-gp activity (Garvan *et al.*, 2000).

Importance of this study:

A number of inborn metabolic diseases of p-gp, exhibiting significant clinical effects, is already known, for example, the carbohydrate deficient glycoprotein syndromes (CDGS). It is quite feasible that other p-gp proteins might too prove to be defective and that in fact, defects in all of these proteins may eventually be discovered. The identification of defects of the p-gp proteins is dependent on the detection and characterisation of specific metabolites or metabolic patterns indicative of these defects.

This study was designed to identify such metabolites and to elucidate these metabolic patterns, which may in future be utilised to establish p-gp deficiencies. With this objective in mind, grapefruit juice, which contains the known p-gp inhibitors like bergamottin and naringin was administered to test subjects, followed by the analysis of an extensive range of metabolites.

The aims of this study were to:

-study the effect of grapefruit juice on the intestinal p-glycoprotein by elucidating the metabolic profile of the subjects to whom grapefruit juice were administered,

-compare these profiles with the metabolic markers of some genetic disorders by identifying molecular markers for screening and evaluation of predisposition to diseases, prevention, and monitoring of diseases and treatment monitoring,

-and in future, develop novel diagnostic and therapeutic options for metabolic disorders resulting from defects of p-gp.

Chapter 2

ABC transporters

2.1 GENERAL

The family of adenosine triphosphate (ATP)-binding cassette (ABC) transporters is the largest gene family known that is found in all organisms. This family comprises a large number of either import or export pumps (no bidirectional ABC transporters have been identified so far). While some ABC transporters translocate single substances across membranes with high specificity, others are involved in the transport of a wide diversity of compounds including sugars, ions, peptides and complex organic molecules (Higgins, 1995).

2.2 MECHANISM OF ACTION

A large number of biological activities like DNA modifications, vectorial pumping of molecules or ions across membranes and directed movement or assembly of macromolecules is energetically unfavourable and proteins involved in these activities need energy to function. Usually, these proteins have evolved to harness the chemical energy provided by the hydrolysis of the β-γ phosphate bond of nucleotides (Repke, 1996), mainly ATP or GTP, to trigger conformational modifications essential for their cellular function. ABC (ATP-binding cassette) transporters are an example of proteins that obtain the energy required for function from nucleotide hydrolysis.

ABC transporters bind ATP and use the energy to drive the transport of various molecules across all cell membranes (Higgins, 1992; Childs & Ling, 1994; Dean & Allikmets, 1995). The stoichiometry of transport is estimated to be close to one substrate molecule for every hydrolysed ATP molecule. These transporters have been termed "traffic ATPases" with respect to their bifunctional action: they hydrolyse adenosine triphosphate to adenosine diphosphate (ADP) and inorganic phosphate

(P_i) and they transport a wide array of molecules or conduct the transport of molecules by stimulating other translocation mechanisms (Efferth, 2001).

2.3 FUNCTIONS OF ABC TRANSPORTERS

ABC transporters are central to many physiological and pathophysiological processes, including the uptake of nutrients, the non-classical secretion of signaling molecules and toxins, multidrug resistance and they are also implicated in a number of human diseases. Furthermore, many clinically relevant transporters belong to this family, such as the chloride channel CFTR (cystic fibrosis transmembrane conductance regulator) and the multidrug resistance (MDR) p-glycoprotein (Riordan *et al.*, 1989; Gros *et al.*, 1986).

2.4 ABC GENES AND HUMAN GENETIC DISEASE

Many ABC genes were originally discovered during the positional cloning of human genetic disease genes. As expected from the diverse functional roles of ABC genes, the genetic deficiencies that they cause also vary widely. Genetic variation in these genes is the cause of, or a contributor to a wide variety of human disorders with Mendelian and complex inheritance, including cystic fibrosis, neurological disease, retinal degeneration, cholesterol and bile transport defects, anaemia and drug response (Dean *et al.,* 2001). Because ABC genes typically encode structural proteins, most of the disorders are recessive, and are attributable to a severe reduction or lack of function of the protein.

Another typical example is tangier disease. It is characterised by deficient efflux of lipids from peripheral cells, such as macrophages, and a very low level of high density lipoproteins (HDL). The disease is caused by alterations in the ABCA1 gene, implicating this protein in the conversion of cholesterol and phospholipids to HDL, the first step in the pathway of their removal (Young & Fielding, 1999). Patients with hypolipidemia have also been described that are heterozygous for ABCA1 mutations, suggesting that ABCA1 variations may have a role in regulating the level of HDLs in the blood (Marcil *et al.*, 1999). Thus, ABC transporters may serve as target for novel therapeutic options, i.e. target-specific drugs or gene-therapeutic approaches.

2.5 STRUCTURE OF ABC TRANSPORTERS

Despite their large number and overwhelming substrate diversity, ABC proteins have a typical sequence and organisation of their two cytoplasmic ATP-binding hydrophilic domains, also known as nucleotide–binding folds (NBFs) and two transmembrane (TM) hydrophobic domains, also known as membrane-spanning domains (MSDs), which form a functional transporter (Figure 2.1). The NBFs, which are located in the cytoplasm, transfer the energy to transport the substrate across the membrane. The transmembrane domains contain 6-11 membrane-spanning ∝-helices and vary considerably between different transporters, whereas the ATP binding domains are highly conserved. The substrate specificity is believed to be determined by the transmembrane domains, including the loops connecting the individual helices (Higgins, 1992).

The eukaryotic ABC genes are organised either as full transporters containing two TMs (or MSDs) and two NBFs, or as half transporters (Hyde *et al.*, 1990). The latter must form either homodimers or heterodimers to constitute a functional transporter.

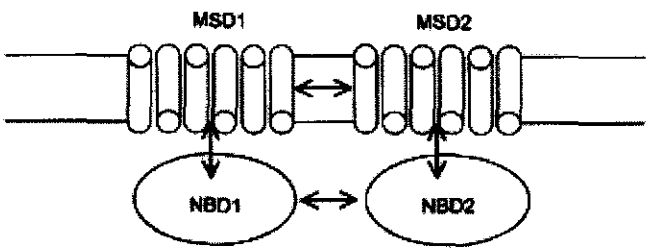


Figure 2.1: *Schematic representation of ABC transporter membrane topology: transporters typically contain two intracellular nucleotide-binding domains (NBDs) and two multiple membrane-spanning domains (MSDs).*

2.6 HUMAN ABC PROTEIN FAMILIES

Human ABC genes can be divided into subfamilies based on similarity in gene structure (half vs. full transporters), order of the domains, and on sequence homology in the NBF and TM domains. In humans, 49 ABC proteins, classified into 7

subfamilies (A-G), are currently known (Dean, 2003). The synonyms of the subfamilies are written in the brackets and they are as discussed below.

2.6.1 ABCA (ABC1) family

Out of the 12 ABCA family members, four are assumed to transport lipophilic substrates: ABCA1: phospholipids, cholesterol, ABCA2: estramustine (a sterol derivative), ABCA4: N-retinylidene-phosphatidylethanolamine (a phospholipid derivative) and ABCA7: presumably lipids. ABCA4 protein performs a crucial step in the visual cycle by transporting vitamin A derivatives in the outer segments of photoreceptor cells. The substrates of the other ABCA members are not known (Broccardo *et al.*, 1999).

2.6.2 ABCB (MDR/TAP) family

The ABCB subfamily is unique in that it contains both full transporters and half transporters. Four full transporters and seven half transporters are currently identified as members of this subfamily. The members of the ABCB family show highly varied specificities (ABCB1: amphiphilic compounds, ABCB2 and ABCB3 (TAP) are half transporters that form a heterodimer to transport peptides, ABCB6,7,8,10: iron, ABCB4: phosphatidylcholine, ABCB11: bile salts) (Dean *et al.*, 2001).

2.6.3 ABCC (CFTR/MRP) family

The ABCC subfamily contains 12 full transporters with a diverse functional spectrum that includes ion transport, cell surface receptor and toxin secretion activities. The CFTR protein is a chloride ion channel that has a role in all exocrine secretions, and mutations in CFTR cause cystic fibrosis. Major functions of ABCC proteins are, among others, the protection against toxic compounds and the secretion of organic anions (Quinton, 1999).

2.6.4 ABCD (ALD) family

All known members of the ABCD family have been implicated in the transport of fatty acids. The genes encode half transporters that are located in the peroxisome, where they function as homo- and / or heterodimers in the regulation of very long chain fatty acid transport (Dean, 2003).

2.6.5 ABCE (OABP) family

ABCE1 is the unique member of the ABCE family. It exhibits an unusual domain organisation, consisting of two ABC domains and completely lacking transmembrane domains. The ABCE subfamily is comprised solely of the oligo-adenylate binding protein, a molecule that recognises oligo-adenylate that is produced in response to infection by certain viruses. This gene is found in multicellular eukaryotes, but not in yeast, suggesting it is part of innate immunity (Dean *et al.*, 2001).

2.6.6 ABCF (GCN 20) family

Like ABCE, ABCF protein family members consist of two ABC domains and lack transmembrane domains. Their functions and substrates are not fully known (Dean, 2003).

2.6.7 ABCG (WHITE) family

A number of ABCG proteins are thought to be involved in the transport of sterols (ABCG1, 5, 8). The mammalian ABCG1 gene is involved in cholesterol transport regulation. Additionally, some members transport phospholipids (ABCG1) and toxins (ABCG2). The functions of ABCG3 and ABCG4 genes are unknown (Klucken *et al.*, 2000).

A member of the ABCB family (p-glycoprotein) was the first human ABC transporter cloned and characterised through its ability to confer a multidrug resistance phenotype to cancer cells (Dean *et al.*, 2001). It is still one of the best known and

most intensively studied proteins of the family. It is also apparently the most ubiquitous in tissue distribution as well as in recognition of substrates.

2.7 P-GLYCOPROTEIN

2.7.1 General properties of p-glycoprotein

P-glycoprotein (p-gp) is a phosphorylated and glycosylated plasma membrane protein belonging to the ABC superfamily of transport proteins. It is a 170kDa transporter with a broad spectrum of amphiphilic substrates. It is also known as a transmembrane adenosine triphosphate (ATP)-dependent efflux pump located in the intestinal villi of jejunal enterocytes, the primary site of absorption, and in other critical transport sites, such as the blood-brain barrier (Fromm, 2000).

As a member of the ABC superfamily of transporters, p-gp possesses two ATP binding sites and uses ATP (via hydrolysis) as the source of energy for 'translocating' substrates (Sharom *et al.*, 1993). The substrates enter from the lipid bilayer, and can bind to two (or more) nonidentical sites (Wang *et al.*, 2000).

2.7.2 P-glycoprotein and multidrug resistance

While the primary function of this protein is unknown, its ability to confer resistance to a wide variety of structurally and chemically unrelated compounds remains impressive. P-gp expression was demonstrated in several malignancies and is one mechanism by which cells acquire multidrug resistance (Gottesman *et al.*, 1996). Multidrug resistance (MDR) is responsible for a decrease in sensitivity of tumour cells to unrelated, naturally occuring anticancer drugs. P-gp can be found in many tumour tissues. In tumours, it is one of the proteins principally responsible for both intrinsic and acquired multidrug resistance. This is of major clinical relevance, as multidrug resistance is the main limitation for systemic antitumour chemotherapy, occuring in about 90% of all metastasising tumours treated with cytostatic drugs (Gottesman, 1993).

2.7.3 Localisation and activity of p-glycoprotein in the blood-brain barrier

P-glycoprotein is predominantly found in the blood luminal membrane of the brain capillary endothelial cells that make up the blood-brain barrier. Since p-gp can actively transport a huge variety of hydrophobic and amphipathic drugs out of the cell, it was hypothesised that it might be responsible for the very poor penetration of many relatively large (>400 Da) hydrophobic drugs in the brain, by performing active back-transport of these drugs to the blood (Schinkel, 1999). Extensive experiments with *in vitro* models and with knockout mice lacking blood-brain barrier p-gp or other animal models treated with blockers of p-gp have fully confirmed this hypothesis. Absence of functional p-gp in the blood-brain barrier leads to highly increased brain penetration of a number of important drugs. Depending on the pharmacological target of these drugs in the central nervous system (CNS), this can result in dramatically increased neurotoxicity, or fundamentally altered pharmacological effects of the drug. Given the variety of drugs affected by p-gp transport, it may be of tremendous therapeutic value to apply these insights to the development of drugs that should have either very poor or very good brain penetration, whichever is preferred for pharmacotherapeutic purposes.

2.7.4 P-glycoprotein gene and tissue distribution

Apart from the expression in the blood-brain barrier, drug-transporting p-gp occur in a range of other tissues. The most prominent sites are the apical membrane of intestinal epithelial cells of small and large intestine, the biliary canalicular membrane of hepatocytes, and the luminal membrane of proximal tubular epithelial cells in the kidney (Thiebaut *et al.*, 1987). These locations suggest that p-gp may excrete its substrates into intestinal lumen, bile, and urine, respectively, thus eliminating toxic compounds from the body. High levels were further found in the adrenal gland of mice and humans (Thiebaut *et al.*, 1987). In addition, moderate levels of p-gp were found in a range of other tissues.

In contrast to man, which has only one drug-transporting p-gp gene, MDR1, mice and other analysed rodents have two drug-transporting p-gp genes, mdr1a (also called mdr3) and mdr1b (also called mdr1) (Schinkel, 1999). The substrate specificity of mdr1a and mdr1b p-gp in the mouse is different but partly overlapping, and together the two mouse genes are expressed in roughly the same set of organs as the single

human MDR1 gene. This suggests that the mdr1a and mdr1b p-gps together perform the same set of functions in the mouse as MDR1 p-gp in man.

2.7.5 P-glycoprotein activities in the tissues

The presence of p-gp in the adrenal gland and in steroid-producing cells of the endometrium suggests it may also have a role in the handling of steroids, possibly providing a protective function for the plasma membranes of steroid-producing cells. Furthermore, it has been found that p-gp expressing epithelial monolayers of cells are able to transport steroids and that some lymphoid cells expressing p-gp are resistant to the cytotoxic effects of steroids (Delph, 2002).

P-gp appears to have a role in cholesterol metabolism. Cholesterol esterification is one of the mechanisms that cells use to control the amount of toxic free cholesterol. Under conditions of excess cholesterol, cholesterol is transported from the plasma membrane to the endoplasmic reticulum (ER) where it is esterified. P-gp functions to increase esterification of cholesterol derived from plasma membrane by facilitating the movement of cholesterol from the plasma membrane to the ER.

One important physiological role of p-gp, the protection of the organism against toxins, is achieved by exporting these compounds from the body, e.g. into the bile, urine, or gut. P-gp is suggested to be a transmembrane pump which removes unusual compounds including drugs from the cell membrane and cytoplasm. ATP hydrolysis provides the energy for active drug transport, which can occur against steep concentration gradients.

Expression of p-gp on the luminal surfaces of the epithelial cells of the small and large intestine, biliary ductules, and proximal tubules of the kidney, suggest a role in decreasing the absorption from the gut and/or the excretion of exogenous hydrophobic and amphipathic toxins. One favoured (though as yet unproven) model proposes that p-gp transports its substrates mainly by flipping them actively from the inner to the outer leaflet of the plasma membrane, which would result in a net efflux of the substrates (Higgins & Gottesman, 1992) (Figure. 2.2).

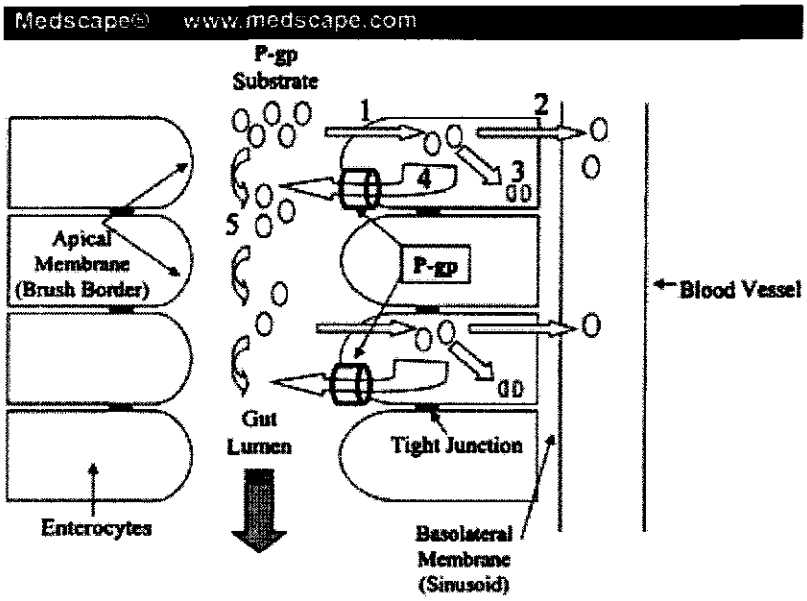


Figure 2.2: *Schematic of the role of p-gp intestinal disposition of substrate. (1) Absorption of p-gp substrate from intestinal lumen into enterocyte. (2) Absorption from enterocyte into the circulation. (3) Metabolism of substrate in the enterocyte. (4) Secretion of substrate back into the intestinal lumen facilitated by p-gp. (5) Movement of substrate through the intestinal lumen for elimination in faeces.*

The intestine, primarily regarded as an absorptive organ, is also prepared for the elimination of certain organic acids, bases and neutral compounds depending on their affinity to intestinal carrier systems.

Recently, an appreciation of the role of transporter p-gp and other transporters present in the intestinal epithelium as major determinants of absorption evolved. For example, two ABC proteins, ABCG5 and ABCG8, have been associated with the accumulation of dietary cholesterol in the sterol storage disease, sitosterolemia (Albrecht *et al.*, 2002). Sitosterolemia is a rare, autosomal recessive sterol storage disease characterised by tendon and tuberous xanthomas and by a strong predisposition to premature coronary atherosclerosis (Lee *et al.*, 2001). Increased levels of phytosterols (plant sterols) such as sitosterol and campesterol are found in blood, plasma, erythrocytes and especially in xanthomas and arteries of affected subjects. Increased intestinal absorption of phytosterols as well as decreased biliary and feacal excretion of cholesterol and phytosterols contribute to the abnormal lipid composition of blood and tissues from these patients. These two 'half-transporters"

ABCG5 and ABCG8, are assumed to dimerize to form the complete sitosterol transporter which reduces the absorption of sitosterol and related molecules in the intestine by pumping them back into the lumen. Cholesterol feeding up-regulates their expression in mice, implicating them in the intestinal absorption of sterols.

It is speculated that defective p-gp may cause ineffective transport of xenobiotics in the brain and carbohydrates and the other compounds in the intestine. A number of diseases involving abnormalities in the synthesis and degradation of glycoproteins have been recognised. Glycoproteins, like most other biomolecules, undergo both synthesis and degradation. Genetically determined defects of the activities of the enzymes involved in the degradation can occur, resulting in abnormal degradation of glycoproteins and this can eventually lead to various diseases. Based on the broad substrate specificity and tissue distribution of p-gp, modulation of p-gp activity, either intentionally or unintentionally, may result in substantial alterations in the disposition of p-gp substrates (Mayer *et al.*, 1997).

2.7.6 P-glycoprotein substrates and blockers

The number and variety of drugs and other compounds that can be transported by p-gp is truly staggering. The use of inhibitors for MDR proteins has allowed the demonstration of individual transporters being involved in the transport of specific substrates. It is yet unclear how p-gp can recognise and transport such a structurally diverse set of compounds ranging in size from about 250 Da (cimetidine (Pan *et al.*, 1994)) to more than 1850 Da (*gramicidin D*). Whatever the precise molecular mechanism of substrate transport, p-gp activity can mediate very effective extrusion of its substrates penetrating the plasma membrane, which results in very low intracellular levels of such substrates.

An interesting aspect of p-gp is the interaction with drug metabolising enzymes, specifically the 3A4 isoenzyme of CYP (CYP3A4). P-gp and CYP3A4 share many substrates and inhibitors and have a common tissue distribution (Wacher *et al.*, 1995; Watkins, 1992). They are both expressed in the intestinal mucosa. The isoenzyme of CYP3A4 accounts for approximately 70% of the total CYP activity in the intestine, and p-gp may act in concert with CYP3A4 to reduce systemic exposure to certain xenobiotics (Wacher *et al.*, 1995). It is likely that because p-gp can influence the intracellular concentration of many CYP3A4 substrates, it may also affect the availability of those substrates to CYP3A4 and therefore the extent of CYP3A4

metabolism of those substrates. P-gp thus plays an important role in modulating expression of CYP3A4 with respect to the amount of CYP3A4 substrates that it makes available, and the inhibition of p-gp is likely to complicate the prediction of drug and other compounds interactions among drugs and compounds that are substrates for both p-gp and CYP3A4 systems.

Many marketed drugs inhibit p-gp function, and several compounds are under development as p-gp inhibitors. Similarly, numerous drugs can induce p-gp expression. While p-gp induction does not yet have a significant therapeutic role, p-gp inhibition is an attractive therapeutic approach to reverse multidrug resistance.

Examples of drugs known as inhibitors of p-gp are verapamil, cyclosporine, erythromycin, ketoconazole and tamoxifen. The major drawback to the use of these inhibitors in a clinical setting stems from the relatively low p-gp inhibitory potency of these compounds, which results in the requirement for administration of high doses to inhibit p-gp function. At high systemic concentrations, the principal pharmacologic effect of these compounds (eg., cardiotoxicity from calcium channel blockers, immunosuppression from cyclosporine) becomes problematic. This limitation led to the development of novel (second-generation) compounds that exhibit less intrinsic toxicity at p-gp inhibitory concentrations than that of their "first-generation" counterparts. Although effective at restoring drug sensitivity in multidrug-resistant tumour cells, these second-generation p-gp inhibitors also inhibit p-gp in normal tissues. Early clinical trials of second-generation p-gp inhibitors administered concomitantly with chemotherapy have enhanced toxicity, including neutropenia and neurotoxicity.

Certain foods, such as grapefruit juice, are known to substantially alter the bioavailability of some drugs, therefore concomitant intake of grapefruit juice will alter the pharmacokinetics of such drugs. These effects may be mediated by interactions with enzyme systems, such as CYP P450, or with active transporter systems, such as p-gp. The exact constituents in grapefruit juice that are responsible for drug metabolism inhibition are not perfectly known. Grapefruit juice composition varies from brand to brand and from lot to lot and also depends on the preparation method (Ho *et al.*, 2000). However, in all cases, grapefruit juice contains different components, in the majority flavonoids and also furanocoumarin derivatives.

Studies have shown that there is no impact of oral grapefruit juice load on systemic clearance of a number of p-gp substrates indicating that grapefruit juice inhibitory effect on p-gp is localised at the intestine.

2.8 GRAPEFRUIT JUICE

Grapefruit juice, a beverage consumed in large quantities by the general population, carries the American Heart Association's healthy "heart-check" food mark and contains compounds that may both reduce atherosclerotic plaque formation (Cerda *et al.*, 1994), and inhibit cancer cell proliferation (So *et al.*, 1996; Guthrie & Carroll, 1998).

2.8.1 Chance discovery

Almost 14 years have passed since investigators by chance, observed an interaction between felodipine and grapefruit juice in a study of felodipine and ethanol that used grapefruit juice to mask the taste of ethanol (Bailey *et al.*, 1989). Subsequent studies confirmed that grapefruit juice significantly increased the oral bioavailability of felodipine (Bailey *et al.*, 1991; Edgar *et al.*, 1992). Thus a decade of grapefruit juice was launched. Investigation has focused on the mechanism of action, the substrates with which it interacts, and the specific components of grapefruit juice.

2.8.2 Mechanism of action

After uptake by the enterocytes, many endogenous substances, xenobiotics and toxins are either metabolised by CYP3A4 or pumped back into the lumen by the p-gp transporter. Thus CYP3A4 and p-gp may act in tandem as a barrier to oral delivery of many drugs.

A lot of compounds, especially medications such as itraconazole, ketoconazole, cyclosporine, diltiazem, and erythromycin inhibit both intestinal CYP3A4 and hepatic CYP3A4. Thus, the reduced presystemic drug metabolism increases the quantity of drug absorbed (increased oral bioavailability) (Kivisto *et al.*, 1997; Kivisto *et al.*, 1998; Floren *et al.*, 1997; Azie *et al.*, 1998). Grapefruit juice has now been recognised as an inhibitor of this intestinal enzyme system (Lundahl *et al.*, 1997). Given the overlap

in the substrate specificity between p-gp and CYP3A4, grapefruit juice might be expected to interact with this protein transporter also.

It has been shown that the effects of grapefruit juice on cyclosporin seemed independent of a reduction of intestinal CYP3A4 and suggested that there was *in vivo* inhibition of p-gp (Edwards *et al.*, 1999). Also, extracts of grapefruit juice have been alleged to inhibit p-gp function based on permeability in caco-2 cell monolayers (Takanaga *et al.*, 1998). Talinolol permeability increased in caco-2 monolayers when grapefruit juice was administered and binding data suggest some grapefruit juice components affect the binding of substrates to p-gp (Langguth *et al.*, 1998). Many, if not all, of the known clinical interactions with grapefruit juice are with compounds that are substrates of p-gp (Gottesman *et al.*, 1996).

Several findings indicate that grapefruit juice acts on the CYP system and p-gp at the intestinal level, not at the hepatic level. First, the medications and other compounds that interact with grapefruit juice undergo metabolism by the CYP3A4 enzyme system in the small bowel. Second, grapefruit juice increases the area under the plasma concentration-time curve (AUC), probably the best measure of the body's exposure to a drug, but minimal if any change in clearance or half-life occurs. Third, in standard doses, grapefruit juice has no effect on the pharmacokinetics of these medications when they are given intravenously (Lundahl *et al.*, 1997; Rashid *et al.*, 1995; Kupferschmidt *et al.*, 1998; Kupferschmidt *et al.*, 1995).

In a study of intravenous cyclosporin A (CsA) vs. oral, it was demonstrated that co-administration with grapefruit juice increased the oral absorption while the systemic clearance was unaffected, indicating that the effect of grapefruit juice was due to diminished enterocytes metabolism of CsA and inhibition of the intestinal p-gp (Bistrup *et al.*, 2001).

The effects of grapefruit juice on the intestinal expression of p-gp have been investigated and, unlike CYP3A4 immunoreactive protein, there was no difference in p-gp levels pre- and post-exposure (Lown *et al.*, 1997). Therefore, if any grapefruit juice constituents are capable of modulating p-gp function, this would be via direct competition for the efflux pump rather than down regulation of protein expression.

The majority of pharmacokinetic studies evaluating interactions between drugs and grapefruit juice have been performed using a single glass of juice (usually 200 ml). Many early studies, however, used frozen juice reconstituted with half the

recommended water ("double-strength" juice) as well as multiple glasses of juice (Bailey *et al.*, 1991; Bailey *et al.*, 1993). Rogers *et al.*, (1999) showed that the effects of the grapefruit juice seen on increased lovastatin bioavailability are much less with a single glass of juice taken approximately 12 hours before the drug than with 3 glasses of double-strength juice per study day.

2.8.3 Grapefruit juice components

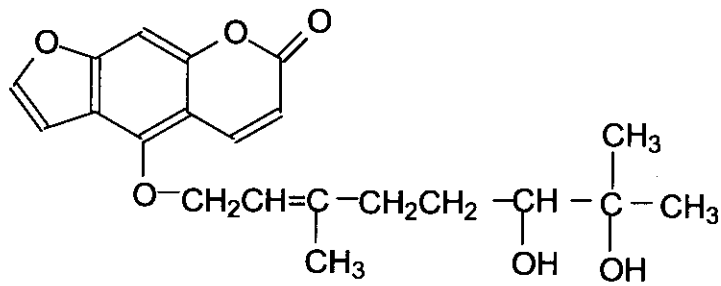
Many compounds have been proposed to be the active ingredients in grapefruit juice but the exact constituents that are responsible for drug metabolism inhibition have not been fully determined. The composition of the juice varies widely depending on the genetic background of the plant, environmental conditions during growth, fruit maturity and also on the preparation method (Ho *et al.*, 2000). Most of the components are found not only in grapefruit juice but also in other citrus fruits. As drug interactions are observed with grapefruit juice from different species, it appears that the substances responsible for the interaction are consistently present in the juice (Fuhr, 1998), though considerable variability in the concentrations of inhibitory substances has been demonstrated among commercial brands and batches of grapefruit juice (Fukuda *et al.*, 2000).

As mentioned earlier, the particular components responsible for the grapefruit juice interactions have not been fully elucidated. Initially, the predominant flavonoid in the juice (naringin) and its aglycone, naringenin (Figure 2.3), were suggested as potential inhibitors. Grapefruit contains several flavonoids, mainly as glycosides, which are hydrolysed by intestinal microflora, to the corresponding aglycones and sugar (Fuhr & Kummert, 1995). These molecules are polyphenolic and electron-rich, implying a potential to act as substrate inhibitors for the CYP enzymes. Naringin constitutes up to 10% of the dry weight of grapefruit juice with a concentration in juice of 450 μg/ml. Naringin is the compound that gives grapefruit juice its distinctive smell and bitter taste and it is not found in other citrus or fruit juices. The aglycone, naringenin, thought to be formed from naringin in the intestine after oral administration (Ameer *et al.*, 1996), has been shown to be a more potent inhibitor of CYP3A4-mediated metabolism (*in vitro*) than naringin (Ho *et al.*, 2000). Naringenin also inhibits p-gp action. It was found by Mitsunaga *et al.*, (2000) that naringenin increased the uptake of vincristine into MBEC4 cells, indicating inhibition of p-gp activity. It has also been found that naringenin decreased the basolateral/apical transport of cyclosporine

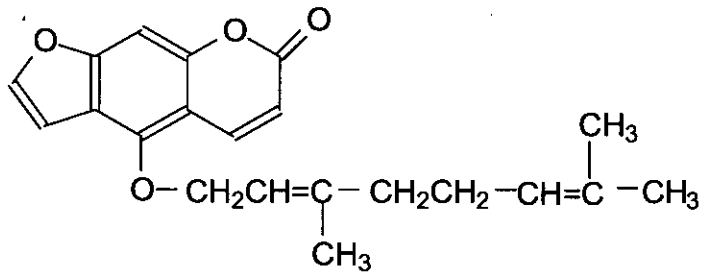
across caco-2 cells in a concentration dependent manner (Janse van Vuuren, 2000:62).

Flavonoids are, however, not the only group of compounds in grapefruit juice that have been implicated in the grapefruit interaction with the intestinal p-gp and CYP enzyme. The second group, the furanocoumarins (eg bergamottin, 6',7'-dihydroxybergamottin, 8-methoxypsoralen and bergapten), are mechanism-based or suicide inhibitors of CYP3A4. According to Edwards *et al.*, (1999), of these compounds, 6',7'-dihydroxybergamottin (Figure 2.3) or its parent compound bergamottin have been implicated to be the main substrate in grapefruit juice responsible for the interaction. Bergamottin is the furanocoumarin found in the highest concentration in fresh grapefruit. It is present in similar quantities in grapefruit juice and grapefruit segments and to a lesser degree in peel extract (He *et al.*, 1998; Bailey *et al.*, 2000). Some data from Bailey *et al.*, (1998), however, suggest that 6',7'-dihydroxybergamottin is not the major active ingredient causing drug-grapefruit juice interaction as an inhibitor of CYP3A4. Wang, *et al.*, (2001), found that bergamottin caused concentration dependent inhibition of p-gp function in the G185 cell line. Bergamottin may therefore be a major cause (or contributor) of the known clinical interactions previously suspected to be CYP3A4 mediated (Wang *et al.*, 2001). This is in accordance to the findings of Janse van Vuuren, (2000:73).

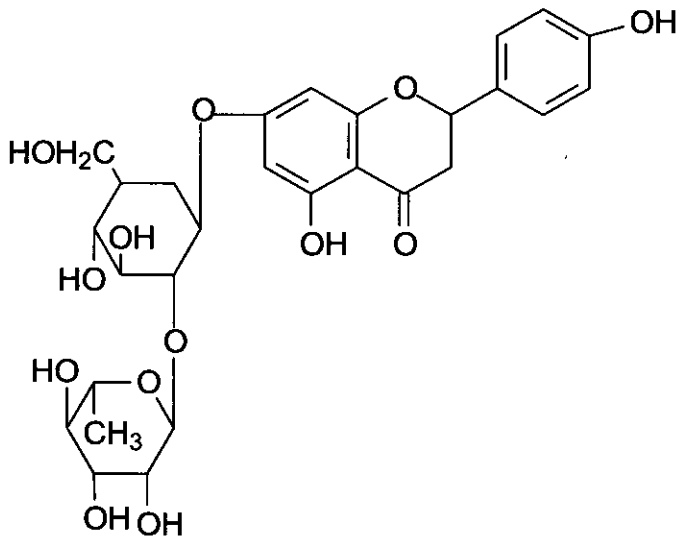
Many of these constituents of grapefruit juice are present as a mixture of chiral isomers that vary markedly in proportion and concentration, depending on the maturity of the fruit and the method of juice extraction and purification (Schmiedlin-Ren *et al.*, 1997; He *et al.*, 1998; Vanakoski *et al.*, 1996). It is possible that the inhibition of first–phase intestinal metabolism and p-gp transporter by grapefruit juice is mediated by a combination of flavonoid and furanocoumarin compounds and does not occur in isolation.



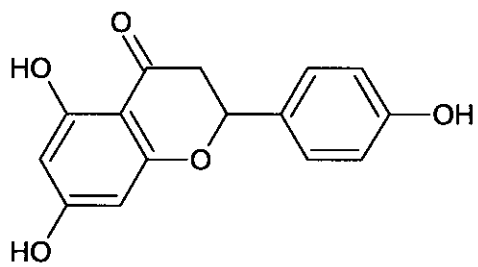
6',7'-Dihydroxybergamottin



Bergamottin



Naringin



Naringenin

Figure 2.3: *Chemical structure of some grapefruit components.*

Chapter 3

An overview of metabolism and biochemical energy

3.1 GENERAL

Metabolism comprises the sum total of the various chemical transformations in the body. The fate of dietary components after digestion and absorption constitutes intermediary metabolism. Metabolic pathways fall into three categories as discussed below.

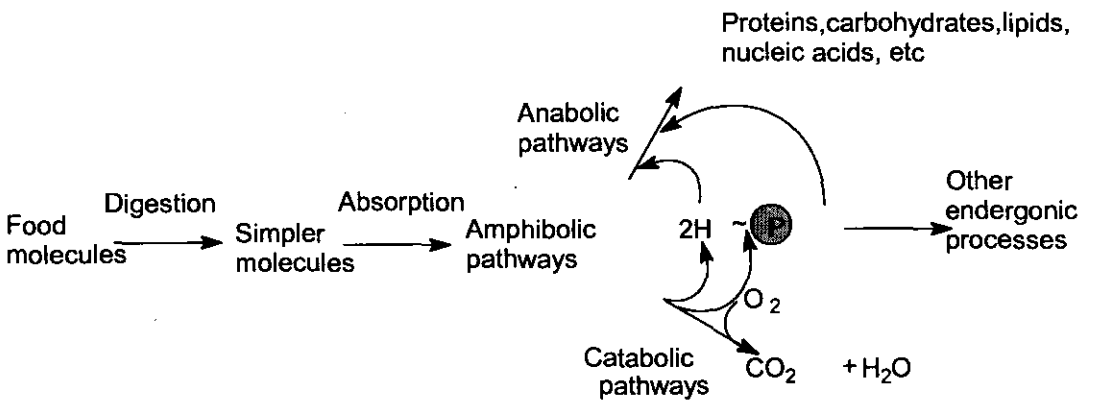


Figure 3.1: *The three major categories of metabolic pathways. Catabolic pathways release free energy in the form of reducing equivalents (2H) or high-energy phosphate (~P) to power the anabolic pathways. Amphibolic pathways acts as links between the other two categories of pathways.*

3.1.1 **Anabolic pathways** are those involved in the synthesis of the compounds constituting the body's structure and machinery. The free energy required for these processes comes from the catabolic pathways.

3.1.2 **Catabolic pathways** involve oxidative processes that release free energy, usually in the form of high-energy phosphate or reducing equivalents, eg, the respiratory chain and oxidative phosphorylation.

3.1.3 **Amphibolic pathways** have more than one function and occur at the "crossroads" of metabolism, acting as links between the anabolic and catabolic pathways, eg, the citric acid cycle.

Knowledge of metabolism in the normal animal is a prerequisite to a sound understanding of abnormal metabolism underlying many diseases (Murray *et al.*, 1996:158).

3.2 DIGESTION

Most foodstuffs are ingested in forms that are unavailable to the organism, since they cannot be absorbed from the digestive tract until they have been broken down into smaller molecules. This disintegration of the naturally occurring foodstuffs into assimilable forms constitutes the process of digestion, thus digestion involves splitting of food molecules by hydrolysis into smaller molecules that can be absorbed through the epithelium of the gastrointestinal tract. The chemical changes incident to digestion are accomplished with the aid of hydrolase enzymes of the digestive tract that catalyse the hydrolysis of native proteins to amino acids, of starches to monosaccharides, and of triacylglycerols to monoacylglycerols, glycerol, and fatty acids. In the course of these digestive reactions, the minerals and vitamins of the foodstuffs are also made more assimilable (Murray *et al.*, 1996:635).

3.3 ABSORPTION FROM THE GASTRO-INTESTINAL TRACT

The small intestine is the main absorptive organ. About 90% of the ingested foodstuffs are absorbed in the course of passage through the small intestine. There

are two pathways for the transport of materials absorbed by the intestine: the hepatic portal system, which leads directly to the liver, transporting water-soluble nutrients and the lymphatic vessels, which lead to the blood by way of the thoracic duct and transport lipid-soluble nutrients (Murray *et al.*, 1996:641).

3.3.1 Absorption of products of carbohydrate digestion

The products of carbohydrate digestion are absorbed from the jejunum into the blood of the portal venous system in the form of monosaccharides - chiefly as hexose (glucose, fructose, mannose, and galactose) and as pentose sugars (ribose).

Two mechanisms are responsible for the absorption of monosaccharides: "active transport" against a concentration gradient and "simple diffusion". The brush border of the enterocyte contains several transporter systems, some very similar to those of the renal brush border membranes, which specialize in the uptake of the different amino acids and sugars. A sodium-dependent glucose transporter (SLGT 1) binds both glucose and Na^+ at separate sites and transports them through the plasma membrane of the intestinal cell. It is envisaged that both glucose and Na^+ are released into the cytosol, allowing the transporter to take up more "cargo". The Na^+ is transported down its concentration gradient and at the same time causes the transporter to carry glucose against its concentration gradient. The free energy required for this active transport is obtained from the hydrolysis of ATP linked to a sodium pump that expels Na^+ from the cell in exchange for K^+ (Murray *et al.*, 1996:641; Morgan, 1986:232).

3.3.2 Absorption of products of lipid digestion

The 2-monoacylglycerols, fatty acids, and small amounts of 1-monoacylglycerols leave the oil phase of the lipid emulsion and diffuse into the mixed micelles and liposomes consisting of bile salts, phosphatidylcholine, and cholesterol, furnished by the bile. Because the micelles are soluble, they allow the products of digestion to be transported through the aqueous environment of the intestinal lumen to the brush border of the mucosal cells where they are absorbed into the intestinal epithelium (Murray *et al.*, 1996:642).

3.3.3 Absorption of products of protein digestion

Under normal circumstances, dietary proteins are almost completely digested to their constituent amino acids, and these end products of protein digestion are then rapidly absorbed from the intestine into the portal blood. Amino acids are transported through the brush border by a multiplicity of carriers (transporters), many having Na^+ - dependent mechanisms similar to the glucose transporter system. Of the Na^+-dependent carriers, there is a neutral amino acid carrier, a phenylalanine and methionine carrier, and a carrier specific for imino acids such as proline and hydroxyproline. Na^+-independent carriers specialising in the transport of neutral and lipophilic amino acids (eg, phenylalanine and leucine) or of cationic amino acids (eg, lysine) have been characterised (Murray *et al.*, 1996:643).

It has been documented that undigested polypeptides may cause antigenic reactions. Individuals in whom an immunologic response to ingested protein occurs must be able to absorb some unhydrolysed protein, since digested protein is nonantigenic (Murray *et al.*, 1996:646). The absorption of the unhydrolysed protein may be due to defective dipeptide carriers or p-gp since such compounds would have been effluxed back into the gut normally.

There is increasing support for the hypothesis that basic defect in "nontropical sprue" is located within the mucosal cells of the intestine and permits the polypeptides resulting from the peptic and tryptic digestion of gluten, the principal protein of wheat, not only to exert a local harmful effect within the intestine but also to be absorbed into the circulation and thus to elicit the production of antibodies. It has been established that circulating antibodies to wheat gluten or its fractions are frequently present in patients with nontropical sprue. The harmful entity is a peptide composed of six or seven amino acids (Murray *et al.*, 1996:646).

3.4 THE BASIC METABOLIC PATHWAYS PROCESS THE MAJOR PRODUCTS OF DIGESTION

The nature of the diet sets the basic pattern of metabolism in the tissues. Mammals such as humans need to process the absorbed products of digestion of dietary

carbohydrate, lipid and protein. All the products of digestion are processed by their respective metabolic pathways to a common product, acetyl-CoA, which is then completely oxidised by the citric acid cycle (Figure 3.2).

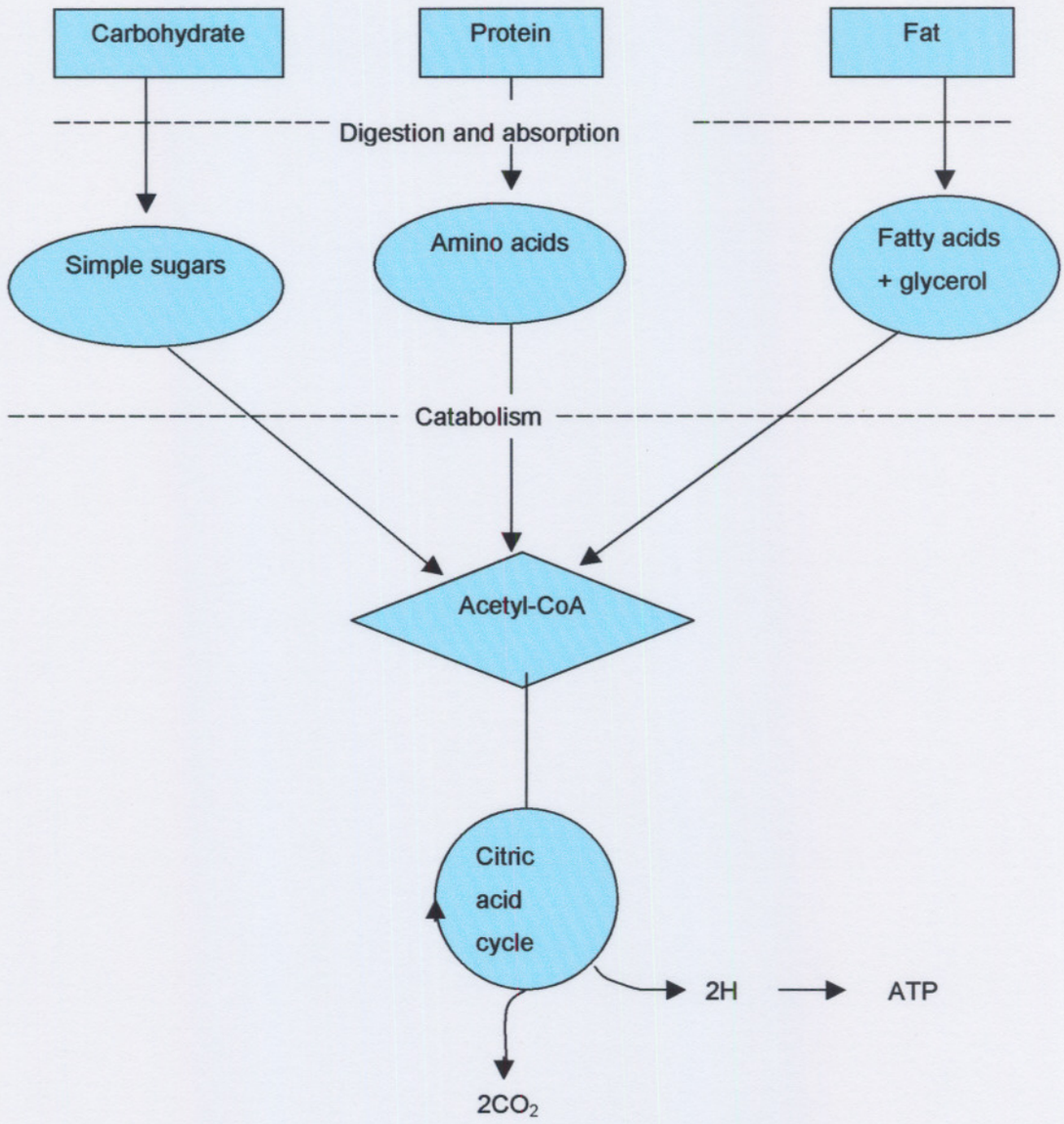
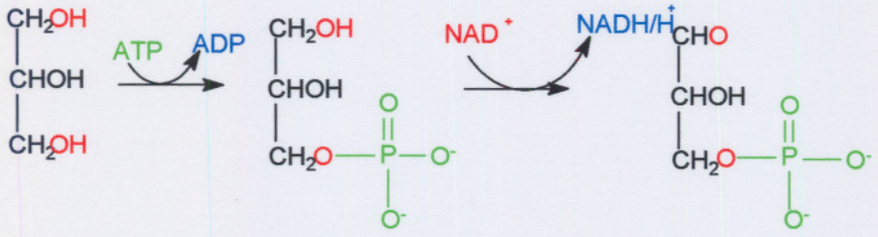


Figure 3.2: *Outline of the pathways for the catabolism of dietary carbohydrate, protein, and fat. All the pathways lead to the production of acetyl-CoA, which is oxidised in the citric acid cycle, ultimately yielding ATP in the process of oxidative phosphorylation.*

3.4.1 Catabolism of fats: β-Oxidation

The catabolism of fats and oils (triacylglycerols) begins with their digestion in the intestine in which ester bonds are hydrolysed to give 2-monoacylglycerols, fatty acids, and 1-monoacylglycerols. Glycerol is then first phosphorylated by reaction with ATP after which it is oxidised to yield glyceraldehyde 3-phosphate, which enters the carbohydrate catabolic pathway.



Glycerol **Glycerol monophosphate** **Glyceraldehyde 3-phosphate**

Fatty acids are catabolised by a repetitive four-step sequence of enzyme-catalysed reactions called the *fatty acid spiral*, or the **β-oxidation pathway**. Each passage along the pathway results in the cleavage of a two-carbon acetyl group from the end of the fatty acid chain, until the entire molecule is ultimately degraded. As each acetyl group is produced, it enters the citric acid cycle and is further catabolised (McMurry, 1996:1177). The process is illustrated as follows:

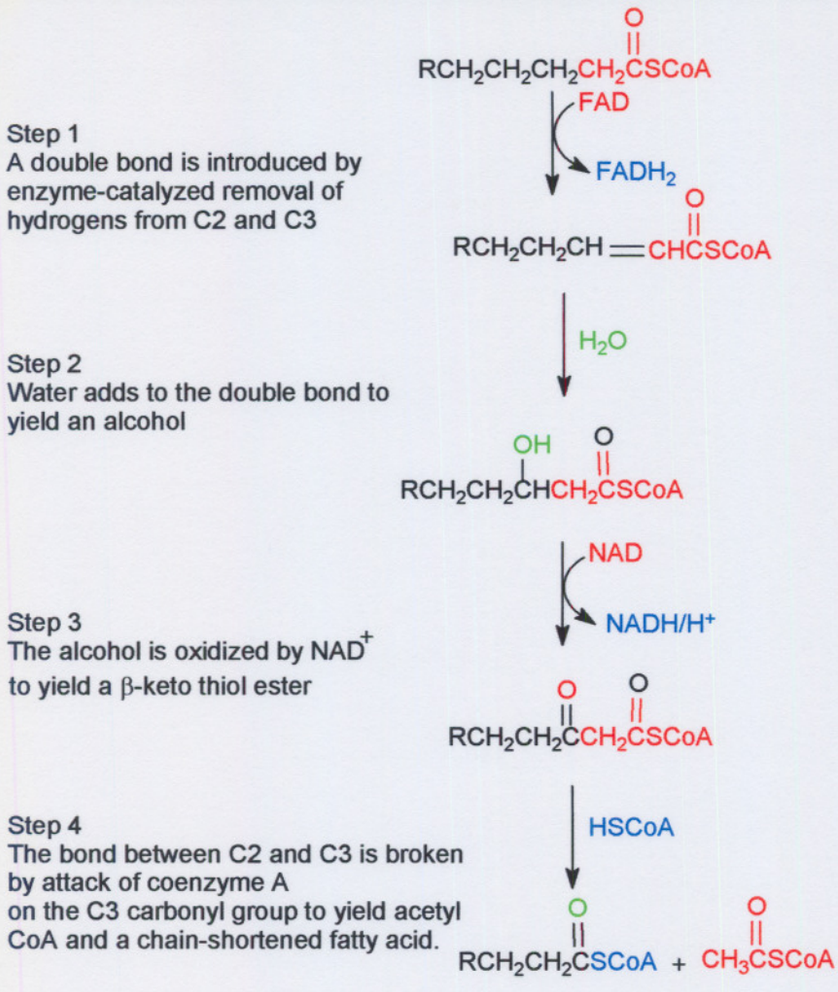
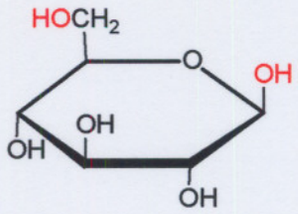


Figure 3.3: *The four steps of the β-oxidation pathway, resulting in the cleavage of a two-carbon acetyl group from the end of the fatty acid chain.*

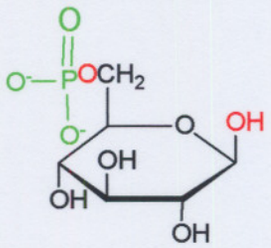
3.4.2 Catabolism of carbohydrates: Glycolysis

Glycolysis is the conversion of glucose (or of other hexoses) to pyruvate. It is a series of 10 enzyme-catalyzed reactions that break down glucose into two equivalents of pyruvate, $CH_3COCO_2^-$ (McMurry, 1996:1182). Glycolysis is a key reaction of metabolism. It takes place in almost all living cells. In consecutive metabolic steps, pyruvate is oxidised in the citrate cycle, or with insufficient oxygen supply, converted to lactate or to ethanol in order to reconstitute NAD^+, which is required for further progress of glycolysis.

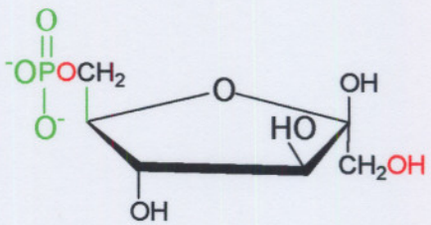


Glucose

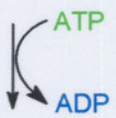
Step 1
Glucose is phosphorylated by reaction with ATP to yield glucose 6-phosphate.

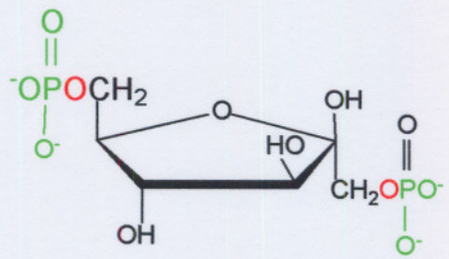


Step 2
Glucose 6-phosphate is isomerized to fructose 6-phosphate.

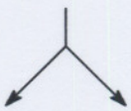


Step 3
Fructose 6-phosphate is phosphorylated by reaction with ATP to yield fructose 1,6-bisphosphate.

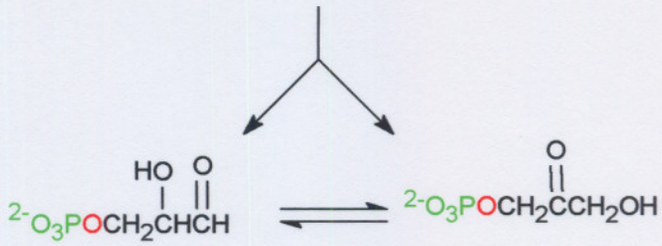




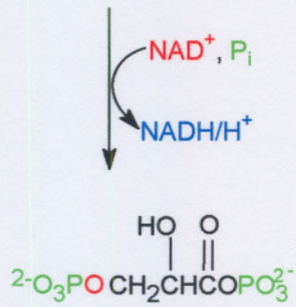
Step 4
Fructose 1,6-bisphosphate is cleaved into two three-carbon pieces by the enzyme aldolase.



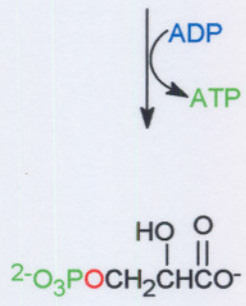
Step 5
Dihydroxyacetone phosphate, one of the products of step 4, is isomerized to glyceraldehyde 3-phosphate, the other product of step 4.



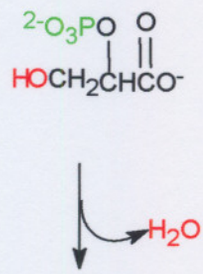
Step 6
Glyceraldehyde 3-phosphate is oxidized and phosphorylated to yield 3-phosphoglyceroyl phosphate.



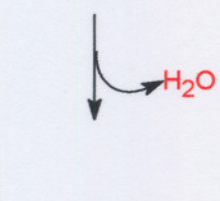
Step 7
A phosphate is transferred from the carboxyl group to ADP, resulting in synthesis of an ATP and yielding 3-phosphoglycerate.



Step 8
A phosphate group is transferred from the C3 hydroxyl to the C2 hydroxyl, giving 2-phosphoglycerate.



Step 9
Dehydration occurs to yield phosphoenolpyruvate (PEP)



Step 10
A phosphate is transferred from PEP to ADP, yielding pyruvate and ATP

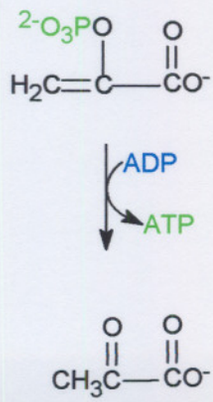
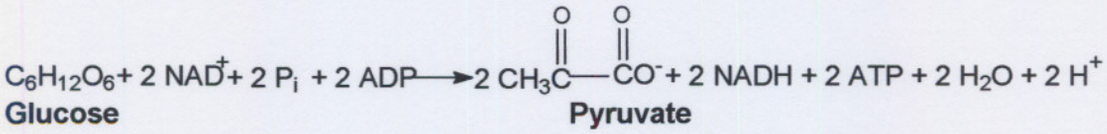


Figure 3.4: *The ten-step glycolysis pathway for catabolizing glucose to pyruvate.*

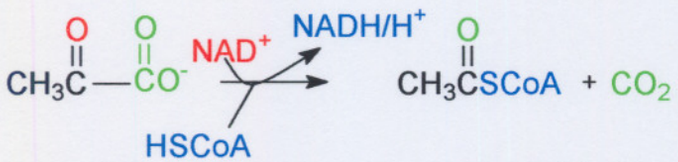
The net result of glycolysis can be summarised by the following equation:



Glucose **Pyruvate**

3.4.3 The conversion of pyruvate to Acetyl CoA

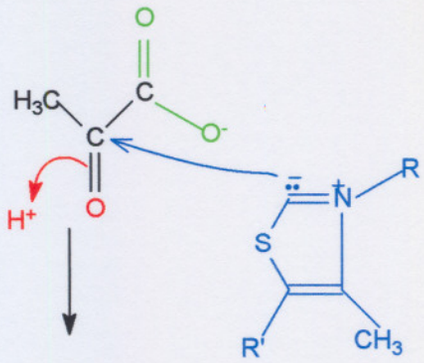
Pyruvate produced in the catabolism of glucose can undergo several further transformations depending on the conditions and on the organism. Most commonly, pyruvate is converted to Acetyl CoA through a multistep sequence of reactions that requires three different enzymes and four different coenzymes (Morgan, 1986:46).



Pyruvate **Acetyl CoA**

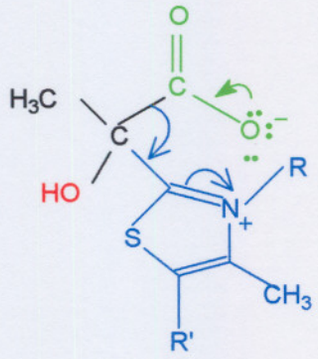
The process is illustrated in figure 3.5.

Step 1
Addition of thiamine pyrophosphate to the ketone carbonyl group of pyruvate yields an intermediate addition product.

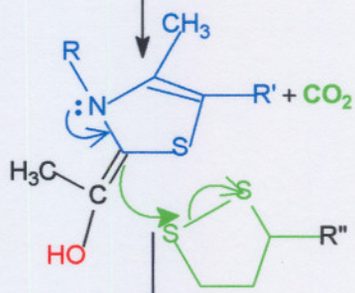


Thiamine pyrophosphate

Step 2
Decarboxylation occurs, yielding an enamine intermediate.

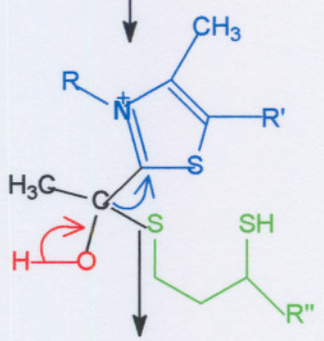


Step 3
The enamine double bond attacks a sulfur atom of lipoamide.

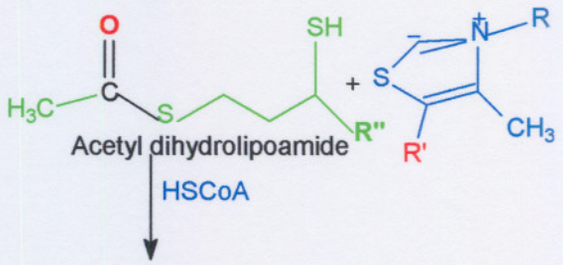


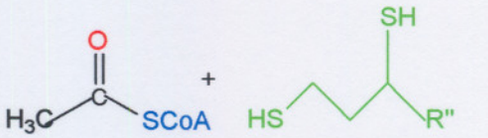
Lipoamide

Step 4
Elimination of thiamine pyrophosphate from the tetrahedral intermediate then yields acetyl dihydrolipoamide.



Step 5
Reaction with coenzyme A gives acetyl CoA and dihydrolipoamide.





Acetyl CoA Dihydrolipoamide

Figure 3.5: *Mechanism of the conversion of pyruvate to acetyl CoA. The multistep sequence of reactions requires three different enzymes and four different coenzymes.*

3.4.4 The citric acid cycle

The first two stages of catabolism result in the conversion of fats and carbohydrates into acetyl groups that are bonded through a thiol ester link to coenzyme A. These acetyl groups then enter the third stage of catabolism – the **citric acid cycle**, also called the *tricarboxylic acid (TCA) cycle* or *Krebs cycle* (Morgan, 1986:49).

The citric acid cycle is a closed loop of reactions in which the product of the final step is a reactant in the first step. The intermediates are constantly regenerated and flow continuously through the cycle, which operates as long as the oxidising coenzymes NAD^+ and FAD are available. To meet this condition, the reduced coenzymes NADH and $FADH_2$ must be reoxidised via the respiratory chain, which in turn relies on oxygen as the ultimate electron acceptor. The net result of the cycle can be summarised as:

$$\text{Acetyl CoA} + 3\ NAD^+ + FAD + ADP + P_I + 2\ H_2O \rightarrow HSCoA + 3\ NADH + 3\ H^+ + FADH_2 + ATP + 2\ CO_2$$

The cycle is illustrated as follows:

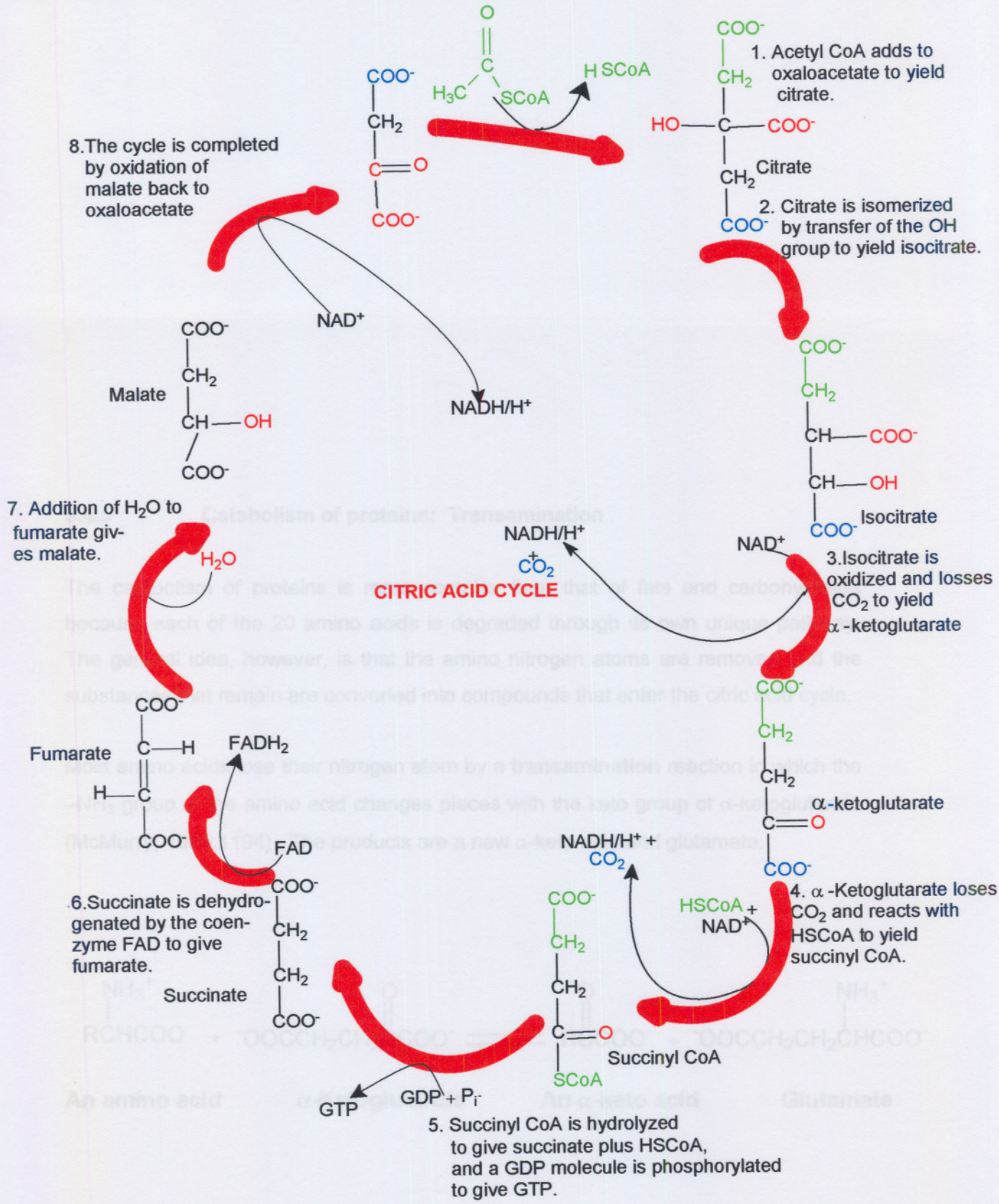


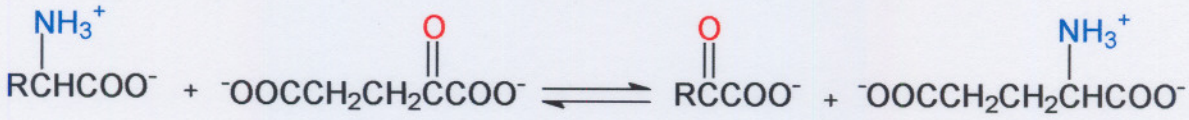
Figure 3.6: *The citric acid cycle, an eight step series of reactions that results in the conversion of an acetyl group into two molecules of CO_2 plus reduced coenzymes.*

which now contains the nitrogen atom of the former amino acid, next undergoes an oxidative deamination to yield ammonium ion and regenerated α-ketoglutarate.

3.4.5 Catabolism of proteins: Transamination

The catabolism of proteins is more complex than that of fats and carbohydrates because each of the 20 amino acids is degraded through its own unique pathway. The general idea, however, is that the amino nitrogen atoms are removed and the substances that remain are converted into compounds that enter the citric acid cycle.

Most amino acids lose their nitrogen atom by a **transamination** reaction in which the $-NH_2$ group of the amino acid changes places with the keto group of α-ketoglutarate (McMurry, 1996:1194). The products are a new α-keto acid and glutamate:



An amino acid **α-Ketoglutarate** **An α-keto acid** **Glutamate**

Transaminations use pyridoxal phosphate, a derivative of vitamin B_6, as cofactor. The key step in transamination is the addition of the amino acid $-NH_2$ group to the pyridoxal aldehyde group to yield a product that is again hydrolysed to yield pyruvate and a nitrogen-containing derivative of pyridoxal phosphate. Pyruvate is converted into acetyl CoA, which enters the citric acid cycle for further catabolism. The pyridoxal phosphate derivative transfers its nitrogen atom to α-ketoglutarate thereby forming glutamate and regenerating pyridoxal phosphate for further use. Glutamate, which now contains the nitrogen atom of the former amino acid, next undergoes an oxidative deamination to yield ammonium ion and regenerated α-ketoglutarate.

3.5 ORGANIC ACIDS IN URINE

The term "organic acid" refers to a broad class of compounds used in fundamental metabolic processes of the body. Unlike amino acids and fatty acids, the category of compounds called organic acids contains no essential nutrients. Instead of tests that measure nutrient concentrations, abnormal concentrations of organic acids in urine provide functional markers for the metabolic effects of vitamin inadequacies, toxic exposure, neuroendocrine activity, and bacterial overgrowth. The ultimate tool for laboratory evaluations in nutritional medicine is a simple, sensitive test that can reveal evidence of functional inadequacy of specific nutrients. The promise of such a tool is found in profiling of organic acids in urine (Bralley & Lord, 2003:4).

All bodily functions are powered by the release of chemical energy. The energy is released through a process of controlled oxidation where chemical bonds are broken and energy is released. Fats, carbohydrates, and amino acids are converted into carboxylic acids before they flow on to the final conversion to carbon dioxide. The organic acids that are formed as intermediates in this process are normally absent from urine or present at very low concentrations. Some of the organic acids that can be found in urine are mentioned below:

Fatty Acid Oxidation	Adipate
	Suberate
	Ethylmalonate
Carbohydrate Metabolism	Pyruvate
	Lactate
	α-Hydroxybutyrate
	β-Hydroxybutyrate
Energy Production (Citric Acid Cycle)	Citrate
	cis-Aconitate
	Isocitrate
	α-Ketoglutarate
	Succinate
	Fumarate

	Malate
	Hydroxymethylglutarate
B-complex Vitamin Markers	α-Ketoisovalerate
	α-Ketoisocaproate
	α-Keto-β-methylvalerate
	Xanthurenate
	β-Hydroxyisovalerate
Detoxication Indicators	Orotate
	2-Methylhippurate
	Glucarate
	Pyroglutamate
	Sulfate
Methylation Cofactor Markers	Methylmalonate
	Formiminoglutamate
Neurotransmitter Metabolism	Vanilmandelate
	Homovanillate
	5-Hydroxindoleacetate
	Kynurenate
	Quinolinate
Oxidative Damage and Antioxidant Markers	p-Hydroxyphenyllactate
	8-Hydroxy-2'-deoxyguanosine

When specific reactions are blocked due to the absence of sufficient enzyme or cofactor, or an abnormal substrate/compound is present, the intermediates that are formed or that precede the blocked step accumulate and spill into urine.

Urinary organic acid analysis for metabolic profiling has traditionally been used for detection of neonatal inborn errors of metabolism. Organic acid profiling has been useful in identification of the source of toxicants from the environment (Ong *et al.*, 1994) and the gut (Goodwin *et al.*, 1994).

3.5.1 Intestinal Dysbiosis Markers

The organic acids termed as intestinal dysbiosis markers are normally found only in the faeces. Their appearance in urine is an indication of overgrowth of microbes that inhabit the lumen of the gut or as it is being investigated in this study, genetic disorder of the system (mainly p-gp), that extrudes toxic materials from the epithelial wall of the intestine. The abnormal overgrowth of unfavourable microflora in the small and large intestine is referred to as "gut dysbiosis".

Most ingested food is absorbed from the small intestine. The residue including the food that is not completely digested passes into the large intestine. Here, considerable absorption of water takes place, and the semiliquid intestinal contents gradually become more solid. During this period, considerable bacterial activity occurs. By fermentation and putrefaction, the bacteria produce various gases, such as CO_2, methane, hydrogen, nitrogen, and hydrogen sulphide, as well as acetic, lactic, propionic, butyric acids and other organic acids as discussed below. The specific compounds that are formed depend on the starting material and the species of organism present. The presence of the following compounds in significant concentration in the urine is indicative of pathological conditions.

3.5.1.1 Hydroxyphenylacetate

Clostridium difficile is known to be involved in transamination and decarboxylation to form p-hydroxyphenylacetate (HPA) and p-cresol respectively. *Proteus vulgaris* can do only the transamination, so HPA increases when *Proteus vulgaris* is the predominant organism. When *P. vulgaris* is accompanied by overgrowth of a newly identified strain of *lactobacillus*, however, p-cresol will be the major product to accumulate (Bralley & Lord, 2003:24). HPA is elevated in a wide variety of conditions involving direct intestinal pathology or digestive organ failure.

3.5.1.2 Benzoate and Hippurate

Bacterial deamination of the amino acid phenylalanine produces benzoate, which is conjugated with glycine in the liver to form hippurate. Glycine and pantothenic acid are the limiting factors in this process (Temellini *et al.*, 1993).

3.5.1.3 Phenylacetate and Phenylpropionate

Intestinal bacterial action on phenylalanine also causes the formation of phenylacetate and phenylpropionate. Excretion of these compounds in urine is markedly increased after the gastrointestinal tracts of germ-free rats are inoculated with faecal micro-organisms, indicating their microbial origin (Goodwin *et al.*, 1994). For individuals with normal, healthy intestinal function, phenylacetate and phenylpropionate should not appear at more than background concentrations in urine. They are products of unidentified, specific strains of bacteria, marking an abnormal state when they appear elevated in urine.

3.5.1.4 p-Cresol, p-Hydroxybenzoate, and p-Hydroxyphenylacetate

Tyrosine from dietary protein is the parent compound from which p-cresol, p-hydroxybenzoate, and p-hydroxyphenylacetate are formed. The compounds are not products of normal human metabolism but are produced by bacteria and protozoa that can populate the gut. Cresol has a chemical structure very similar to phenol and is highly toxic. Cresol excretion is not affected by dietary protein intake, suggesting that the bacteria responsible reside in the lower portion of the intestine (Bures *et al.*, 1990). Strains of *Escherichia coli* can produce p-hydroxybenzoate from glucose (Barker & Frost, 2001).

p-Hydroxyphenylacetic aciduria has been found useful in detecting small bowel disease associated with *Giardia lamblia* infestation, ileal resection with blind loop, and other diseases of the small intestine associated with anaerobic bacterial overgrowth (Chalmers *et al.*, 1979).

3.5.1.5 Indican

Bacteria in the upper bowel produce the enzymes that catalyse the conversion of tryptophan to indole. Absorbed indole is converted in the liver to indoxyl, which is then sulfated to allow for urinary excretion. Indoxyl sulfate (also known as indican) is present in urine at low levels in healthy subjects because the upper bowel is sparsely populated with bacteria.

The interpretation of indican results can be complicated by impaired protein digestion or malabsorption, which increases the tryptophan available for bacterial action.

Patients with normal intestinal bacterial populations can show increased postprandial indican excretion when they fail to digest dietary protein. Indican evaluation has been used to assess intestinal absorption of tryptophan in scleroderma (Stachow *et al.*, 1976). Some degree of malabsorption was detected in 30% of an elderly population by combinations of indican with the shilling and other tests (Montgomery *et al.*, 1978).

3.5.1.6 D-Lactate

Lactobacillus acidophilus is highly competitive under carbohydrate-rich, acidic conditions and a major product of its growth is D-lactic acid. D-Lactate entering portal circulation can undergo hepatic conversion to carbon dioxide, but this pathway has limited capacity. This limitation is in contrast to the extremely large capacity for metabolism of the L-lactate isomer produced in skeletal muscle and other tissues.

Jejunoileostomy patients have the highest risk of developing D-lactic acidosis and the accompanying encephalopathy (Dahlquist *et al.*, 1984; Halverson *et al.*, 1984). They usually have some degree of carbohydrate malabsorption.

3.5.1.7 Tricarballylate

Tricarballylate (tricab) is produced by a strain of aerobic bacteria that quickly repopulates in the gut of germ-free animals (McDevitt & Goldman, 1991). Tricarb contains three carboxylic acid groups that are ionised at physiological pH to give a small molecule with three negative charges akin to the structure of the powerful chelating agent EDTA. Magnesium is bound so tightly by tricarb that magnesium deficiency results from overgrowth of tricarb-producing intestinal bacteria in ruminants (Schwartz *et al.*, 1988). This condition known as "grass tetany", is also accompanied by lower levels of calcium and Zinc, all of which can form divalent ion complexes with tricarb.

3.5.1.8 Dihydroxyphenylpropionate

Cases of confirmed *Clostridium* overgrowth show elevated levels of dihydroxyphenylpropionate. While other organisms may produce this compound, *clostridium* is the most commonly encountered genera. Various compounds closely related to dihydroxyphenylpropionate also are produced by the genus *Clostridium* (Elsden *et al.*, 1976).

3.5.1.9 Arabinitol

Among pathogenic yeasts and fungi, *Candida spp.* are of widest clinical concern because of their transmission by direct invasion of the gastro-intestinal tract and their ability to rapidly overwhelm immune responses in many hospitalised patients. Most species of *Candida* grow best on carbohydrate substrates. D-arabinitol (DA) is a metabolite of most pathogenic *Candida* species, in vitro as well as *in vivo* (Bralley & Lord, 2003:27)

3.5.1.10 Citramalate, β-Ketoglutarate, and Tartarate

Citramalate has been reported to be associated with fermentative intestinal overgrowth and is elevated in cerebrospinal fluid of patients with bacterial meningitis (Yeo *et al.*, 2000). The structures of these compounds are closely related to those of intermediates of normal human metabolism, and they are sometimes called anti-metabolites because they may block metabolic pathways through molecular mimicry. Tartarates have known toxicity related to metabolic interference (Robertson & Lonell, 1968). The pathways that they impact are those of central energy production.

Chapter 4

Experimental procedure

4.1 GENERAL

It was hypothesised that defects in the p-gp may be involved in many of the metabolic disorder diseases, since defective p-gp may cause the absorption of harmful substances, resulting in the development of diseases. To test this hypothesis, it was necessary to inhibit p-gp and to establish the effect of this inhibition on the metabolism of subjects.

Grapefruit juice has been recognised as an inhibitor of p-gp. The mechanism of the inhibition has been proven to be via direct competitive binding with p-gp at the intestinal level, but not at the hepatic level (Kupferschmidt *et al.*, 1998).

To effect inhibition of the intestinal p-gp, grapefruit juice was administered to healthy volunteers for five days. Urine samples were collected and analysed and compared with urine samples collected before the administration of the grapefruit juice (control). A significant difference was expected in the carbohydrate and organic acid urinary profile of the control and samples collected after administration of the grapefruit juice.

4.2 PROTOCOL

The protocol for this study and the informed consent form were approved by the medical ethical committee of the North-West University, Potchefstroom campus.

4.2.1 Selection of subjects

Subjects were randomly selected. Before participation, all subjects received written and oral information about the purpose and design of the study and their consent, or for the minor participants, their parents' or guardians' consent was obtained.

4.2.2 Inclusion and exclusion criteria

None of the subjects had a history of drug allergy, drug or alcohol abuse, or was on medication of any sort. Only non-smoking volunteers were included in the study. Individuals were excluded if they had used medications with long half-lives, such as astemizole, within 60 days prior to participation.

4.2.3 Subject responsibilities

In addition to the information given to volunteers about the purpose and design of the study, the following written instructions were also given to them before their participation in the study.

1) Please avoid intake of any medication three days before starting, during the participatory period and a week after the participatory period.

2) Should you require medication before one week had expired after taking the last grapefruit juice, first consult with your physician or pharmacist.

3) Avoid citrus juices seven days before and during the participatory period. Grapefruit juice consumption outside the protocol is prohibited during the study.

4) Avoid any caffeine containing food, snacks and drinks one day before and during the participatory period. Some of the caffeine containing products are: dark chocolate, chocolate milk beverage, milk chocolate, chocolate flavoured syrup, beverages from cocoa or cocoa products, Coca-Cola soft drink, tea and coffee.

5) Please avoid alcohol or xanthine - containing substances for the full duration of the study, and for at least 72 hours prior to the experimental days.

6) Please collect the first urine in the morning on any day before the intake of the grapefruit juice.

7) The grapefruit juice should preferably be taken in the evening and urine sample collected about eight hours after or all samples pooled up to eight hours from the time the juice is taken.

8) Please note down in writing the time the grapefruit juice is taken and the time the urine sample is collected.

4.2.4 Number of subjects

This study included twelve healthy volunteers consisting of six children and six adults of both sexes. Subjects ranged from 6 to 54 years of age.

4.2.5 Study design

4.2.5.1 Administration of grapefruit juice

A control urine sample was collected from each adult volunteer after which each subject took 500 ml of grapefruit juice every evening for the five day study period. Child volunteers were treated in the same way but received 250 ml of grapefruit juice. The grapefruit juice taken by all the volunteers for the study was from the same batch.

4.2.5.2 Collection of urine specimens

When only chemical examination of the urine is to be performed, there is no need for extensive procedures designed to guard against bacterial contamination. The morning urine specimen is the most concentrated urine obtainable and it has also become widely accepted for primary study of metabolic disorders. The first morning urine usually contains a greater array of metabolic by-products than at any other time of the day and is relatively free of the metabolic effects of strenuous muscle activity (Bralley & Lord, 2003:5).

The urine samples from each volunteer were collected just after eight hours or all samples pooled up to eight hours from the time in the evening the juice is taken on the first, third and fifth day of the study period into urine sample container. The samples were stored immediately after collection at -20^{o} C and thawed at room temperature just before analysis.

4.3 Materials

The grapefruit juice was from Magaliesberg Citrus Co-operative Limited, RSA. 3-Phenylbutyric acid, trimethylchlorosilane (TMCS) (Sigma T4252) and *bis*-(3-methylsilyl)-trifluoroacetamide (BSTFA) (Sigma T1506) were purchased from Sigma Chemical Co., St. Louis, MO., U.S.A. Anhydrous sodium sulphate, hydrochloric acid, ethylacetate and diethylether were purchased from Merck, Darmstadt, Germany. Butanol and sulphuric acid were purchased from Rochelle Chemicals, Johannesburg, R.S.A. Acetic acid was purchased from LabChem (pty) Ltd, Edenvale, Johannesburg, R.S.A., acetone from Associated Chemical Enterprises, Glenvista, R.S.A., while orcinol monohydrate was purchased from Acros Organics, New Jersey, USA.

All chemicals were of analytical grade.

4.4 INSTRUMENTATION

For thin layer chromatography (TLC), the equipment used were: TLC-silica plates (5553), glass developing tank with lid, Hamilton syringes, hair dryer, and oven (100° C).

The GC-MS system consisting of software, a 5973 Mass Selective Detector and a 6890 series GC system were all from Hewlett Packard, Palo Alto, CA. A Permabond^{R} SE30 fused silica capillary column, 0.25 μm (25 m x 0.32 mm ID) was used.

The mobile phase for the GC-MS system was helium at a flow rate of 2 ml/min. During the analysis, the temperature was programmed from 70° C (2 min) to 280° C at 5° C/min. The final temperature was maintained for 3 min. An ionisation energy of 70 eV was used.

4.5 URINARY ORGANIC ACID ANALYSIS

The analysis of organic acids by gas chromatography-mass spectrometry (GC-MS) has become well established as an important procedure for the diagnosis of disorders

of organic acid metabolism (Thompson & Markey, 1975). The important steps of the procedure are:

1) isolation of the organic acids from physiological fluids,

2) formation of volatile derivatives, and

3) GC-MS analysis.

The value of the analysis is enhanced if quantitative as well as qualitative results are obtained using international units of mmol/mol creatinine for urine. The most critical step for quantitative analysis is the method of isolation of the acids. This is commonly accomplished by solvent extraction (Thompson & Markey, 1975; Tanaka *et al.*, 1980) or anion-exchange chromatography (Chalmers & Watts, 1972). Anion-exchange chromatography does not give uniformly good recoveries of the acids because of their widely differing physical properties. In addition there may be interference from urea, sulphate, and phosphate with anion-exchange. It has been shown that diethyl ether extracts (a commonly used solvent) produce cleaner chromatograms (Gerson, 1985; Bowers & Canafax, 1984).

Volatile trimethylsilyl (TMS) derivatives of the dried extracted organic acids are formed by heating with *N,O*-bis-(trimethylsilyl)trifluoracetamide (BSTFA) which gives an earlier solvent front than the less volatile *N,O*-bis-(trimethylsilyl)acetamide (BSA). The TMSs are still the most useful and versatile derivatives for the wide range of chemical functional groups in organic acids (Ramsdell & Tanaka, 1977).

The most definitive identification of the acids is by GC-MS where the mass spectra provide a wealth of information. In addition, GC-MS enables the quantification of compounds from the areas of extracted ion chromatograms of ions unique (or nearly unique) for each compound, which effectively increases the chromatographic resolution of overlapping peaks in the total ion chromatogram (Gates *et al.*, 1978a,b; Hoffman *et al.*, 1989).

4.5.1 Gas Chromatography

Gas chromatography (GC) – specifically gas-liquid chromatography, is one of the most extensively used instrumental procedures in analytical work. It is, in essence, a form of column chromatography used for the separation of gases or vaporised chemicals, with some inert carrier gas as the mobile phase. As the carrier gas passes from the column, it enters a detector, which detects a substance that differs from the inert carrier gas to give evidence of that particular material. The sample is introduced into a stream of heated carrier gas (the mobile phase), where its components are volatilised and swept through the chromatographic column, which contains a stationary phase. In the GC column, the components are selectively retarded according to their interactions with the stationary phase. Generally, the detector picks up the signal. Samples can be identified by their time of detection under particular conditions, as compared to known materials.

4.5.1.1 Instrumental components

4.5.1.1.1 Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependent upon the type of detector that is being used (Chasteen, 2003).

4.5.1.1.2 Sample injection port

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour – slow injection of large samples causes band broadening and loss of resolution. The most common injection method is where a microsyringe is used to inject the sample through a rubber septum into a flash vapouriser port at the head of the column. The temperature of the sample port is usually about 50° C higher than the boiling point of the least volatile component of the sample. For capillary GC, split/splitless injection is used (Chasteen, 2003).

4.5.1.1.3 Columns

There are two general types of columns, packed and capillary (also known as open tubular). Packed columns contain a finely divided, inert, solid support material (commonly based on diatomaceous earth) coated with liquid stationary phase. Most packed columns are 1.5 – 10 m in length and have an internal diameter of 2 – 4 mm.

Capillary columns have an internal diameter of a few tenths of a millimeter. They can be one of two types; wall-coated open tubular (WCOT) or support-coated open tubular (SCOT). Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase. In support-coated columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns (Chasteen, 2003).

A new type of WCOT column was later devised – the Fused Silica Open Tubular (FSOT) column. This has much thinner walls than the glass capillary columns, and are strengthened by a polyimide coating. This column is flexible and can be wound into coils. It has the advantages of physical strength, flexibility and low reactivity (Chasteen, 2003).

4.5.1.1.4 Column temperature

For precise work, column temperature must be controlled to within tenths of a degree. The optimum column temperature is dependent upon boiling point of the sample. As a rule of thumb, a temperature slightly above the average boiling point of the sample results in an elution time of 2 – 30 minutes. Minimal temperatures give good resolution, but increase elution times. If a sample has a wide boiling range, then temperature programming can be used, where the column temperature is increased continuously or stepwise as the analysis proceeds (Chasteen, 2003).

4.5.1.1.5 Detectors

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. A non-selective detector responds to all compounds except the carrier gas. A selective detector responds to a range of compounds with a common physical or chemical property and a specific detector responds to a single chemical compound. Detectors can also be grouped into concentration dependent detectors and mass flow dependent detectors. The signal from a concentration dependent detector is related to the concentration of solute in the detector and does not usually destroy the sample. Dilution with make-up gas will lower the detectors response. Mass flow dependent detectors usually destroy the sample and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependent detector is unaffected by make-up gas (Sheffield Hallam University, 1998).

Some common GC detectors include flame ionisation detector (FID), thermal conductivity detector (TCD), electron capture detector (ECD), flame photometric detector (FPD), photo-ionisation detector (PID) and hall electrolytic conductivity. A few more unusual or very expensive detectors include the atomic emission detector (AED) and the ozone- or fluorine-induced chemiluminescence detectors. Another GC detector that is also very expensive but powerful is a scaled down version of the mass spectrometer. When coupled to a GC the detection system itself is often referred to as the mass selective detector or more simply the mass detector. This powerful analytical technique belongs to the class of hyphenated analytical instrumentation (since each part had a different beginning and can exist independently) and is called gas chromatography-mass spectrometry (GC-MS) (Sheffield Hallam University, 1998).

4.5.2 Mass Spectrometry

Mass spectrometry is a powerful tool for analysis and may be a viable clinical alternative for other analytical methods (Wu *et al.*, 1997). Mass spectrometric detectors can acquire multiple signals, but each signal is produced by an ion with a specific mass-to-charge (m/z) ratio. MS detection can help confirm peak identity using molecular weight information in addition to structural information from fragmentation and elemental information from the isotonic distribution. As alternative,

mass spectrometry offers a variety of advantages over other techniques: it is mass specific, rapid, and sensitive for picomole to femtomole quantities of material.

Placed at the end of a chromatographic column in a manner similar to the other GC detectors, the mass detector is more complicated than, for instance, the FID because of the mass spectrometer's complex requirements for the process of generation, filteration and detection of gas phase ions. A capillary column is most often used in the chromatograph because the entire MS process must be carried out at very low pressures (~10^{-5} torr) and in order to meet this requirement a vacuum is maintained via constant pumping using a vacuum pump. It is difficult for packed GC columns to be interfaced to an MS detector because they have carrier gas flow rates that cannot be as successfully pumped away by normal vacuum pumps, however, capillary columns' carrier flow is 25 or 30 times less and therefore easier to "pump down."

The high cost for the pump, ionisation source, mass filter or separator, ion detector, and computer instrumentation and software has limited the wide application of this system as compared to the less expensive GC detectors. However, the power of this technique lies in the production of mass spectra from each of the analytes detected instead of merely an electronic signal that varies with the amount of analyte. These data can be used to determine the identity as well as the quantity of unknown chromatographic components with an assuredness simply unavailable by other techniques (Sheffield Hallam University, 1998).

4.5.3 METHOD

4.5.3.1 Creatinine determinations

The concentrations of the organic acids were expressed in terms of the creatinine content of the urine. Therefore, creatinine was determined prior to urine organic acid extractions. A urine sample was diluted 20 times and the creatinine level measured according to the prescriptions of the manufacturer (Miles Inc., Tarrytown, NY) on a Technicon RA-100 analyser.

4.5.3.2 Organic acid extractions

The mg% creatinine value of the urine sample is used to determine the volume of urine taken for the analysis and it is calculated by multiplying the mmol/l creatinine determined by the technicon RA-100 analyser by 11.312. If the mg% creatinine value was less than 5 mg%, then 2 ml of urine was used. If the creatinine value was higher than 5 mg% and less than 100 mg%, then 1 ml of urine was used. And if the creatinine value was higher than 100 mg%, then 0.5 ml of urine was used. 3-Phenylbutyric acid (the volume used (µl) was calculated by multiplying the mg% creatinine value by 5) was added as internal standard into a 15 ml glass tube. The solution was acidified with 6 drops of 5 M hydrochloric acid and 6 ml distilled ethylacetate was added. The mixture was shaken for 30 minutes at 60 rpm with the aid of the roto-torque and centrifuged for 3 minutes (1000 rpm). The ethylacetate (organic) phase was aspirated into another clean 15 ml glass tube with a Pasteur pipette. 3 ml distilled diethylether was added to the remaining aqueous (lower phase) mixture which was again shaken for 10 minutes at 60 rpm with the aid of the roto-torque and centrifuged for 3 minutes (1000 rpm). The diethylether (organic) phase was aspirated into the tube containing the aspirated ethylacetate and two spatulas of anhydrous sodium sulphate was added to remove water from the combined organic phase. This tube was then vortexed for some seconds, centrifuged for 3 minutes (1000 rpm), decanted into a 10 ml glass tube and evaporated to dryness in a stream of dry nitrogen.

The dried residue was derivatized to the corresponding trimethylsilane (TMS) esters and ethers for GC-MS analysis with N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA), (the volume used (µl) was calculated by multiplying the mg% creatinine value by 3) and 3-methylchlorosilane (TMCS) (the volume used (µl) was calculated by multiplying the mg% creatinine value by 0.6). Derivatization took place as the mixture was incubated at 70° C for 45 minutes.

4.5.3.3 Qualitative and quantitative determination of the organic acids present in the urine.

After the incubation process, 1 µl of air was injected into the GC-MS followed by 1 µl external standard. Then another 1 µl of air was injected after which 0.4 µl of the derivatized compounds was injected. An ionisation energy of 70 eV was used.

The mass spectra obtained from the GC-MS analysis of the extracted and derivatized compounds in the urine were qualitatively and quantitatively elucidated with the use of the *Automated Mass Spectral Deconvolution and Identification System (AMDIS)* - a software programme that has been developed for spectral extraction and compound identification by GC-MS.

AMDIS is an integrated set of procedures that first extracts pure component spectra and related information from complex chromatograms and then uses this information to determine whether the component can be identified as one of the compounds represented in a reference library. The Whiley's MS-library was used to identify the compounds. The practical goal is to reduce the effort involved in identifying compounds by GC-MS while maintaining the high level of reliability associated with traditional analysis.

4.6 ANALYSIS OF SUGARS IN THE URINE

Simple sugars such as lactose, fructose and galactose are important compounds in human nutrition and metabolic activities in the body. The presence of a reducing substance other than glucose in the urine indicates abnormal metabolism of these sugars. Thin layer chromatography is one of the chromatographic techniques that has been used to identify sugars and it is the most suitable test for screening (Prinz *et al.*, 1978).

4.6.1 Thin layer chromatography

Despite the fact that thin layer chromatography has been supplanted to a large extent by more sophisticated techniques, it still finds application in the screening of sugars and some other analyses. One dimensional thin layer chromatography is a simple screening technique used in the identification of sugars present in urine. Sugars separate according to their mobility in a solvent as they are carried through a stationary phase. Thin layer chromatography as a primary diagnostic tool has the advantage of being a rapid, inexpensive method and only microlitre sample volumes are required. No pre-treatment is required for urine samples analysis.

4.6.2 Methods

The TLC-silica plate is cut into blocks of 10 cm x 10 cm and pencil lines of 1 cm in length, 0.5 cm apart, 1.5 cm from each side of the plate and 0.5 cm from the bottom of the plate are drawn. The volume of urine required for the analysis is applied to the pencil line with a Hamilton syringe and the spot dried continuously with a hair-dryer.

The mg% creatinine value of the urine sample is used as a standard to determine the volume of urine to be applied onto the TLC-plate using the formula below:

$2F / C \times 100 = \mu l$

F = Age factor (see below)

C = Creatinine concentration in mmol/L

	F
0 – 1 years	0.075
1 – 2 years	0.100
2 – 8 years	0.150
> 8 years	0.200

Standard solution (4 µl) is applied to the 1 cm line in the centre of TLC-plate and the spot also dried with the hair-dryer. The standard solution is composed of:

1 mg/ml xylose
1 mg/ml glucose
1 mg/ml galactose
1 mg/ml sucrose
1 mg/ml lactose

The TLC-plate is developed in a closed chamber saturated with the mobile phase consisting of freshly prepared butanol (50 ml), acetic acid (25 ml), and distilled water (25 ml) and removed as soon as the mobile phase reaches the top of the plate. It is allowed to dry for one hour and then replaced in the same mobile phase and the process repeated in the same direction of flow. The dried plate is then placed in the staining reagent (freshly prepared orcinol H_2SO_4-solution) and allowed to dry for 8 – 10 minutes in an oven at 100° C.

Chapter 5

Results and discussion

5.1 GENERAL

Correct treatment depends upon diagnosis. An error in, or an absence of, diagnosis leaves manifestations naked, pathogenesis is not reversed and cause continues to initiate the disease. This has led to a lot of deaths and chronic diseased state that could have been prevented were the treatment commenced early enough. An early and exact diagnosis of genetic metabolic diseases is in some cases essential for successful treatment and in all instances, it is important for good medical and psychosocial care of the patient and the family.

Mutations in genes are known to be causative in numerous diseases in the metabolic mosaic that underlies health (Blau *et al.*, 1996:XV). For example, the ability of p-gp to catalyse the efflux of xenobiotics has led to the proposal that the transporter normally functions in detoxification (Metherall *et al.*, 1996), therefore, any defect or mutation in the p-gp may lead to the accumulation of foreign compounds in the cell which may eventually lead to the emergence of diseases.

5.2 INHIBITION OF P-GLYCOPROTEIN IN THE INTESTINE

This study was designed to investigate the effect of intestinal p-gp inhibition on the urinary metabolic profile of subjects by examining the pattern of the results obtained with urine samples collected after grapefruit juice administration when compared to the controls. Deductions were to be made on the observations. It was hypothesized that defective p-gp could result in the manifestation of diseases because of the accumulation of toxic substances in the cells that might result from the defect and other several genetic disorders.

Inhibition of p-gp has been an attractive therapeutic approach to reverse some of the p-gp functions, one of which is multidrug resistance. There is now a considerable body of *in vitro* data suggesting that multidrug resistance due to p-gp can be effectively modulated by a range of drugs (Yanagisawa *et al.,* 1999). Many marketed drugs inhibit p-gp functions, examples of which are: i) calcium channel blockers (dihydropyridine analogues, i.e., verapamil) and ii) calmodulin antagonists (phenothiazines and thioxanthenes, i.e., trifluoperazine) (Ambudkar *et al.*, 1999). Almost all the compounds used to inhibit the functions of p-gp have the major setback that they cannot be administered to healthy subjects because of the manifestation of their intrinsic pharmacological actions at the concentration with which they can inhibit p-gp and this is not always desirable. The inhibition of p-gp in this study is effected by the administration of grapefruit juice to healthy human subjects, being a natural beverage commonly taken.

The inhibitory effect of grapefruit juice was first noticed serendipitously by Bailey and co-workers in 1989, during a trial in which the effect of ethanol on the haemodynamics of felodipine was investigated, using a grapefruit juice vehicle to mask the taste of ethanol (Bailey *et al.*, 1989). The administration of grapefruit juice as an inhibitor of p-gp is believed to have minimal adverse effects, if any, on the subjects. Its effect on p-gp has been proven to be limited to the intestine, thus it does not affect the p-gp functions in other tissues (Lundahl *et al.*, 1997) as other inhibitory agents of p-gp mentioned above. Grapefruit juice effects on the intestinal expression of p-gp has also been investigated by Lown *et al.*, (1997) and it was found that the mechanism of action is via direct competition for the efflux pump rather than down regulation of protein expression. This substantiates that grapefruit juice does not have the adverse effect of damaging the intestinal p-gp and that the inhibition is reversible. Wang *et al.,* 2001 documented that the presence of bergamottin, one of the constituents of grapefruit juice, causes a concentration-dependent increase in the rate of ATP hydrolysis relative to baseline rate, which indicates that it is a comparatively rapid substrate for p-gp.

The involvement of only healthy subjects, not taking any medication, was to assure that the p-gp in the intestine of these subjects was not down regulated and that no medication was present which might have competed with the grapefruit juice for the binding with p-gp or could act as a substrate for p-gp. Six adults (four males and two females) and six children (three females and three males) were included in the study. The ages of the children ranged from 5 years to 11 years and that of the adults from 26 years to 56 years as shown in tables 5.1 and 5.2.

Table 5.1: List of subjects (adult group) with their ages, sex and code used to identify each subject in this study.

Subjects (Adults)	Age (years)	Sex	Code
1	26	F	AF26
2	29	M	AM29
3	32	M	AM32
4	32	F	AF32
5	43	M	AM43
6	56	M	AM56

Table 5.2: List of subjects (children group) with their ages, sex and code used to identify each subject in this study.

Subjects (Children)	Age (years)	Sex	Code
7	5	F	CF5
8	7	F	CF7
9	7	M	CM7
10	9	F	CF9
11	10	M	CM10
12	11	F	CF11

5.3 RESULTS OF THE ANALYSIS OF SUGARS IN THE URINE

Carbohydrates play an important role in human metabolism. In addition to the role of glucose in energy homeostasis, many simple carbohydrates are derived from the diet. The presence of carbohydrates in the urine, other than a certain amount of glucose, is usually indicative of disorders in the metabolism of the sugars.

To identify the sugars present in the urine samples collected from subjects before and after the administration of grapefruit juice, a unidimensional thin layer chromatographic method was employed. TLC-silica plates, on which carbohydrates have been spotted and developed, may contain several bands, even in normal subjects. The normal excretion of sugars is almost negligible, hence attention should be paid to any marked increase of sugars in the urine. However, abnormalities are often the result of impaired intestinal absorption.

In this study, a standard solution comprising of different sugars as mentioned in the method was spotted along with the samples to qualitatively determine the carbohydrate content in the samples.

The TLC-plates did not reveal significant concentrations of any carbohydrate. Also, the chromatograms of the urine samples after grapefruit administration were neither different from one another nor from the control. This may indicate that inhibition of p-gp or defective p-gp does not result in the presence of carbohydrates in the urine. It should be noted though, that despite all the advantages of the thin layer chromatographic method, it still has the limitation of insensitivity - especially when the small volumes of urine samples spotted in this study are considered. A TLC-plate representative of the results is shown in figure 5.1.

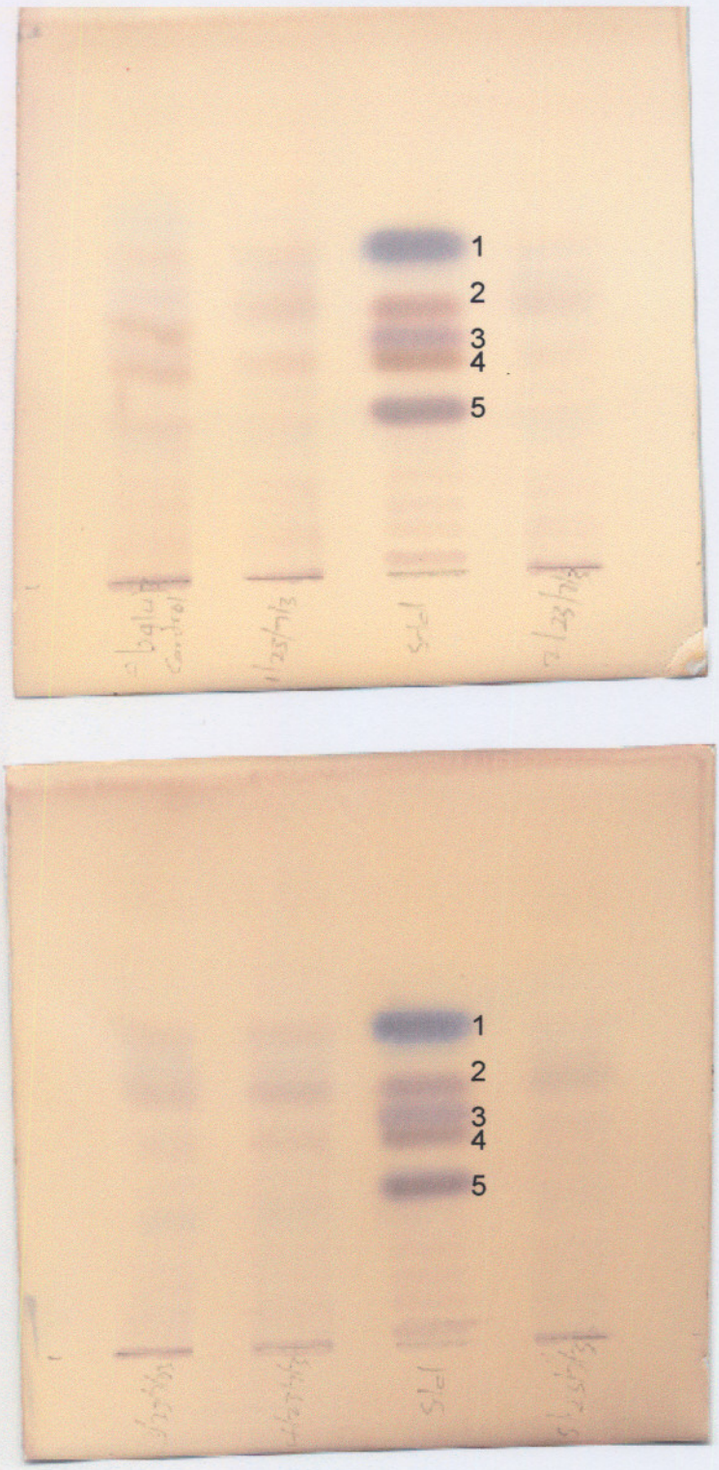


Figure 5.1: *The developed TLC-plate spotted with the urine samples (control and samples after grapefruit juice administration) of subject CM 7 and standard solution (second from the right on each plate: 1=xylose, 2=glucose, 3=galactose, 4=sucrose, 5=lactose).*

5.4 RESULTS OF THE ANALYSIS OF ORGANIC ACIDS IN THE URINE

Organic acids comprise key metabolites of virtually all pathways of intermediary metabolism as well as exogenous compounds. Comprehensive quantitative analysis of organic acids in body fluids has therefore the potential of yielding information on the physiological and pathophysiological status of different metabolic pathways, as well as their interrelationships (Blau et al, 1996:31). For example, the first indication of a β-oxidation defect or organic aciduria often comes from a urinary organic acid profile obtained during the acute episode (Scriver et al., 1995).

Since grapefruit juice inhibition of p-gp occurs exclusively in the intestine, unique organic acids that originate from the intestine are chosen as markers for this study. One important physiological role of p-gp is the protection of organisms against toxins, achieved by exporting these compounds from the body. In the intestine, p-gp effluxes amphipathic and exogenous hydrophobic toxins, including the intestinal dysbiosis markers absorbed from the gut back into the intestinal lumen for elimination in the faeces (Higgins & Gottesman, 1992).

The intestinal dysbiosis markers are organic acids produced from bacterial metabolic action on the food residue in the gut and they are normally found only in faeces. Thus, these microbial metabolites originate from the lumen of the gut and only spill to the urine when there is an abnormal overgrowth of microbes that inhabit the lumen of gut. The abnormal overgrowth of unfavourable microflora in the intestine is referred to as "gut dysbiosis" (Bralley & Lord, 2003:4). The presence of these microbial metabolites in the urine when there is overgrowth of microbes can be attributed to the saturation of the transport system, p-gp, by high concentrations of its substrates in the intestinal lumen. So, with the inhibition of or defective p-gp, more of these microbial metabolites are expected to be in the urine than when the p-gp is functioning normally in the absence of abnormal overgrowth of the microbes. Thus, these intestinal dysbiosis markers were chosen as markers in this study to monitor the effect of the inhibition of p-gp by grapefruit juice.

Most organic acid analyses are done on urine, since metabolic disorders show characteristic patterns in the organic acid profile in urine. Urine is known to contain a wealth of information about human metabolism. Urinary organic acid analysis for

metabolic profiling has traditionally been used for detection of neonatal inborn errors of metabolism.

The organic acids were isolated from the urine samples by solvent extraction with ethyl acetate and diethyl ether after acidification with hydrochloric acid. GC-MS was used as a reliable method to analyse these acids. Organic acids are unsuitable for gas chromatographic analysis because of their high polarity, low volatility and thermal instability. Thus, it is necessary to convert the carboxylic acid group in the organic acids into a non-polar, volatile, thermally stable derivative (such as an ester) prior to analysis by gas chromatograph. Liquid chromatography is not advantageous since there is an inadequacy of detection methods for organic acids. The representatives of the chromatograms obtained are shown in Fig 5.2 and 5.3. All the other chromatograms, with the identification of the compounds and their concentration in the control and samples of the subjects are included in the appendix.

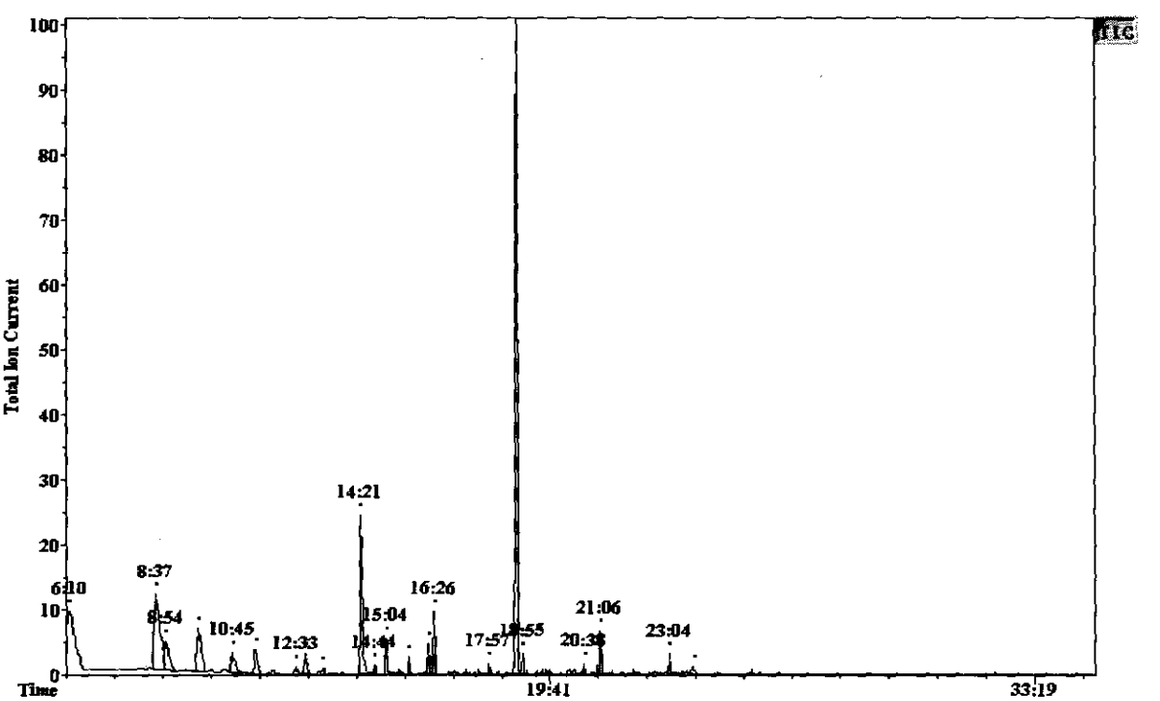


Figure 5.2: *A representative GC-MS total ion chromatogram of the control sample of subject AM 29*

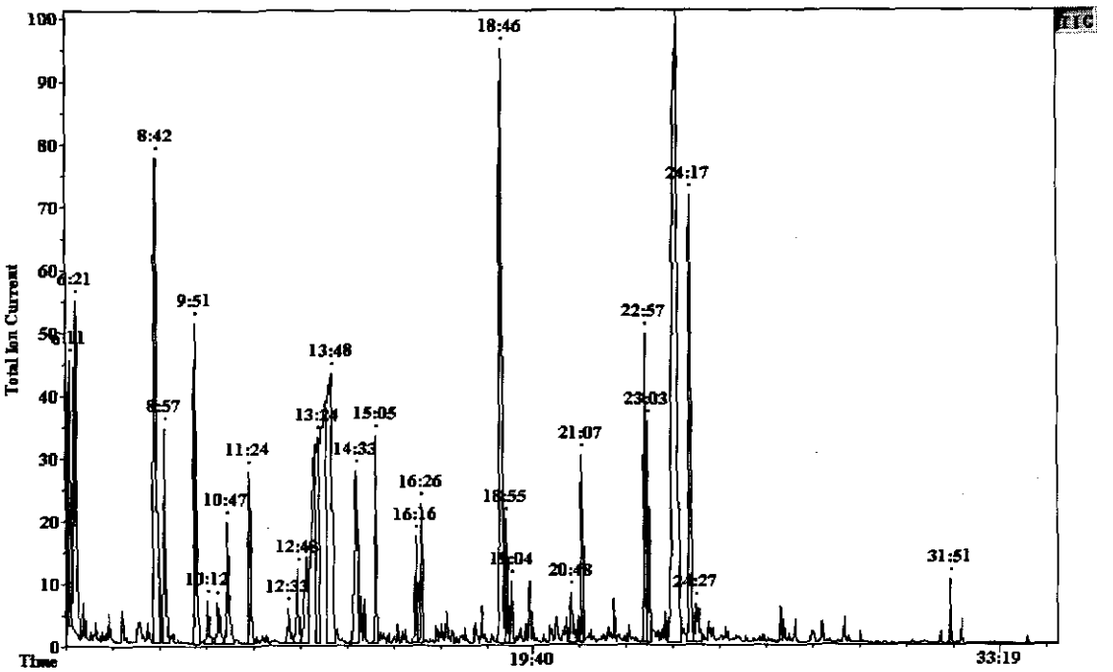


Figure 5.3: *A representative GC-MS total ion chromatogram of the urine sample on day one after the intake of grapefruit juice of subject AM 29.*

Table 5.3: Interpretation of GC-MS spectrum of control sample of subject AM 29.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.191	5	19636	19.50
LACTIC-DITMS	8.64	94	37200	36.94
GLYCOLIC-DITMS	8.934	105	15372	15.27
1,2-DIHYDROXYBUTANE-DITMS	9.849	139	21900	21.75
p-CRESOL-TMS	10.79	173	8720	8.66
2-HYDROXY-ISO-VALERIC-DITMS	10.875	176	983	0.98
3-HYDROXYISOBUTYRIC-DITMS	11.435	197	7880	7.83
3-HYDROXY-ISO-VALERIC-DITMS	12.835	248	8256	8.20
BENZOIC-TMS	13.228	263	1210	1.20
3-ETHYLHYDRACRYLIC-DITMS	13.34	267	2319	2.30
PHOSPHORIC-TRITMS	14.393	305	67352	66.89
GLYCERIN-TRITMS	14.781	319	4097	4.07
SUCCINIC-DITMS	15.114	332	16380	16.27
4-HYDROXY-2-METHYLVALERIC-DITMS	15.463	344	1200	1.19
ITACONIC-DITMS	15.755	355	7882	7.83
4-DEOXYTETRONIC-TRITMS	16.298	375	14528	14.43

THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.462	381	30436	30.23
2,3-DIHYDROXYBUTANE-DITMS	16.962	399	834	0.83
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.618	497	1104	1.09
m-HYDROXYBENZOIC-DITMS	21.045	549	1521	1.51
p-HYDROXYPHENYLACETIC-DITMS	21.152	553	3985	3.96
LAURIC-DITMS	21.455	564	598	0.59
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.091	624	12156	12.07
HIPPURIC-TMS	23.746	648	2450	2.43
MANNONIC-1,4-LACTONE-TMS	24.458	674	1479	1.47
HEXACOSANE-TMS	26.15	736	713	0.71
DIOCTYLPHTALATE-TMS	32.218	958	563	0.56
18.80 min INTERNAL STANDARD	18.774	466	264328	262.5

Table 5.4: Interpretation of GC-MS spectrum of urine sample of day one of subject AM 29.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.41	13	471158	255.07
1,2-DIHYDROXYPROPANE-DITMS	7.352	47	16847	9.12
2,3-DIHYDROXYBUTANE-DITMS	8.482	89	16329	8.84
LACTIC-DITMS	8.728	98	444305	240.53
GLYCOLIC-DITMS	8.987	107	196355	106.30
1,2-DIHYDROXYBUTANE-DI-TMS	9.882	140	329880	178.58
OXALIC-DITMS	10.239	153	42905	23.23
2-HYDROXYBUTYRIC-DITMS	10.55	164	26065	14.11
p-CRESOL-TMS	10.816	174	95491	51.70
3-HYDROXYPROPIONIC-DITMS	10.873	176	11858	6.42
3-HYDROXYISOBUTYRIC-DITMS	11.446	197	108870	58.94
3-HYDROXY-ISO-VALERIC-DITMS	12.837	248	44334	24.00
BENZOIC-TMS	13.236	263	8315	4.50
3-ETHYLHYDRACRYLIC-DITMS	13.348	267	49928	27.03
UREA-DITMS	13.857	286	218966	118.54
OCTANOIC-TMS	13.979	290	6622	3.59
PHOSPHORIC-TRITMS	14.569	312	135482	73.35
GLYCERIN-TRITMS	14.793	320	37746	20.43
SUCCINIC-DITMS	15.125	332	187737	101.63
1,2-DIHYDROXYBENZENE-DITMS	15.325	339	18279	9.90
4-HYDROXY-2-METHYLVALERIC-DITMS	15.474	345	7114	3.85
URACIL-DITMS	15.636	351	1570	0.85
ITACONIC-DITMS	15.756	355	14818	8.02
FUMARIC-DITMS	15.969	363	7355	3.98
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.463	381	121072	65.54
GLUTARIC-DITMS	17.035	402	8592	4.65
3-METHYLGLUTACONIC-DITMS	17.718	427	7847	4.25
3,4-DIHYDROXYBUTYRIC-TRITMS	18.012	438	25104	13.59
CITRAMALIC-TRITMS	18.754	465	7189	3.89
ADIPIC-DITMS	18.945	472	69961	37.87
PYROGLUTAMIC-DITMS	19.112	478	46515	25.18
3-METHYLADIPIC-DITMS	19.491	492	8692	4.71

5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.622	497	37213	20.15
ERYTHRITOL-TMS	19.71	500	8348	4.52
2-HYDROXYGLUTARIC-TRITMS	20.389	525	16536	8.95
ERYTHRONIC-TETRATMS	20.446	527	10456	5.66
PIMELIC-DITMS	20.604	533	2319	1.26
3-HYDROXYPHENYLACETIC-DITMS	20.665	535	29276	15.85
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.905	544	3785	2.05
m-HYDROXYBENZOIC-DITMS	21.041	549	13690	7.41
p-HYDROXYPHENYLACETIC-DITMS	21.15	553	230113	124.57
ISOCITRICLACTON-DITMS	21.898	580	9064	4.91
SUBERIC-DITMS	22.084	587	19602	10.61
LYXOSE-TETRATMS(i)	22.523	603	14136	7.65
XYLOSE-TETRATMS(iv)	22.523	603	15354	8.31
t-ACONITIC-TRITMS	22.989	620	247480	133.98
VANILLIC-DITMS	23.066	623	2118	1.15
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.093	624	192479	104.20
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.12	625	5756	3.12
p-HYDROXYMANDELIC-TRITMS	23.497	639	6198	3.36
AZELAIC-DITMS	23.563	641	11495	6.22
HIPPURIC-TMS	23.929	655	487719	264.03
a-RESORCYLIC-TRITMS	24.019	658	1262	0.68
CITRIC-TETRATMS	24.311	669	305134	165.19
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.535	677	15869	8.59
VANILGLYCOLIC-TRITMS	24.838	688	13212	7.15
VANILLYLMANDELIC-TRITMS	24.84	688	16882	9.14
p-HYDROXYPHENYLLACTIC-TRITMS	25.217	702	3152	1.71
VANILGLYCOL-TRITMS	26.313	742	2771	1.50
PALMITIC-TMS	26.916	764	15555	8.42
o-HYDROXYHIPPURIC-DITMS	27.022	768	8384	4.54
p-HYDROXYHIPPURIC-DITMS	28.795	833	16468	8.92
DIOCTYLPHTALATE-TMS	32.208	958	14454	7.83
18.80 min INTERNAL STANDARD	18.815	467	484889	262.5

The dysbiosis markers (unique metabolites from microbes) for all of the subjects were selected from the chromatograms of the GC-MS analyses of the urine samples and compared with the controls. These results are depicted in Table 5.5.

Table 5.5: The concentration in mg/g creatinine of each dysbiosis marker present in the control and urine samples after grapefruit juice administration on day one, three and five.

Subject (AM43)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	0	2.34	2.86	1.71
m-HYDROXYBENZOIC ACID	0	7.11	5.72	3.54
p-HYDROXYPHENYLACETIC ACID	0	61.01	4.29	31.8
HIPPURIC ACID	55.19	201.12	240.17	66.98
p-CRESOL	4.94	5.39	4.08	0
3-HYDROXYPHENYLACETIC ACID	0	68.73	17.5	23.82
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	0	1.1	4.51	1.95
CITRAMALATE	0	0	0	0
m-HYDROXYPHENYLPROPIONIC ACID	0	0	2.74	1.04
3-HYDROXYPROPIONIC ACID	0	2.02	1.85	1.81

Subject (AM29)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	1.2	4.5	8.36	3.61
m-HYDROXYBENZOIC ACID	1.51	7.41	2.01	2.21
p-HYDROXYPHENYLACETIC ACID	3.95	124.57	21.7	0
HIPPURIC ACID	2.43	264.03	241.79	238.07
p-CRESOL	8.65	51.69	12.4	19.01
3-HYDROXYPHENYLACETIC ACID	0	15.84	9.45	17.44
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	0	3.11	1.36	2.25
CITRAMALATE	0	3.89	0	0
m-HYDROXYPHENYLPROPIONIC ACID	0	0	0	0
3-HYDROXYPROPIONIC ACID	0	6.41	2.2	4.56

Subject (AM 32)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	2.09	4.63	6.52	15.46
m-HYDROXYBENZOIC ACID	4.71	14.53	16.18	19.71
p-HYDROXYPHENYLACETIC ACID	1.92	10.86	16.04	54.32
HIPPURIC ACID	60.97	267.35	233.04	165.27
p-CRESOL	24.77	43.07	121.48	133.72
3-HYDROXYPHENYLACETIC ACID	0	16.3	9.78	21.6
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	0	3.07	0	3.87
CITRAMALATE	0	3.07	4.38	0
m-HYDROXYPHENYLPROPIONIC ACID	0	0	0	0
3-HYDROXYPROPIONIC ACID	0	3.19	0	0

Subject (AM 56)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	2.77	4.19	9.74	9.2
m-HYDROXYBENZOIC ACID	2.27	1.28	7.11	2.78
p-HYDROXYPHENYLACETIC ACID	61.69	126.2	111.36	17.02
HIPPURIC ACID	142.82	244.24	233.14	88.4
p-CRESOL	6.31	30.4	6.25	10.06
3-HYDROXYPHENYLACETIC ACID	0	25.9	23.55	7.33
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	0	4.3	3.74	1.37
CITRAMALATE	0	0	0	0
m-HYDROXYPHENYLPROPIONIC ACID	1.49	1.49	0	0
3-HYDROXYPROPIONIC ACID	0	2.79	0	0.97

Subject (AF 32)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	0	3.66	3.33	3.36
m-HYDROXYBENZOIC ACID	3.86	3.25	2.3	3.58
p-HYDROXYPHENYLACETIC ACID	26.3	0	11.83	6.48
HIPPURIC ACID	104.97	206.86	220.53	260.78
p-CRESOL	43.08	95.41	27.43	68.38
3-HYDROXYPHENYLACETIC ACID	0	5.01	3.98	18.24
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	0	2.66	1.39	3.72
CITRAMALATE	0	3.61	0	0
m-HYDROXYPHENYLPROPIONIC ACID	0	0	0	0
3-HYDROXYPROPIONIC ACID	0	2.99	1.65	3.09

Subject (AF 26)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	8.85	9.67	7.1	4.1
m-HYDROXYBENZOIC ACID	3.72	11.83	0	0
p-HYDROXYPHENYLACETIC ACID	30.91	46.28	64.33	89.55
HIPPURIC ACID	192.74	87.76	81.06	81.82
p-CRESOL	36.04	116.18	14.6	18.82
3-HYDROXYPHENYLACETIC ACID	17.8	0	25.27	33.18
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	0	0	0	0
CITRAMALATE	0	26.28	0	0
m-HYDROXYPHENYLPROPIONIC ACID	10.14	15.28	3.53	2.59
3-HYDROXYPROPIONIC ACID	0	26.31	6.33	6.99

Subject (CM 10)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	18.27	6.29	3.52	2.9
m-HYDROXYBENZOIC ACID	9.44	21.28	5.72	10.07
p-HYDROXYPHENYLACETIC ACID	71.52	242.61	9.74	1.83
HIPPURIC ACID	118.57	292.53	231.78	116.12
p-CRESOL	17.14	26.11	6.76	10.77
3-HYDROXYPHENYLACETIC ACID	0	12.19	23.23	7.18
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	0	6.2	5.18	7.12
CITRAMALATE	0	0	0	0
m-HYDROXYPHENYLPROPIONIC ACID	0	0	0	0
3-HYDROXYPROPIONIC ACID	16.55	6.08	3.3	4.32

Subject (CF 9)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	10.44	12.12	0	0
m-HYDROXYBENZOIC ACID	18.04	2.96	4.83	1.72
p-HYDROXYPHENYLACETIC ACID	166.12	20.37	43.7	15.07
HIPPURIC ACID	209.56	146.99	193.86	143.22
p-CRESOL	80.93	0	9.16	4.8
3-HYDROXYPHENYLACETIC ACID	24.23	0	0	2.32
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	17.8	0	0	0
CITRAMALATE	0	0	0	0
m-HYDROXYPHENYLPROPIONIC ACID	0	8.51	4.23	0.66
3-HYDROXYPROPIONIC ACID	0	0	0	0.98

Subject (CF 7)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	4.16	5.44	3.09	3.35
m-HYDROXYBENZOIC ACID	3.11	5.47	4.32	0
p-HYDROXYPHENYLACETIC ACID	8.87	210.76	243.53	365.12
HIPPURIC ACID	188.82	172.57	161.82	159.19
p-CRESOL	12.77	85.15	103.46	162.51
3-HYDROXYPHENYLACETIC ACID	27.4	0	0	0
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	1.45	5.9	4.29	0
CITRAMALATE	0	0	0	0
m-HYDROXYPHENYLPROPIONIC ACID	0	0	0	0
3-HYDROXYPROPIONIC ACID	2.8	0	0	0

Subject (CF 5)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	0	0	4.13	1.2
m-HYDROXYBENZOIC ACID	7.34	4.95	6.25	10.3
p-HYDROXYPHENYLACETIC ACID	82	46.43	35.65	62.2
HIPPURIC ACID	214.75	144.72	192.53	322.04
p-CRESOL	46.46	40.52	67.43	27.19
3-HYDROXYPHENYLACETIC ACID	19.23	15.8	15.35	10.6
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	0	0	0	9.68
CITRAMALATE	0	0	0	11.75
m-HYDROXYPHENYLPROPIONIC ACID	4.54	0	0	0
3-HYDROXYPROPIONIC ACID	0	0	0	8.01

Subject (CM 7)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	55.28	8.23	18.14	15.37
m-HYDROXYBENZOIC ACID	29.4	9.22	4.85	6.72
p-HYDROXYPHENYLACETIC ACID	99.08	12.72	51.56	45.15
HIPPURIC ACID	327.19	177.29	182.06	137.07
p-CRESOL	31.74	61.53	0	73.82
3-HYDROXYPHENYLACETIC ACID	35.58	22.28	0	13.69
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	6.2	0	0	0
CITRAMALATE	0	0	0	0
m-HYDROXYPHENYLPROPIONIC ACID	0	4.07	0	0
3-HYDROXYPROPIONIC ACID	3.9	0	0	0

Subject (CM 11)	CONTROL	1st day	3rd day	5th day
BENZOIC ACID	3.09	5.69	10.97	-
m-HYDROXYBENZOIC ACID	6.48	12.37	15.21	-
p-HYDROXYPHENYLACETIC ACID	0	39.1	74.73	-
HIPPURIC ACID	215.56	224.09	216.56	-
p-CRESOL	21.1	166.63	85.25	-
3-HYDROXYPHENYLACETIC ACID	26.2	25.86	14.07	-
4-HYDROXY-3-METHOXYPHENYLACETIC ACID	4	0	0	-
CITRAMALATE	0	0	0	-
m-HYDROXYPHENYLPROPIONIC ACID	0	4.73	0	-
3-HYDROXYPROPIONIC ACID	0	0	0	-

5.4.1 Statistical analysis

All statistical analyses were one sided, that is, to determine if the amounts of the compounds in the urine samples after the administration of the grapefruit juice were higher than in the controls. The analyses were based on a $P<0.05$ level of confidence. P-values less than 0.05 indicated that the groups being compared were statistically different, with a confidence interval of 95%. The raw data were analysed by computing the mean, standard deviation and P-values for the difference in the concentration of the samples and the controls for each compound in all subjects on each day the samples were collected and the mean of the differences on all the three days. This was done for the adults and the children group. The difference in the concentration of the samples and the controls for each compound in all subjects on each day in the two groups were also compared to determine if they were significantly different from each other.

The difference in the concentration (difcon) was calculated by the substraction of the concentration of each compound in the control from the same compound in the urine samples after grapefruit juice administration. The mean of the difference of each compound of all the subjects in each group on the three days (pooled together) were then computed along with the standard deviation and P–value. Difcon11 means the difference of compound 1 on day one, difcon31 means the difference of compound 1 on day three and difcon51 means the difference of compound 1 on day five. The compounds are listed in the order they are written in table 5.3. The P–value was computed using the paired t–test for comparison within the group and student's t–test for computation of the comparison between the groups. The statistical results for each compound are presented below:

5.4.1.1 BENZOIC ACID:

Table 5.6: Statistical evaluation of the difference in the concentration of benzoic acid (compound 1) in the control and samples after grapefruit juice administration on each of the days, of all the subjects and the mean of all the three days, of all the subjects in the adult and children group.

VARIABLE	N	MEAN OF DIFFERENCE	STD DEV OF DIFFERENCE	P - VALUE
ADULT GROUP				
difcon11	6	2.3466667	1.0826388	0.0016
difcon31	6	3.8333333	3.2752323	0.0175
difcon51	6	3.7550000	5.9680139	0.0819
Mean1	6	4.1400000	2.3563578	0.0038
CHILDREN GROUP				
difcon11	6	-8.8616667	19.3345746	0.1563
difcon31	6	-8.5650000	16.4077820	0.1286
difcon51	5	-13.0660000	16.4812994	0.0755
Mean1	6	-4.2500000	10.1679483	0.1764

The paired t-test analysis of the difference in amount of benzoic acid in the controls and urine samples after the intake of grapefruit juice on each day in the adult group (table 5.6) revealed that the difference was statistically significant ($P<0.05$) on day one and day three but not on day five. This means that the increase in the amount of benzoic acid in the urine samples after grapefruit juice intake on day one and day three of all the subjects put together was statistically significant, but the increase on day five was not significant in the adult group.

The mean of the difference in the concentration of all the days (that is, day one, three and five) pooled together revealed that the difference was statistically significant in the adult group, indicating that the amount of benzoic acid significantly increased on average after the administration of grapefruit juice in the adult group.

In the children group, the paired t-test analysis showed that the difference in the amount of benzoic acid in the urine samples and controls on each day, (day one, three and five), of all the child subjects put together was not statistically significant. This indicates that the increase in the amount of this compound in the urine samples after the intake of grapefruit juice compared with the control was not statistically significant in the children group.

Also the mean of the difference in the amount of benzoic acid in the urine samples and controls on all the days (that is, day one, three and five) pooled together was not statistically significant for the children group, showing that the average increase in the amount of benzoic acid after the administration of grapefruit juice in the children group was not statistically significant.

5.4.1.2 m-HYDROXYBENZOIC ACID:

Table 5.7: Statistical evaluation of the difference in the concentration of m-hydroxybenzoic acid (compound 2) in the control and samples after grapefruit juice administration on each of the days, of all the subjects and the mean of all the three days, of all the subjects in the adult and children group.

VARIABLE	N	MEAN OF DIFFERENCE	STD DEV OF DIFFERENCE	P - VALUE
ADULT GROUP				
difcon12	6	4.8900000	4.5923023	0.0239
difcon32	6	2.8750000	5.5611716	0.1306
difcon52	6	2.6250000	6.4939164	0.1838
Mean2	6	4.3561111	4.8617283	0.0398
CHILDREN GROUP				
difcon12	6	-2.9266667	12.4048856	0.2942
difcon32	6	-5.4383333	11.7682393	0.1545
difcon52	5	-7.7040000	11.2115713	0.0996
Mean2	6	-0.7816667	8.1496955	0.4118

The paired t-test analysis of the difference in amount of m-hydroxybenzoic acid in the controls and urine samples after the intake of grapefruit juice on each day in the adult group (table 5.7) revealed that the difference was statistically significant ($P<0.05$) only on day one, but not on day three and day five. This means that the increase in the amount of m-hydroxybenzoic acid in the urine samples after grapefruit juice intake on day one of all the subjects put together was statistically significant, but the increase on day three and day five was not significant in the adult group.

The mean of the difference in the concentration of all the days (that is, day one, three and five) pooled together revealed that the difference was statistically significant in the adult group, indicating that the amount of m-hydroxybenzoic acid significantly increased on average, after the administration of grapefruit juice in the adult group.

In the children group, the paired t-test analysis showed that the difference in the amount of m-hydroxybenzoic acid in the urine samples and controls on each day, (day one, three and five), of all the child subjects put together was not statistically significant. This indicates that the increase in the amount of m-hydroxybenzoic acid in the urine samples after the intake of grapefruit juice compared with the control was not statistically significant in the children group.

Also the mean of the difference in the amount of m-hydroxybenzoic acid in the urine samples and controls on all the days (that is, day one, three and five) pooled together was not statistically significant for the children group, showing that the average increase in the amount of m-hydroxybenzoic benzoic acid after the administration of grapefruit juice in the children group was not statistically significant.

5.4.1.3 p-HYDROXYPHENYLACETIC ACID:

Table 5.8: Statistical evaluation of the difference in the concentration of p-hydroxyphenylacetic acid (compound 3) in the control and samples after grapefruit juice administration on each of the days, of all the subjects and the mean of all the three days, of all the subjects in the adult and children group.

VARIABLE	N	MEAN OF DIFFERENCE	STD DEV OF DIFFERENCE	P - VALUE
ADULT GROUP				
difcon13	6	40.6916667	51.9729250	0.0566
difcon33	6	17.4633333	22.3446599	0.0568
difcon53	6	15.3066667	40.8903576	0.2006
Mean3	6	31.4188889	23.0526523	0.0103
CHILDREN GROUP				
difcon13	6	24.0666667	140.0366242	0.3456
difcon33	6	5.2200000	129.3802889	0.4625
difcon53	5	12.3560000	198.1943442	0.4479
Mean3	6	40.1113889	121.9854613	0.2285

The paired t-test analysis of the difference in amount of p-hydroxyphenylacetic acid in the controls and urine samples after the intake of grapefruit juice on each day in the adult group (table 5.8) revealed that the difference was not statistically significant ($P>0.05$) on day one, three and five. This means that the increase in the amount of p-hydroxyphenylacetic acid in the urine samples after grapefruit juice intake on day one, three and five of all the subjects put together was not statistically significant in the adult group.

The mean of the difference in the concentration of all the days (that is, day one, three and five) pooled together revealed that the difference was significant statistically in the adult group, indicating that the amount of p-hydroxyphenylacetic acid significantly increased on average after the administration of grapefruit juice in the adult group.

In the children group, the paired t-test analysis showed that the difference in the amount of p-hydroxyphenylacetic acid in the urine samples and controls on each day, (day one, three and five) of all the child subjects put together was not statistically significant. This indicates that the increase in the amount of this compound in the urine samples after the intake of grapefruit juice compared with the control was not statistically significant in the children group.

Also the mean of the difference in the amount of p-hydroxyphenylacetic acid in the urine samples and controls on all the days (that is, day one, three and five) pooled together was not statistically significant for the children group, showing that the average increase in the amount of p-hydroxyphenylacetic acid after the administration of grapefruit juice in the children group was not statistically significant.

5.4.1.4 HIPPURIC ACID:

Table 5.9: Statistical evaluation of the difference in the concentration of hippuric acid (compound 4) in the control and samples after grapefruit juice administration on each of the days, of all the subjects and the mean of all the three days, of all the subjects in the adult and children group.

VARIABLE	N	MEAN OF DIFFERENCE	STD DEV OF DIFFERENCE	P - VALUE
ADULT GROUP				
difcon14	6	140.0400000	133.1852309	0.0248
difcon34	6	136.4350000	128.7804317	0.0242
difcon54	6	78.3666667	119.7411508	0.0849
Mean4	6	142.2316667	99.1108640	0.0085
CHILDREN GROUP				
difcon14	6	-19.3766667	109.2111620	0.3410
difcon34	6	-15.9733333	82.2417839	0.3271
difcon54	5	-36.2500000	107.6700759	0.2467
Mean4	6	37.2391667	63.8362078	0.1062

The paired t-test analysis of the difference in amount of hippuric acid in the controls and urine samples after the intake of grapefruit juice on each day in the adult group (table 5.9) revealed that the difference was statistically significant ($P<0.05$) on day one and day three, but not on day five. This means that the increase in the amount of hippuric acid in the urine samples after grapefruit juice intake on day one and day three of all the subjects put together was statistically significant, but the increase on day five was not significant in the adult group.

The mean of the difference in the concentration of all the days (that is, day one, three and five) pooled together revealed that the difference is statistically significant in the adult group, indicating that the amount of hippuric acid significantly increased on average after the administration of grapefruit juice in the adult group.

In the children group, the paired t-test analysis showed that the difference in the amount of hippuric acid in the urine samples and controls on each day, (day one, three and five) of all the child subjects put together was not statistically significant. This indicates that the increase in the amount of this compound in the urine samples after the intake of grapefruit juice compared with the control was not statistically significant in the children group.

Also the mean of the difference in the amount of hippuric acid in the urine samples and controls on all the days (that is, day one, three and five) pooled together was not statistically significant in the children group, showing that the average increase in the amount of hippuric acid after the administration of grapefruit juice in the children group was not statistically significant.

5.4.1.5 p-CRESOL:

Table 5.10: Statistical evaluation of the difference in the concentration of p-cresol (compound 5) in the control and samples after grapefruit juice administration on each of the days, of all the subjects and the mean of all the three days, of all the subjects in the adult and children group.

VARIABLE	N	MEAN OF DIFFERENCE	STD DEV OF DIFFERENCE	P - VALUE
ADULT GROUP				
difcon15	6	42.3983333	40.5422532	0.0252
difcon35	6	16.4150000	40.5191287	0.1833
difcon55	6	27.0400000	41.5288117	0.0853
Mean5	6	33.4927778	29.8890318	0.0203
CHILDREN GROUP				
difcon15	6	28.3000000	76.3745492	0.2028
difcon35	6	10.3200000	60.6740966	0.3471
difcon55	5	18.0100000	84.8214009	0.3298
Mean5	6	34.2027778	61.5835821	0.1159

The paired t-test analysis of the difference in amount of p-cresol in the controls and urine samples after the intake of grapefruit juice on each day in the adult group (table 5.10) revealed that the difference was statistically significant ($P<0.05$) only on day one, but not on day three and day five. This means that the increase in the amount of p-cresol in the urine samples after grapefruit juice intake on day one of all the subjects put together was statistically significant but the increase on day three and day five was not statistically significant in the adult group.

The mean of the difference in the concentration of day one, three and five pooled together, revealed that the difference was statistically significant in the adult group, indicating that the amount of p-cresol significantly increased on average, after the administration of grapefruit juice in the adult group.

In the children group, the paired t-test analysis showed that the difference in the amount of p-cresol in the urine samples and controls on each day, (day one, three

and five), of all the child subjects put together was not statistically significant. This indicates that the increase in the amount of this compound in the urine samples after the intake of grapefruit juice compared with the control was not statistically significant in the children group.

Also the mean of the difference in the amount of p-cresol in the urine samples and controls on day one, three and five pooled together, was not statistically significant in the children group, showing that the average increase in the amount of p-cresol after the administration of grapefruit juice in the children group was not statistically significant.

5.4.1.6 3-HYDROXYPHENYLACETIC ACID:

Table 5.11: Statistical evaluation of the difference in the concentration of 3-hydroxyphenylacetic acid (compound 6) in the control and samples after grapefruit juice administration on each of the days, of all the subjects and the mean of all the three days, of all the subjects in the adult and children group.

VARIABLE	N	MEAN OF DIFFERENCE	STD DEV OF DIFFERENCE	P - VALUE
ADULT GROUP				
difcon16	6	23.3816667	23.3603283	0.0289
difcon36	6	11.9550000	7.2098897	0.0048
difcon56	6	14.3950000	9.0953521	0.0058
Mean6	6	17.5661111	10.2669155	0.0043
CHILDREN GROUP				
difcon16	6	-9.4183333	15.1194542	0.0917
difcon36	6	-13.3316667	21.1622942	0.0917
difcon56	5	-14.5300000	13.9678649	0.0403
Mean6	6	-6.0525000	12.0808094	0.1372

The paired t-test analysis of the difference in amount of 3-hydroxyphenylacetic acid in the controls and urine samples after the intake of grapefruit juice on each day in the adult group (table 5.11) revealed that the difference was statistically significant

($P<0.05$) on day one and day three, but not on day five. This means that the increase in the amount of 3-hydroxyphenylacetic acid in the urine samples after grapefruit juice intake on day one and day three of all the subjects put together was statistically significant, but the increase on day five was not significant in the adult group.

The mean of the difference in the concentration of all the days pooled together revealed that the difference was statistically significant in the adult group, indicating that the amount of 3-hydroxyphenylacetic acid significantly increased on average after the administration of grapefruit juice in the adult group.

In the children group, the paired t-test analysis showed that the difference in the amount of 3-hydroxyphenylacetic acid in the urine samples and controls on day one, and day three of all the child subjects put together was not statistically significant, but on day five, it was statistically significant. This indicates that the increase in the amount of 3-hydroxyphenylacetic acid in the urine samples after the intake of grapefruit juice was only significant on day five in the children group.

The mean of the difference in the amount of 3-hydroxyphenylacetic acid in the urine samples and controls on all the days pooled together was not statistically significant in the children group, showing that the average increase in the amount of 3-hydroxyphenylacetic acid after the administration of grapefruit juice in the children group was not statistically significant.

5.4.1.7 4-HYDROXY-3-METHOXYPHENYLACETIC ACID:

Table 5.12: Statistical evaluation of the difference in the concentration of 4-hydroxy-3-methoxyphenylacetic acid (compound 7) in the control and samples after grapefruit juice administration on each of the days, of all the subjects and the mean of all the three days, of all the subjects in the adult and children group.

VARIABLE	N	MEAN OF DIFFERENCE	STD DEV OF DIFFERENCE	P - VALUE
ADULT GROUP				
difcon17	6	2.3733333	1.5541128	0.0067
difcon37	6	1.8333333	1.8943460	0.0319
difcon57	6	2.1933333	1.4623907	0.0072
Mean7	6	2.1333333	1.0917529	0.0024
CHILDREN GROUP				
difcon17	6	-2.8916667	8.7103626	0.2265
difcon37	6	-3.3300000	8.2419003	0.1839
difcon57	5	-1.7300000	11.0319400	0.3717
Mean7	6	-1.3627778	6.5894355	0.3170

The paired t-test analysis of the difference in amount of 4-hydroxy-3-methoxyphenylacetic acid in the controls and urine samples after the intake of grapefruit juice on each day in the adult group (table 5.12) revealed that the difference was statistically significant ($P<0.05$) on day one, day three and day five, indicating that the increase in the amount of 3-hydroxyphenylacetic acid in the urine samples after grapefruit juice intake on day one, day three and day five, of all the subjects put together was statistically significant.

The mean of the difference in the concentration of all the days pooled together revealed that the difference was significant statistically in the adult group, indicating that the amount of 4-hydroxy-3-methoxyphenylacetic acid significantly increased on average after the administration of grapefruit juice in the adult group.

In the children group, the paired t-test analysis showed that the difference in the amount of 4-hydroxy-3-methoxyphenylacetic acid in the urine samples and controls on each day, (day one, three and five), of all the child subjects put together was not statistically significant. This indicates that the increase in the amount of this compound in the urine samples after the intake of grapefruit juice compared with the control was not statistically significant in the children group.

Also the mean of the difference in the amount of 4-hydroxy-3-methoxyphenylacetic acid in the urine samples and controls on all the days pooled together was not statistically significant for the children group, showing that the average increase in the amount of 4-hydroxy-3-methoxyphenylacetic acid after the administration of grapefruit juice in the children group was not statistically significant.

5.4.1.8 CITRAMALATE:

Table 5.13: Statistical evaluation of the difference in the concentration of citramalate (compound 8) in the control and samples after grapefruit juice administration on each of the days, of all the subjects and the mean of all the three days, of all the subjects in the adult and children group.

VARIABLE	N	MEAN OF DIFFERENCE	STD DEV OF DIFFERENCE	P - VALUE
ADULT GROUP				
difcon18	6	6.1416667	10.0190527	0.0967
difcon38	6	0.7300000	1.7881275	0.1816
difcon58	6	0	0	-
Mean8	6	2.2905556	3.3031510	0.0750
CHILDREN GROUP				
difcon18	6	0	0	-
difcon38	6	0	0	-
difcon58	5	2.3500000	5.2547597	0.1869
Mean8	6	0.6527778	1.5989725	0.1816

The paired t-test analysis of the difference in amount of citramalate in the controls and urine samples after the intake of grapefruit juice on each day in the adult group (table 5.13) revealed that the difference was not statistically significant (P>0.05) on day one and day three. This means that the increase in the amount of citramalate in the urine samples after grapefruit juice intake on day one and day three of all the subjects put together was not statistically significant. On day five there was no P-value. This was because the compound was not present at all in the control and urine samples after the intake of grapefruit juice on day five in the adult group.

The mean of the difference in the concentration of all the days pooled together revealed that the difference was not significant statistically in the adult group, indicating that the amount of citramalate did not significantly increase on average after the administration of grapefruit juice in the adult group.

In the children group, the paired t-test analysis of the difference in amount of citramalate in the controls and urine samples after the intake of grapefruit juice gave no P–value on day one and day three. This was because citramalate was not present at all in the control and urine samples after the intake of grapefruit juice on day one and day three. On day five, the increase was not statistically significant (P>0.05), that is, the amount of this compound in the urine samples after the intake of grapefruit juice when compared with the control was not statistically significant in the children group.

Also the mean of the difference in the amount of citramalate in the urine samples and controls on all the days pooled together was not statistically significant for the children group, showing that the average increase in the amount of citramalate after the administration of grapefruit juice in the children group was not statistically significant.

5.4.1.9 m-HYDROXYPHENYLPROPIONIC ACID:

Table 5.14: Statistical evaluation of the difference in the concentration of m-hydroxyphenylpropionic acid (compound 9) in the control and samples after grapefruit juice administration on each of the days, of all the subjects and the mean of all the three days, of all the subjects in the adult and children group.

VARIABLE	N	MEAN OF DIFFERENCE	STD DEV OF DIFFERENCE	P - VALUE
ADULT GROUP				
difcon19	6	0.8566667	2.0983962	0.1816
difcon39	6	-0.4433333	3.4505227	0.3828
difcon59	6	-1.3333333	3.1507946	0.1737
Mean9	6	0.3394444	0.6468880	0.1275
CHILDREN GROUP				
difcon19	6	2.1283333	4.5780058	0.1532
difcon39	6	-0.0516667	2.7744723	0.4827
difcon59	5	-0.7760000	2.1234594	0.2298
Mean9	6	0.8602778	2.5339741	0.2217

The paired t-test analysis of the difference in amount of m-hydroxyphenylpropionic acid in the controls and urine samples after the intake of grapefruit juice on each day in the adult group (table 5.14) revealed that the difference was not statistically significant ($P>0.05$) on day one, day three and day five, showing that the increase in the amount of m-hydroxyphenylpropionic acid in the urine samples after grapefruit juice intake on day one, day three and day five of all the subjects put together was not statistically significant in the adult group.

The mean of the difference in the concentration of all the days (that is, day one, three and five) pooled together revealed that the difference was not statistically significant in the adult group, indicating that the amount of m-hydroxyphenylpropionic acid did not significantly increase on average, after the administration of grapefruit juice in the adult group.

In the children group, the paired t-test analysis showed that the difference in the amount of m-hydroxyphenylpropionic acid in the urine samples and controls on each day, (day one, three and five), of all the child subjects put together was not statistically significant. This indicates that the increase in the amount of this compound in the urine samples after the intake of grapefruit juice compared with the control was not statistically significant in the children group.

Also the mean of the difference in the amount of m-hydroxyphenylpropionic acid in the urine samples and controls on all the days pooled together was not statistically significant for the children group, showing that the average increase in the amount of m-hydroxyphenylpropionic acid after the administration of grapefruit juice in the children group was not statistically significant.

5.4.1.10 3-HYDROXYPROPIONIC ACID

Table 5.15: Statistical evaluation of the difference in the concentration of 3-hydroxypropionic acid (compound 10) in the control and samples after grapefruit juice administration on each of the days, of all the subjects and the mean of all the three days, of all the subjects in the adult and children group.

VARIABLE	N	MEAN OF DIFFERENCE	STD DEV OF DIFFERENCE	P - VALUE
ADULT GROUP				
difcon110	6	7.2850000	9.4430668	0.0587
difcon310	6	2.0050000	2.3209373	0.0439
difcon510	6	3.3600000	2.1535459	0.0062
Mean10	6	4.2166667	4.5342367	0.0358
CHILDREN GROUP				
difcon110	6	-2.8616667	4.0874462	0.0735
difcon310	6	-3.3250000	5.1435153	0.0871
difcon510	5	-1.9880000	7.3821183	0.2897
Mean10	6	-1.3227778	3.1240802	0.1736

The paired t-test analysis of the difference in amount of 3-hydroxypropionic acid in the controls and urine samples after the intake of grapefruit juice on each day in the adult group (table 5.15) revealed that the difference was statistically significant ($P<0.05$) on day three and day five, but not on day one. This means that the increase in the amount of 3-hydroxypropionic acid in the urine samples after grapefruit juice intake on day three and day five of all the subjects put together was statistically significant, but the increase on day one was not significant in the adult group.

The mean of the difference in the concentration of all the days pooled together revealed that the difference was statistically significant in the adult group, indicating that the amount of 3-hydroxypropionic acid significantly increased on average after the administration of grapefruit juice in the adult group.

In the children group, the paired t-test analysis showed that the difference in the amount of 3-hydroxypropionic acid in the urine samples and controls on each day, (day one, three and five), of all the child subjects put together was not statistically significant. This indicates that the increase in the amount of this compound in the urine samples after the intake of grapefruit juice compared with the control was not statistically significant in the children group.

Also the mean of the difference in the amount of 3-hydroxypropionic acid in the urine samples and controls on all the days (that is, day one, three and five) pooled together was not statistically significant in the children group, showing that the average increase in the amount of 3-hydroxypropionic acid after the administration of grapefruit juice in the children group was not statistically significant.

5.5 DISCUSSION

5.5.1 Effect of metabolic by-products of intestinal microbes on human

Within the crypts between the villi of the small intestine, potentially pathogenic micro-organisms can adhere and grow rapidly. Whether rapidly growing bacterial species are classed as invasive, cytotoxic, or adhesive, they all produce metabolic by-products that can be absorbed. The abnormal overgrowth of microflora in the small and large intestine is referred to as gut dysbiosis in order to distinguish this clinical condition from that of infection. Dysbiosis has been related to a wide variety of symptoms due to pathogenic toxins produced by the populations of microflora. Some compounds that appear in urine are unique metabolic products of the microbes that inhabit the lumen of the gut. These compounds have a wide range of relative toxicities, with p-cresol and tartaric acid at the upper extreme and hippuric acid and benzoic acid at the lower end.

In addition to serving as markers of intestinal dysbiosis, these compounds have important physiological impact. Many of the compounds have toxic effects on the brain, liver, muscle and other tissues. Effects can be as diverse as headache, insomnia, behavioural disorders, joint pain, learning disorders, immune dysfunction, chronic fatigue and nutritional deficiencies. For example, phenylacetic acid is one of the compounds that accumulates in the genetic disorder, phenylketonuria (PKU). The neurotoxicity of phenylacetic acid is probably due to very strong inhibition of synaptic choline acetyltransferase. Phenylacetic acid elevation due to dysbiosis and probably defective p-gp would have the same metabolic effects as elevation due to PKU, where it is linked to behaviour and learning disabilities. 3-Hydroxypropionic acid is also found in large amounts in the urine of patients suffering from disorders of valine-isoleucine metabolism. The clinical presentation of this disorder is characteristic with life-threatening episodes of ketosis and acidosis. The presence of certain metabolites from bacterial origin may therefore lead to erroneous diagnoses of metabolic disorders. This would especially be the case when p-gp inhibition occurs in cases where there are no microbial overgrowth in the gut.

Food materials that are not assimilated can be used by gut microbes for growth. The specific compounds that are formed depend on the starting material and the species of organism present. Each species of microbe has specific activities, yielding one or

more of the products reported. Many of the unique organic acids from bacterial origin mentioned in the literature, were observed in this study (see chromatograms in appendix).

For example, intestinal bacteria that contain L-amino acid decarboxylase enzymes degrade tyrosine to tyramine. The tyramine is subsequently deaminated and oxidized to p-hydroxyphenylacetic acid. This product is excreted unchanged and unconjugated in the urine. Although p-hydroxyphenylacetic acid can be produced in the liver, abnormally high levels in urine are of bacterial origin.

Tyrosine can also be metabolised directly by bacteria and protozoa to give p-cresol, p-hydroxybenzoic acid and p-hydroxyphenylacetic acid as final products. These compounds are not products of normal human metabolism. Strains of *Escherichia coli* can also produce p-hydroxybenzoic acid from glucose.

Bacterial deamination of the amino acid phenylalanine produces benzoic acid, which is conjugated with glycine in the liver to form hippuric acid. Benzoic acid and Hippuric acid have been associated with intestinal bacterial overgrowth, and they can convey additional information. Intestinal dysbiosis with weakened mucosal epithelium is a common reason for toxemia and the resulting up-regulation of the hepatic pathways. Intestinal bacterial action on phenylalanine also causes the appearance of phenylacetic acid in urine.

5.5.2 Discussion on the analysis of organic acids from microbial origin

The results obtained above confirmed that grapefruit juice inhibited intestinal p-gp in healthy adult subjects. Out of the ten compounds analysed, only citramalate and m-hydroxyphenylpropionic acid were not statistically significantly increased on average after grapefruit juice administration. The average increase of the other eight compounds were statistically significant. The fact that the average increase of citramalate and m-hydroxyphenylpropionic acid was not statistically significant might be because citramalate originates from yeast and fungi and m-hydroxyphenylpropionic acid is a product of unidentified, specific strains of bacteria (Goodwin *et al.*, 1994; Yeo *et al.*,2000). The normal flora in the gut are kept in check by competition and symbiosis among the hundreds of microbial species. Repeated use of broad-spectrum antibiotics sets up conditions for opportunistic overgrowth of organisms that are not affected by the drugs or that are able to re-colonize rapidly when treatment has ended and this is one way by which yeast, fungi and unidentified

bacteria may gain access to the gut. Fungal growth is especially prevalent in intensive care wards where combinations of antibacterials and immune suppression frequently lead to disseminated candidiasis. This is particularly true of yeast and fungal organisms. The organisms from which the two compounds, that is, citramalate and m-hydroxyphenylpropionic acid originate from are not always present in the gut of humans, thus the variations and the statistical insignificance in the results obtained from the analysis of the two compounds. The other eight compounds were statistically significantly increased in the adults.

The graphical representations of the difference in the concentration of the organic acids in the control and samples after grapefruit juice administration on each of the days, of all the subjects and the mean of all the three days are presented below (The organic acids included gave a statistically significant increase on average):

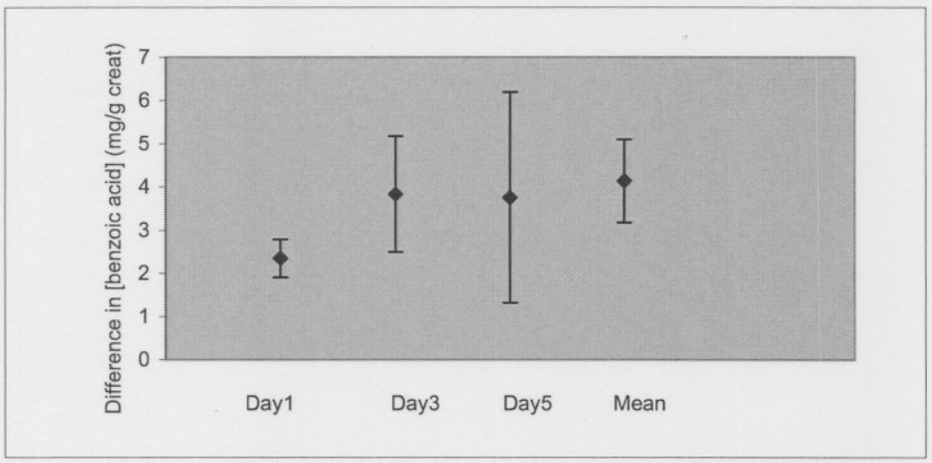


Figure 5.4: *Differences in the concentrations of benzoic acid between control and grapefruit juice treated adult subjects (n=6). Data are presented as mean ± SEM.*

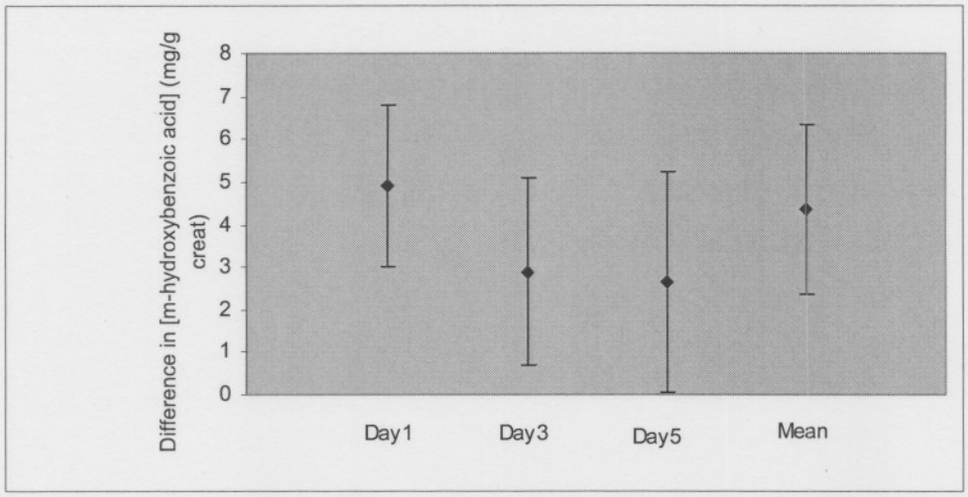


Figure 5.5: *Differences in the concentrations of m-hydroxybenzoic acid between control and grapefruit juice treated adult subjects (n=6). Data are presented as mean ± SEM.*

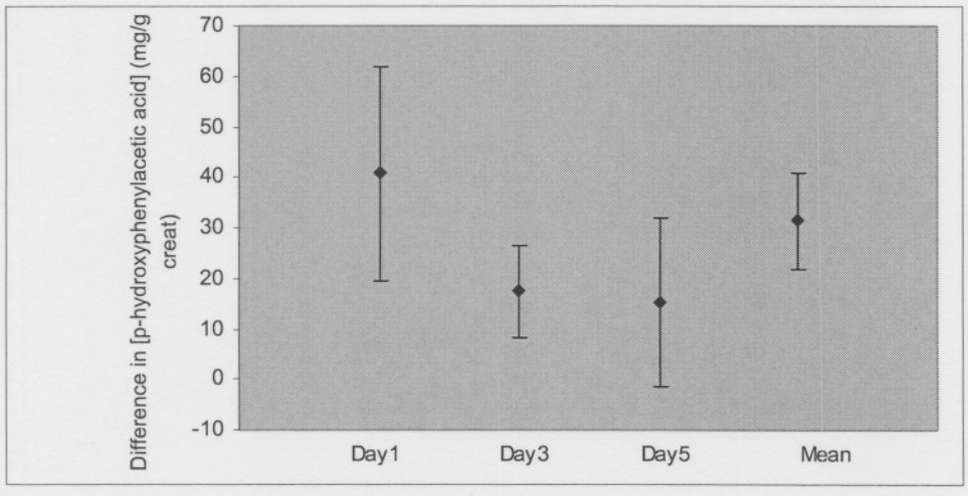


Figure 5.6: *Differences in the concentrations of p-hydroxyphenylacetic acid between control and grapefruit juice treated adult subjects (n=6). Data are presented as mean ± SEM.*

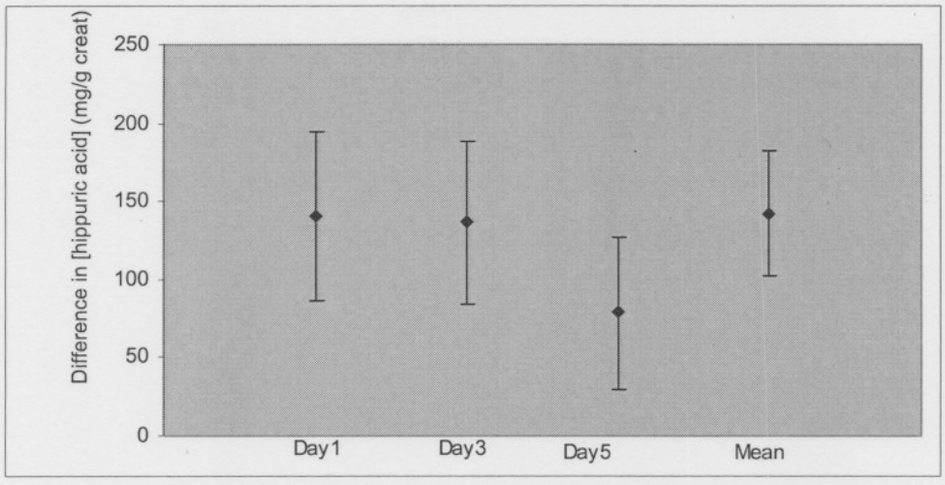


Figure 5.7: *Differences in the concentrations of hippuric acid between control and grapefruit juice treated adult subjects (n=6). Data are presented as mean ± SEM.*

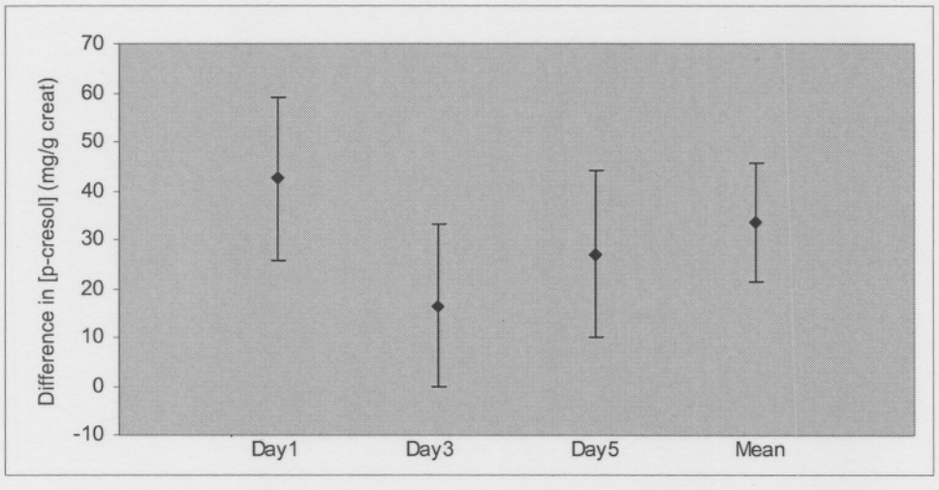


Figure 5.8: *Differences in the concentrations of p-cresol between control and grapefruit juice treated adult subjects (n=6). Data are presented as mean ± SEM.*

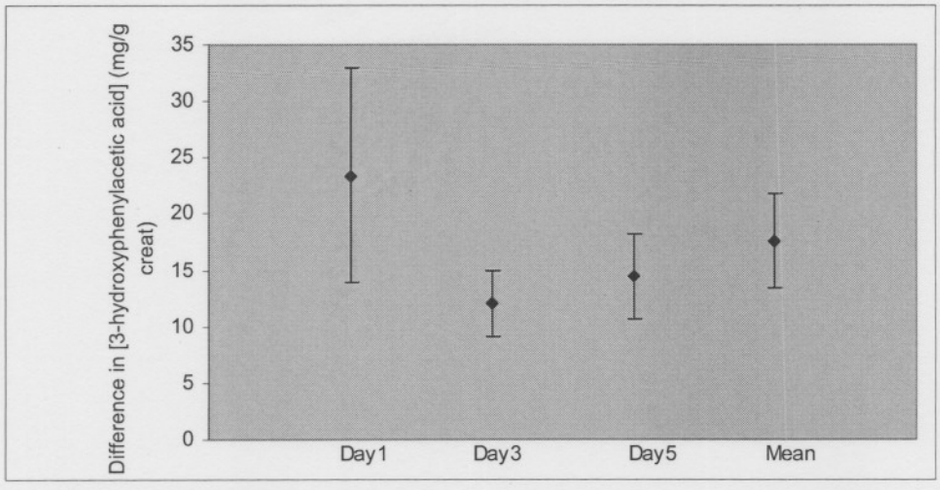


Figure 5.9: *Differences in the concentrations of 3-hydroxyphenylacetic acid between control and grapefruit juice treated adult subjects (n=6). Data are presented as mean ± SEM.*

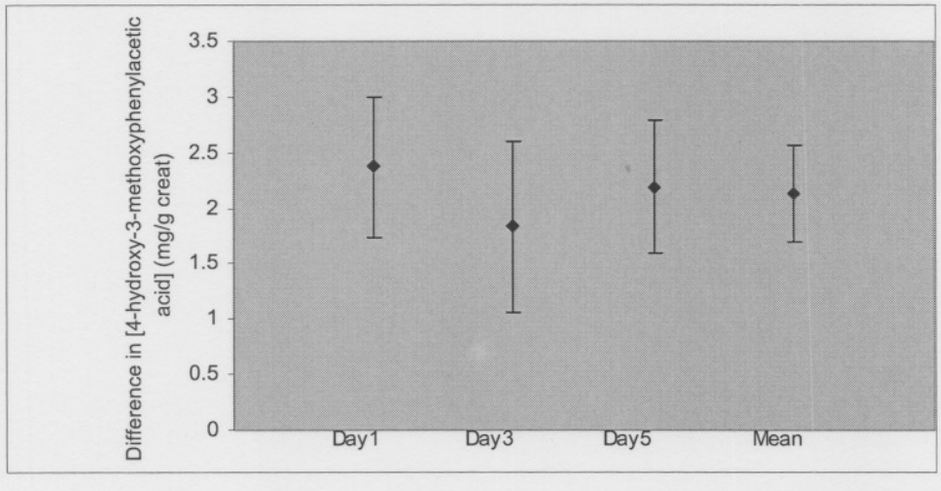


Figure 5.10: *Differences in the concentrations of 4-hydroxy-3-methoxyphenylacetic acid between control and grapefruit juice treated adult subjects (n=6). Data are presented as mean ± SEM.*

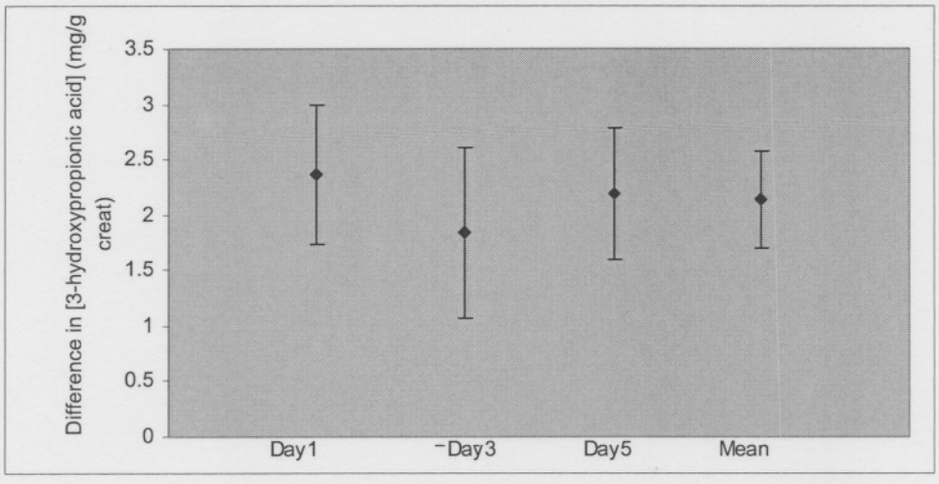


Figure 5.11: *Differences in the concentrations of 3-hydroxypropionic acid between control and grapefruit juice treated adult subjects (n=6). Data are presented as mean ± SEM.*

It is however, apparent that the increase in these metabolites after the grapefruit juice administration was not progressive. This is due to the fact that there was no gut dysbiosis in the subjects participating in the study. The same amount of these metabolites were therefore in the gut and only the p-gp, that normally effluxes them out of the gut wall, were inhibited.

Also, the recent evidence that suggests that there may be considerable substrate overlap between different transport proteins (e.g., p-gp and MRP) (Gutmann *et al.*, 1999) may have contributed to the results obtained from the adults in the study. While the lists of compounds that are substrates for or inhibitors of p-gp are considerable, less is known regarding substrates and inhibitors for other transport proteins other than p-gp (e.g., MRP). Also, little is known about the potential interplay between different transport proteins in terms of substrate transport. For example, in animal models in which a transporter gene is deleted, upregulation of compensatory transporters was observed (Schinkel *et al.*, 1994)

The profiles obtained were not absolutely similar and the variability observed with subjects can mainly be attributed to variation in p-gp expression in the small bowel enterocytes of individuals (Lown *et al.*, 1997b). The number of p-gps in different mammalian species is variable, although the reason for having a larger or smaller number of p-gps is not known (Childs & Ling 1996). Humans are not necessarily created equal in terms of biological make-up. Because of evolutionary and environmental factors, there is a remarkable degree of genetic variability built into the population. Genetic polymorphism of p-gp has been reported and it may represent a major source of individual variability especially with respect to the amount of p-gp substrate found in urine, although interindividual variability of p-gp is not well documented yet. Results obtained from the few studies done, however, revealed that interindividual variability in intestinal p-gp expression may contribute to variability of absorption of p-gp substrates (Lin & Yamazaki, 2003). It was reported by Benedett & Baltes, (2003), that p-gps, mainly those present in the gut, liver and brain are key factors contributing to the differences in drug absorption and drug distribution in humans. This may be genetically related.

The results obtained from the children are not however in correlation with that expected according to the hypothesis made. Considering these results from children, we might speculate that p-gp expression or/and function is related to age. In children p-gp may not be well developed and this may be the reason why metabolic disorders are more observed in children compared to adults.

There is little information on the ontogeny of p-gp in children (NIH Guide, 2000). Although Warrington *et al.,* 2004 showed that there was no apparent change in p-gp expression in the intestine of rats with age, Sievers *et al.*, 1995, the only study that analysed p-gp expression in a large group of children with acute myeloid leukemia (AML) showed that in contrast to adult AML, MDR1 expression was not of prognostic significance. Wuchter *et al.*, 2000 also found no correlation between p-gp expression/function and response to induction chemotherapy in childhood acute lymphoblastic leukemia. There is thus the need for more studies to be conducted on the developmental variations in p-gp and the role of intestinal p-gp in children.

Chapter 6

Conclusion

ABC (ATP binding cassette) transporters comprise one of the largest of all paralogous protein families, amongst which is p-glycoprotein (p-gp), a well characterized efflux pump that is located at high levels in the jejunal villus enterocytes, the primary site of oral absorption.

Apart from the location of p-gp in the intestine, it is also expressed in several other human tissues where it is thought to act as an efflux pump for organic metabolites. Due to this functional importance, it was postulated in this study that mutation of the gene encoding this protein may result in inborn errors of the metabolism. One notable example is where a mutation of the gene encoding the ABC1 protein leads to Tangier diseases.

To test this hypothesis, it was necessary to inhibit p-gp in a way that would not be hazardous to the subjects involved since human subjects were used in this study. Subsequently, the effect of this inhibition on the metabolism of subjects was investigated. Grapefruit juice was chosen as the p-gp inhibitor because of the advantages it has over the other known inhibitors. For example, several findings indicated that grapefruit juice inhibits only p-gp at the intestinal level and not at the hepatic level (Lundahl *et al.*, 1997). Also, in contrast to the other inhibitors, it does not inhibit p-gp function in other tissues. Grapefruit juice is a beverage consumed in large quantities by the general population and has minimal adverse effects, if any, on individuals.

One of the roles of the transporter p-gp is that it is a major determinant of absorption in the intestine. It is speculated that defective p-gp may cause ineffective transport of substances in the intestine. The effect of the inhibition of p-gp at the intestinal level is expected to mimic that of mutation of or defective p-gp in the intestine. This effect

was examined by the analysis of carbohydrates and organic acids in the urine since these methods are traditionally used for detection of metabolic disorders.

Carbohydrates and organic acids were analysed by thin layer chromatography and gas chromatography-mass spectrometry (GC-MS) respectively. Thin layer chromatography is one of the chromatographic techniques that has been used to identify sugars and it is the most suitable test for screening (Prinz et al., 1978). The analysis of organic acids by gas chromatography-mass spectrometry (GC-MS) is also a well-established and important procedure for the diagnosis of disorders of organic acid metabolism (Thompson & Markey, 1975).

Organic acid profiling has been useful in the identification of the source of toxicants from the gut (Goodwin *et al.*, 1994). Moreover, intestinal dysbiosis markers such as organic acids from microbial origin, are found in increased amounts in the urine when there is abnormal overgrowth of these microbes in the intestines. If the concentrations of these markers are excessively high due to microbial overgrowth in the gut, the dysbiosis markers may appear in the urine samples because the p-gp efflux pump may become saturated. It is speculated in this study that these markers may be present in the urine of humans when p-gp is inhibited since p-gp normally acts to efflux them back into the gut.

In this study, no abnormal carbohydrates were detected in the urine following inhibition of p-gp. It may be reasoned that p-gp inhibition does not reflect carbohydrate absorption or that the concentrations of these carbohydrates were too low to be detected by thin layer chromatography.

With the analysis of organic acids by gas chromatography-mass spectrophotometry, and the subsequent statistical analysis of the organic acids from microbial origin, results with the adult group supported the hypothesis. The intestinal dysbiosis markers were present in the urine in increased amounts in adult humans treated with grapefruit juice. Thus, it may be interpreted that inhibition of intestinal p-gp gives rise to a metabolic profile similar to that seen in dysbiosis. The increase in these organic acids from microbial origin in the samples after the intake of grapefruit juice was statistically significant when compared with the controls. The following eight organic acids, solely from microbial origin normally present in the gut were significantly increased when compared with the controls. They were:

1. benzoic acid,
2. m-hydroxybenzoic acid,
3. p-hydroxyphenylacetic acid,
4. hippuric acid,
5. p-cresol,
6. 3-hydroxyphenylacetic acid,
7. 4-hydroxy-3-methoxyphenylacetic acid and
8. 3-hydroxypropionic acid.

These organic acids have also been shown to be elevated in certain metabolic disorders such as phenylketonuria (PKU) (phenylacetic acid) and the disorders of valine-isoleucine metabolism (3-hydroxypropionic acid). These metabolic by-products are also thought to have toxic effects on brain, liver, muscle and other tissues.

It is interesting to note that most of these organic acids found to increase in the urine by p-gp inhibition are phenolic compounds. Since the main purpose of our study was to find a metabolic profile of people with p-gp inhibition, it would be appropriate to strongly speculate that phenolic compounds are substrates of p-gp and so, if there is a mutation or deficiency of p-gp, a rise in phenol and phenolic compounds would be expected in the urine of affected persons.

Thus, to screen for p-gp deficiency, the following protocol is suggested:

Screening of phenolic compounds should first be done by using α-nitrosonaphtol (this is a standard method of analysis). Once elevated phenol and phenolic compounds are found in the urine, GC-MS analysis of the patients should then be done. If the compounds found in this study are present, it can be speculated that their presence is from the following two possibilities:

(i) p-gp deficiency, and

(ii) digestive problems which eventually results in overgrowth of microbes in the intestine.

The next step would be to administer grapefruit juice to these patients. If it is a digestive problem, there would be a further rise in these compounds in the urine. If the problem is from p-gp deficiency, there would be no further rise in the compounds.

This study also revealed that p-gp in children may not be functionally developed since the increase in the amount of the organic acids from microbial origin in the samples after the intake of grapefruit juice, when compared to the controls, were not statistically significant.

The following recommendations for future work are made:

Investigation into the developmental variations in p-glycoprotein expression and functions in children, particularly in the intestines should be made.

Apart from the organic acids from microbial origin, many other compounds were detected in the urine samples after the intake of grapefruit juice that were not in the controls or were in lower amounts in the controls. Further studies should be carried out to identify these compounds and establish their origin.

More p-gp inhibitors with less adverse effects should be developed especially for studies on issues pertaining to p-gp in other tissues in the body.

The effects of inhibition of p-gp expression in the brain, liver and other tissues should be investigated.

Novel diagnostic and therapeutic options should be developed for diseases related to defects or mutation of p-gp.

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Appendix

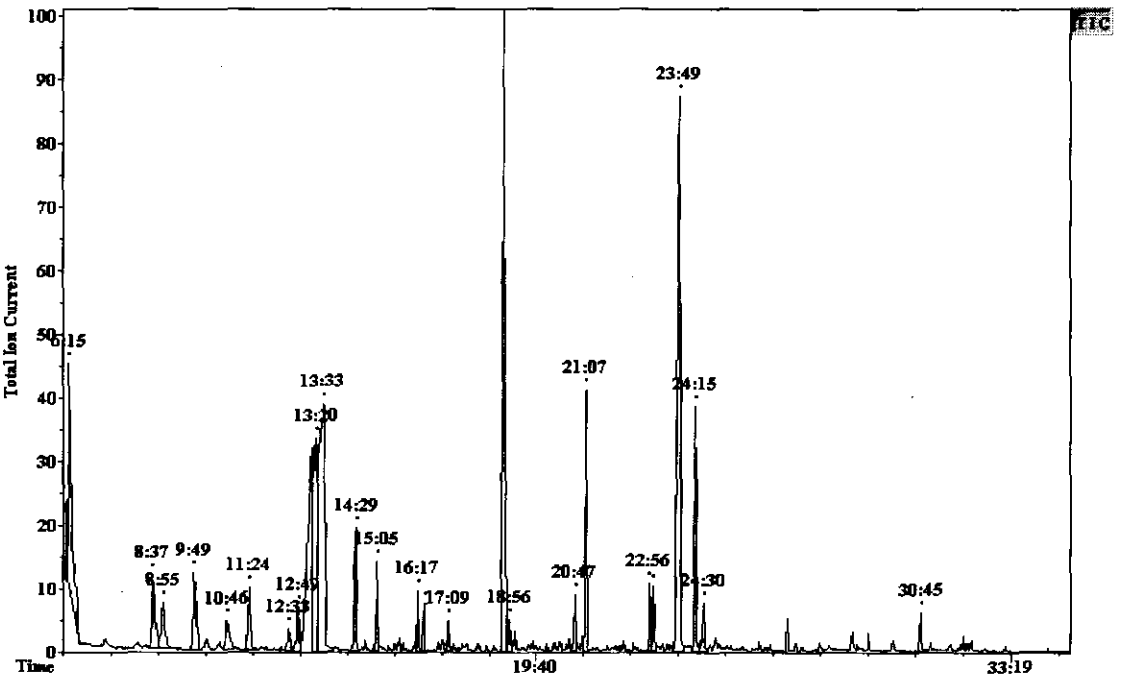

Figure A1: *GC-MS total ion chromatogram of the urine sample on day three after the intake of grapefruit juice of subject AM 29*

Table A1: Interpretation of GC-MS spectrum of urine sample of day three of subject AM 29.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.284	8	301288	162.40
2,3-DIHYDROXYBUTANE-DITMS	8.451	88	3076	1.66
LACTIC-DITMS	8.666	95	62912	33.91
GLYCOLIC-DITMS	8.956	106	46236	24.92
1,2-DIHYDROXYBUTANE-DITMS	9.861	139	73459	39.59
OXALIC-DITMS	10.229	153	11321	6.10
p-CRESOL-TMS	10.805	174	23023	12.41

3-HYDROXYPROPIONIC-DITMS	10.867	176	4084	2.2
3-HYDROXYISOBUTYRIC-DITMS	11.446	197	37869	20.41
3-HYDROXY-ISO-VALERIC-DITMS	12.85	249	35132	18.94
BENZOIC-TMS	13.243	263	15518	8.36
UREA-DITMS	13.606	277	192597	103.81
ETHYLMALONIC-DITMS	14.479	309	11032	5.95
PHOSPHORIC-TRITMS	14.507	310	98836	53.27
GLYCERIN-TRITMS	14.796	320	9074	4.89
SUCCINIC-DITMS	15.126	332	68715	37.04
4-HYDROXY-2-METHYLVALERIC-DITMS	15.478	345	3058	1.65
URACIL-DITMS	15.639	351	2701	1.46
ITACONIC-DITMS	15.772	356	12694	6.84
CITRACONIC-DITMS	15.887	360	6181	3.33
FUMARIC-DITMS	15.985	364	837	0.45
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.479	382	49389	26.62
GLUTARIC-DITMS	17.044	403	4906	2.64
3-METHYLGLUTACONIC-DITMS	17.734	428	2495	1.35
PYROGLUTAMIC-DITMS	19.122	479	17928	9.66
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.624	497	6118	3.3
ERYTHRONIC-TETRATMS	20.464	528	3556	1.92
3-HYDROXYPHENYLACETIC-DITMS	20.677	536	17533	9.45
m-HYDROXYBENZOIC-DITMS	21.059	550	3745	2.02
p-HYDROXYPHENYLACETIC-DITMS	21.168	554	40262	21.7
t-ACONITIC-TRITMS	22.984	620	60244	32.47
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.088	624	61404	33.1
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.135	626	2526	1.36
p-HYDROXYMANDELIC-TRITMS	23.497	639	6523	3.52
HIPPURIC-TMS	23.841	652	448604	241.8
CITRIC-TETRATMS	24.305	669	62107	33.48
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.531	677	45422	24.48
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	24.884	690	3778	2.04
VANILGLYCOL-TRITMS	26.327	743	3083	1.66
PALMITIC-TMS	26.932	765	16358	8.82
p-HYDROXYHIPPURIC-DITMS	28.812	834	11098	5.98
DEHYDROABIETIC ACID	30.796	907	3627	1.96
GALACTONO-1,4-LACTONE-TETRATMS	31.898	947	3967	2.14
DIOCTYLPHTALATE-TMS	32.223	959	6151	3.32
HEXACOSANE-TMS	33.189	994	4009	2.16
18.80 min INTERNAL STANDARD	18.794	467	487015	262.5

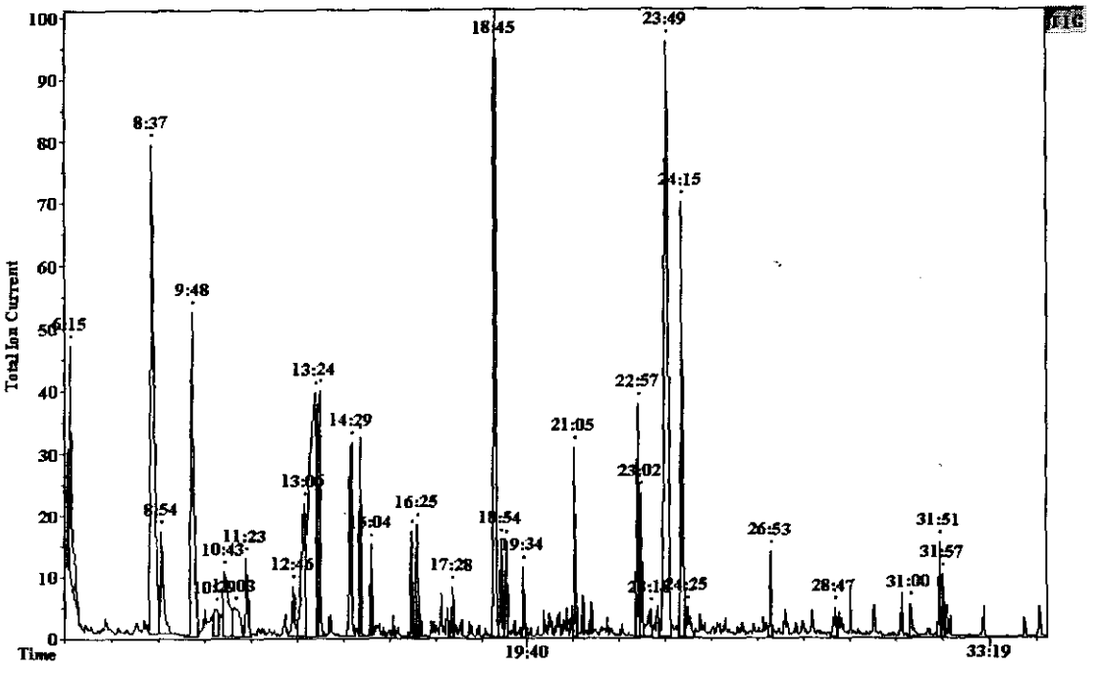

Figure A2: *GC-MS total ion chromatogram of the urine sample on day five after the intake of grapefruit juice of subject AM 29*

Table A2: Interpretation of GC-MS spectrum of urine sample of day five of subject AM 29.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.276	8	323846	192.06
ETHYLENGLYCOL-DITMS	6.803	27	2913	1.73
1,2-DIHYDROXYPROPANE-DITMS	8.426	87	9311	5.52
LACTIC-DITMS	8.658	95	362394	214.92
GLYCOLIC-DITMS	8.933	105	87304	51.78
ETHYLMALONIC-DITMS	9.316	119	2144	1.27
1,2-DIHYDROXYBUTANE-DITMS	9.834	138	285866	169.53
OXALIC-DITMS	10.197	152	19230	11.40
p-CRESOL-TMS	10.767	173	32055	19.01
3-HYDROXYPROPIONIC-DITMS	10.843	175	7691	4.56
3-HYDROXYISOBUTYRIC-DITMS	11.414	196	46010	27.29
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.51	236	4458	2.64
3-HYDROXY-ISO-VALERIC-DITMS	12.811	247	25595	15.18
UREA-DITMS	13.144	260	88644	52.60
BENZOIC-TMS	13.212	262	6099	3.62
PHOSPHORIC-TRITMS	14.509	310	141301	83.80
GLYCERIN-TRITMS	14.771	319	147594	87.53

SUCCINIC-DITMS	15.101	331	70651	41.90
4-HYDROXY-2-METHYLVALERIC-DITMS	15.453	344	4734	2.81
URACIL-DITMS	15.614	350	2082	1.24
ITACONIC-DITMS	15.748	355	17643	10.46
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.291	375	87440	51.86
SERINE-TRITMS	16.544	384	13873	8.23
GLUTARIC-DITMS	17.027	402	4301	2.55
HYDROCHINON-DITMS	17.109	405	1378	0.82
2,4-DIHYDROXYBUTYRIC-TRITMS	17.578	422	6412	3.80
3-METHYLGLUTACONIC-DITMS	17.709	427	1895	1.12
PYROGLUTAMIC-DITMS	19.104	478	82071	48.67
5-PYROLIDON-2-CARBOXYLIC-DITMS	19.104	478	81952	48.60
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.6	496	38469	22.81
ERYTHRITOL-TMS	19.704	500	1939	1.15
2-HYDROXYGLUTARIC-TRITMS	20.367	524	8285	4.91
ERYTHRONIC-TETRATMS	20.438	527	7917	4.70
3-HYDROXYPHENYLACETIC-DITMS	20.654	535	29423	17.45
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.88	543	19815	11.75
m-HYDROXYBENZOIC-DITMS	21.033	549	3736	2.22
TARTARIC-TETRATMS	21.598	569	36678	21.75
ISOCITRICLACTON-DITMS	21.89	580	5616	3.33
SUBERIC-DITMS	22.079	587	7424	4.40
FUCONO-g-LACTONE-PENTATMS	22.188	591	1848	1.10
LYXOSE-TETRATMS(i)	22.518	603	8810	5.23
t-ACONITIC-TRITMS	22.979	620	164669	97.66
3-HYDROXY-4-METHOXYBENZOIC-DITMS	23.059	623	1319	0.78
RIBOSE-PENTATMS	23.072	623	122080	72.40
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.083	624	53814	31.91
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.116	625	3802	2.26
AZELAIC-DITMS	23.555	641	10109	6.00
HIPPURIC-TMS	23.856	652	401438	238.07
CITRIC-TETRATMS	24.287	668	300722	178.34
MYRISTIC-TMS	24.336	670	4019	2.38
LYXOSE-TETRATMS(iv)	24.456	674	23938	14.20
SORBOFURANOSE-TMS	24.456	674	23772	14.10
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	24.862	689	1002	0.59
MANDELIC-DITMS	25.195	701	4899	2.91
VANILLYLMANDELIC-TRITMS	26.306	742	1174	0.70
FUCOSE-PENTATMS(iii)	26.693	756	13253	7.86
PALMITIC-TMS	26.911	764	33451	19.84
FERULIC-DITMS	27.351	780	916	0.54
p-HYDROXYHIPPURIC-DITMS	28.792	833	11383	6.75
DEHYDROABIETIC ACID	30.782	906	24435	14.49
HEXACOSANE-TMS	31.049	916	21034	12.47
DIOCTYLPHTALATE-TMS	32.203	958	11889	7.05
18.80 min INTERNAL STANDARD	18.784	466	442630	262.5

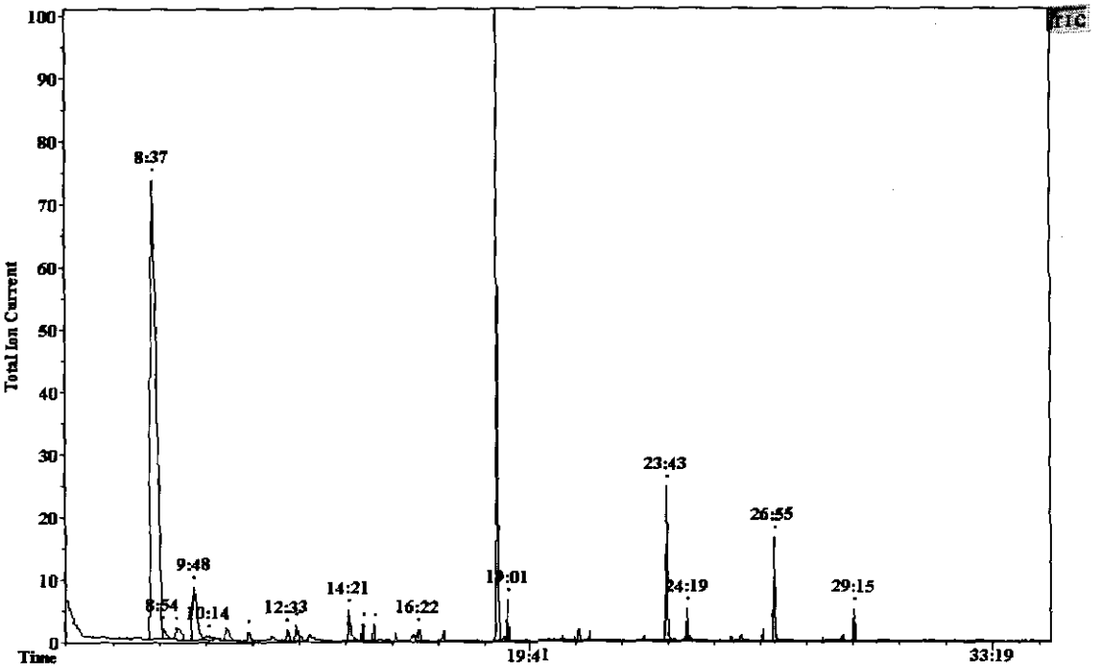

Figure A3: *GC-MS total ion chromatogram of the control sample of subject AM 43*

Table A3: Interpretation of GC-MS spectrum of the control sample of subject AM 43.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.654	95	148018	226.33
1,2-DIHYDROXYBUTANE-DITMS	9.842	138	18522	28.32
P-CRESOL-TMS	10.784	173	3231	4.94
4-HYDROXYBUTYRIC-DITMS	11.431	197	2353	3.6
3-HYDROXY-ISO-VALERIC-DITMS	12.84	248	4519	6.91
PHOSPHORIC-TRITMS	14.39	305	6558	10.03
GLYCERIN-TRITMS	14.792	320	6288	9.62
METHYLMALONIC-DITMS	15.12	332	5284	8.08
2,3-DIHYDROXYBUTANE-DITMS	16.304	375	1860	2.84
4-DEOXYTETRONIC-TRITMS	16.465	381	4474	6.84
SALICYLIC-DITMS	19.076	477	5482	8.38
LAURIC-DITMS	21.46	564	1886	2.88
HIPPURIC-TMS	23.753	648	36100	55.2
MYRISTIC-TMS	24.354	670	5934	9.07
SACCHARIC-1,4LACTONE-TERATMS	24.465	674	1921	2.94
PALMITIC-TMS	26.94	765	17997	27.52
STEARIC-TMS	29.289	851	5491	8.4
18.80 min INTERNAL STANDARD	18.754	465	171673	262.5

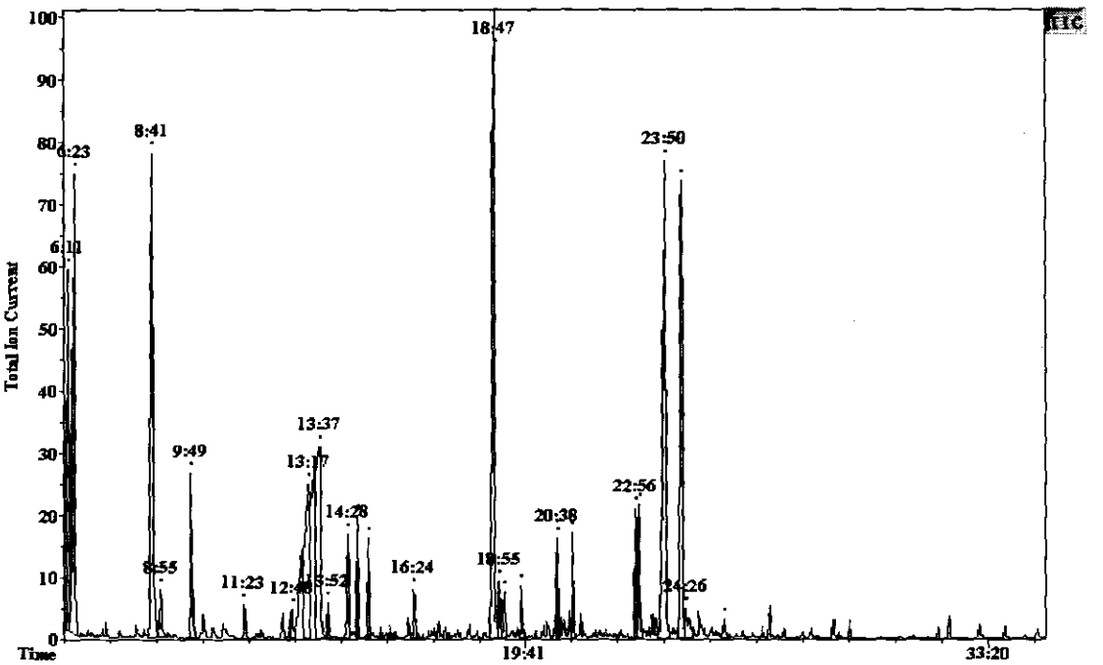

Figure A4: *GC-MS spectra of the urine sample on day one after the intake of grapefruit juice of subject AM 43*

Table A4: Interpretation of GC-MS spectrum of urine sample of day one of subject AM 43.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.412	13	584511	332.91
ETHYLENGLYCOL-DITMS	6.91	31	2180	1.24
LACTIC-DITMS	8.709	97	354409	201.85
2-HYDROXYISOBUTYRIC-DITMS	8.905	104	11947	6.80
GLYCOLIC-DITMS	8.968	107	37436	21.32
1,2-DIHYDROXYBUTANE-DITMS	9.861	139	136726	77.87
OXALIC-DITMS	10.229	153	8878	5.06
p-CRESOL-TMS	10.795	173	9470	5.39
3-HYDROXYPROPIONIC-DITMS	10.866	176	3554	2.02
3-HYDROXYISOBUTYRIC-DITMS	11.428	197	17010	9.69
3-HYDROXY-ISO-VALERIC-DITMS	12.83	248	18744	10.68
BENZOIC-TMS	13.221	262	4123	2.35
UREA-DITMS	13.669	279	132249	75.32
ETHYLMALONIC-DITMS	14.453	307	6194	3.53
PHOSPHORIC-TRITMS	14.502	309	71403	40.67
GLYCERIN-TRITMS	14.775	319	92913	52.92

SUCCINIC-DITMS	15.105	331	73129	41.65
4-HYDROXY-2-METHYLVALERIC-DITMS	15.457	344	6304	3.59
URACIL-DITMS	15.621	350	1934	1.10
ITACONIC-DITMS	15.749	355	11487	6.54
CITRACONIC-DITMS	15.88	360	4830	2.75
4-DEOXYTETRONIC-TRITMS	16.282	374	16640	9.48
2,3-DIHYDROXYBUTANE-DITMS	16.451	380	43153	24.58
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.461	381	7521	4.28
SERINE-TRITMS	16.552	384	3801	2.17
GLUTARIC-DITMS	17.021	401	2607	1.49
3-METHYLGLUTACONIC-DITMS	17.715	427	2569	1.46
5-PYROLIDON-2-CARBOXYLIC-DITMS	19.11	478	38229	21.77
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.607	496	29526	16.82
TREONIC-TETRATMS	20.448	527	709	0.40
PIMELIC-DITMS	20.59	532	5345	3.04
3-HYDROXYPHENYLACETIC-DITMS	20.666	535	120683	68.73
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.887	543	11504	6.55
m-HYDROXYBENZOIC-DITMS	21.029	548	12501	7.12
p-HYDROXYPHENYLACETIC-DITMS	21.136	552	107138	61.02
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	22.525	603	6975	3.97
t-ACONITIC-TRITMS	22.98	620	103296	58.83
c-ACONITIC-TRITMS	22.986	620	48833	27.81
3-HYDROXY-4-METHOXYBENZOIC-DITMS	23.057	622	1689	0.96
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.076	623	120706	68.75
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.119	625	1933	1.10
MANNONIC-1,4-LACTONE-TMS	23.474	638	24570	13.99
AZELAIC-DITMS	23.559	641	7768	4.42
HIPPURIC-TMS	23.854	652	353139	201.13
LYXOSE-TETRATMS(iv)	24.073	660	4685	2.67
CITRIC-TETRATMS	24.343	670	157361	89.62
SORBOFURANOSE-TMS	24.461	674	26087	14.86
FRUCTOFURANOSE-TMS	24.542	677	4803	2.74
ARABINOSE-PENTATMS	24.657	681	1806	1.03
VANILGLYCOL-TRITMS	24.824	687	21476	12.23
FUCOSE-PENTATMS(iii)	25.564	714	6268	3.57
LYXOSE-TETRATMS(i)	25.577	715	14320	8.16
HEXACOSANE-TMS	25.61	716	1589	0.91
PALMITIC-TMS	26.92	764	13857	7.89
GLUCURONIC-PENTATMS	27.163	773	2722	1.55
FERULIC-DITMS	27.356	780	1051	0.60
p-HYDROXYHIPPURIC-DITMS	28.8	833	11667	6.65
STEARIC-TMS	29.264	850	7248	4.13
GALACTONO-1,4-LACTONE-TETRATMS	31.879	946	7658	4.36
DIOCTYLPHTALATE-TMS	32.204	958	10775	6.14
18.80 min INTERNAL STANDARD	18.804	467	460894	262.5

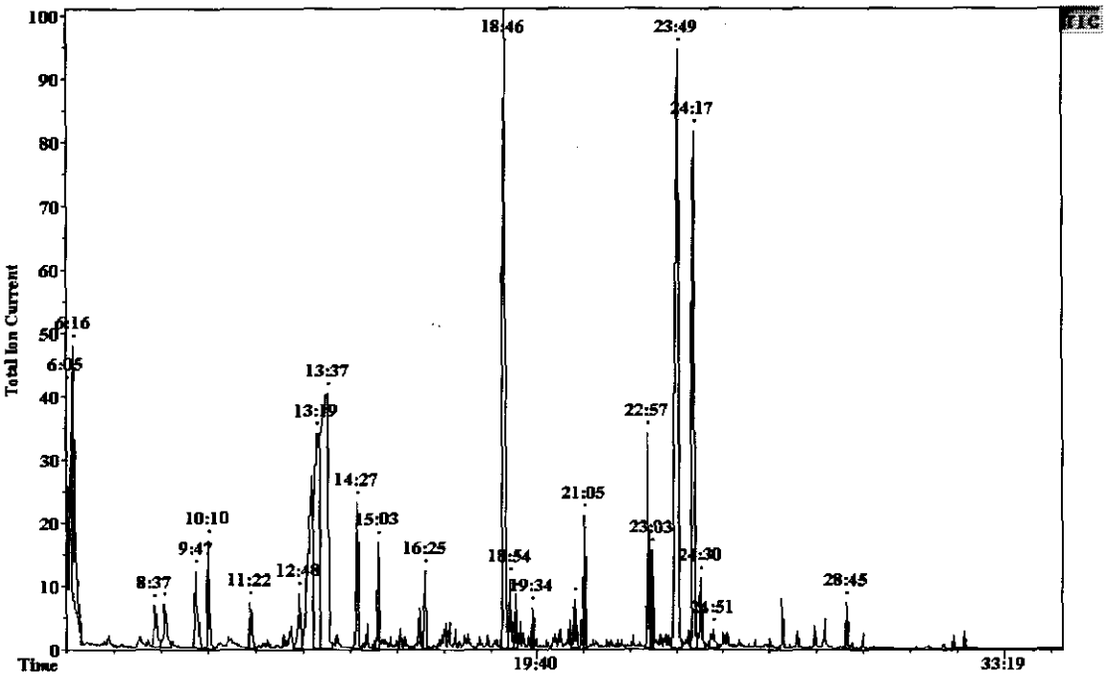

Figure A5: *GC-MS spectra of the urine sample on day three after the intake of grapefruit juice of subject AM 43*

Table A5: Interpretation of GC-MS spectrum of urine sample of day three of subject AM 43.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.303	9	327848	205.07
ETHYLENGLYCOL-DITMS	6.827	28	1836	1.15
LACTIC-DITMS	8.651	95	31485	19.69
GLYCOLIC-DITMS	8.935	105	32303	20.21
1,2-DIHYDROXYBUTANE-DITMS	9.836	138	57513	35.97
OXALIC-DITMS	10.209	152	111126	69.51
p-CRESOL-TMS	10.779	173	6538	4.09
3-HYDROXYPROPIONIC-DITMS	10.847	175	2964	1.85
3-HYDROXYISOBUTYRIC-DITMS	11.417	196	21363	13.36
UREA-TRITMS	12.359	231	6168	3.86
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.558	238	16032	10.03
3-HYDROXY-ISO-VALERIC-DITMS	12.822	248	32442	20.29
UREA-DITMS	13.177	261	107493	67.24
BENZOIC-TMS	13.218	262	4583	2.87
PHOSPHORIC-TRITMS	14.49	309	101525	63.50
GLYCERIN-TRITMS	14.771	319	15439	9.66
SUCCINIC-DITMS	15.104	331	71319	44.61
ETHYLMALONIC-DITMS	15.451	344	5383	3.37
URACIL-DITMS	15.612	350	2020	1.26

ITACONIC-DITMS	15.748	355	13539	8.47
CITRACONIC-DITMS	15.868	359	6254	3.91
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.291	375	27840	17.41
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	17.055	403	17840	11.16
3-METHYLGLUTACONIC-DITMS	17.709	427	4229	2.65
PYROGLUTAMIC-DITMS	19.103	478	38829	24.29
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	19.215	482	8729	5.46
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.6	496	19713	12.33
2-HYDROXYGLUTARIC-TRITMS	20.383	525	9544	5.97
ERYTHRONIC-TETRATMS	20.429	527	4845	3.03
TREONIC-TETRATMS	20.44	527	1168	0.73
3-HYDROXYPHENYLACETIC-DITMS	20.658	535	27984	17.50
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.891	544	1521	0.95
m-HYDROXYBENZOIC-DITMS	21.035	549	9148	5.72
p-HYDROXYPHENYLACETIC-DITMS	21.139	553	6863	4.29
ISOCITRICLACTON-DITMS	21.889	580	2769	1.73
m-HYDROXYPHENYLPROPIONIC-DITMS	22.522	603	4392	2.75
VANILLIC-DITMS	23.057	623	3129	1.96
LYXOSE-TETRATMS(iv)	23.081	624	80816	50.55
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.111	625	7221	4.52
p-HYDROXYMANDELIC-TRITMS	23.485	639	5072	3.17
HIPPURIC-TMS	23.846	652	383979	240.18
CITRIC-TETRATMS	24.312	669	162265	101.50
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.531	677	26719	16.71
VANILGLYCOL-TRITMS	24.826	688	12235	7.65
ISOVANILGLYCOLIC-TRITMS	24.828	688	8561	5.36
VANILGLYCOLIC-TRITMS	24.828	688	8505	5.32
MANDELIC-DITMS	25.194	701	10696	6.69
1,6-ANHYDRO-b-d-GALACTOPYRANOSE-TRITMS	27.153	773	2972	1.86
FERULIC-DITMS	27.344	780	6266	3.92
HEXACOSANE-TMS	28.765	832	1104	0.69
p-HYDROXYHIPPURIC-DITMS	28.79	833	25480	15.94
DIOCTYLPHTALATE-TMS	32.2	958	9152	5.73
18.80 min INTERNAL STANDARD	18.792	467	419666	262.5

4-DEOXYTETRONIC-TRITMS	16.159	370	14622	6.64
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.323	376	21001	9.54
PYROLCARBOXYLIC-DITMS	16.568	385	11413	5.18
GLUTARIC-DITMS	16.898	397	5591	2.54
3-METHYLGLUTACONIC-DITMS	17.585	422	3225	1.46
ADIPIC-DITMS	18.812	467	5744	2.61
SALICYLIC-DITMS	18.941	472	7618	3.46
3-METHYLADIPIC-DITMS	19.375	488	1385	0.63
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.489	492	10079	4.58
ARABINOSE-TETRATMS	19.596	496	2777	1.26
2-HYDROXYGLUTARIC-TRITMS	20.275	521	4331	1.97
ERYTHRONIC-TETRATMS	20.324	523	3180	1.44
3-HYDROXYPHENYLACETIC-DITMS	20.55	531	52469	23.82
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.788	540	1781	0.81
m-HYDROXYBENZOIC-DITMS	20.927	545	7801	3.54
p-HYDROXYPHENYLACETIC-DITMS	21.019	548	70051	31.81
LAURIC-DITMS	21.341	560	4884	2.22
TARTARIC-TETRATMS	21.502	566	5583	2.54
SUBERIC-DITMS	21.968	583	3896	1.77
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	22.413	599	8731	3.97
m-HYDROXYPHENYLPROPIONIC-DITMS	22.424	600	2305	1.05
t-ACONITIC-TRITMS	22.866	616	43013	19.53
c-ACONITIC-TRITMS	22.869	616	35323	16.04
VANILLIC-DITMS	22.948	619	3930	1.79
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	22.97	620	20021	9.09
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.003	621	4308	1.96
m-HYDROXYMANDELIC-TRITMS	23.382	635	8018	3.64
TECEPHALIC-DITMS	23.461	638	12717	5.77
HIPPURIC-TMS	23.655	645	147518	66.98
a-RESORCYLIC-TRITMS	23.892	654	2263	1.03
CITRIC-TETRATMS	24.177	664	244142	110.86
MYRISTIC-TMS	24.231	666	15043	6.83
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.42	673	57603	26.16
VANILGLYCOLIC-TRITMS	24.722	684	6210	2.82
?? VANILLYLMANDELIC-TRITMS	24.722	684	6169	2.8
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	24.752	685	2534	1.15
p-HYDROXYPHENYLLACTIC-TRITMS	25.099	698	4227	1.92
LYXOSE-TETRATMS(i)	25.458	711	8464	3.84
PENTADECANOIC-TMS	25.546	714	4171	1.89
PALMITOLEIC-TMS	26.468	748	4753	2.16
PALMITIC-TMS	26.817	761	55023	24.98
HEPTADECANOIC-TMS	28.001	804	1183	0.54
OLEIC-TMS	28.833	835	7119	3.23
STEARIC-TMS	29.163	847	13478	6.12
DIOCTYLPHTALATE-TMS	32.093	954	36579	16.61
18.80 min INTERNAL STANDARD	18.66	462	578109	262.5

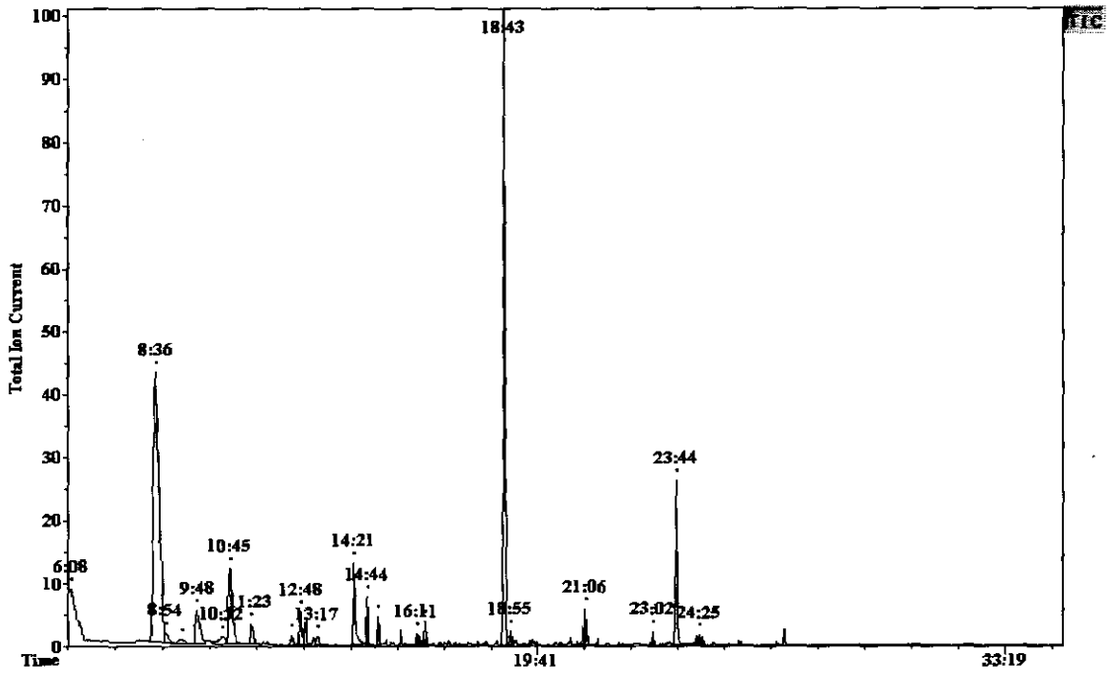

Figure A7: *GC-MS total ion chromatogram of the control sample of subject AM 32*

Table A7: Interpretation of GC-MS spectrum of the control sample of subject AM 32.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.167	4	11937	11.35
LACTIC-DITMS	8.635	94	134221	127.57
2,3-DIHYDROXYBUTANE-DITMS	8.644	95	110471	104.99
1,2-DIHYDROXYBUTANE-DITMS	9.842	138	18240	17.34
p-CRESOL-TMS	10.772	173	26069	24.78
4-HYDROXYBUTANOIC-TRITMS	11.422	196	6191	5.88
3-HYDROXY-ISO-VALERIC-DITMS	12.835	248	13300	12.64
UREA-DITMS	12.991	254	8436	8.02
BENZOIC-TMS	13.22	262	2209	2.10
3-ETHYLHYDRACRYLIC-DITMS	13.329	266	2886	2.74
PHOSPHORIC-TRITMS	14.385	305	25610	24.34
GLYCERIN-TRITMS	14.776	319	22338	21.23
SUCCINIC-DITMS	15.103	331	12571	11.95
ITACONIC-DITMS	15.753	355	6880	6.54
1,4-DIHYDROXYBUTANE-DITMS	16.383	378	914	0.87
4-DEOXYTETRONIC-TRITMS	16.459	381	12882	12.24
PYROGLUTAMIC-DITMS	19.105	478	2120	2.02
m-HYDROXYBENZOIC-DITMS	21.045	549	4960	4.71
p-HYDROXYPHENYLACETIC-DITMS	21.146	553	2028	1.93
MANNONIC-1,4-LACTONE-TMS	23.087	624	2008	1.91
HIPPURIC-TMS	23.771	649	64152	60.97

SACCHARIC-1,4LACTONE-TERATMS	24.459	674	5976	5.68
LYXOSE-TETRATMS(i)	25.573	715	1338	1.27
DIOCTYLPHTALATE-TMS	32.214	958	536	0.51
18.80 min INTERNAL STANDARD	18.756	465	276196	262.5

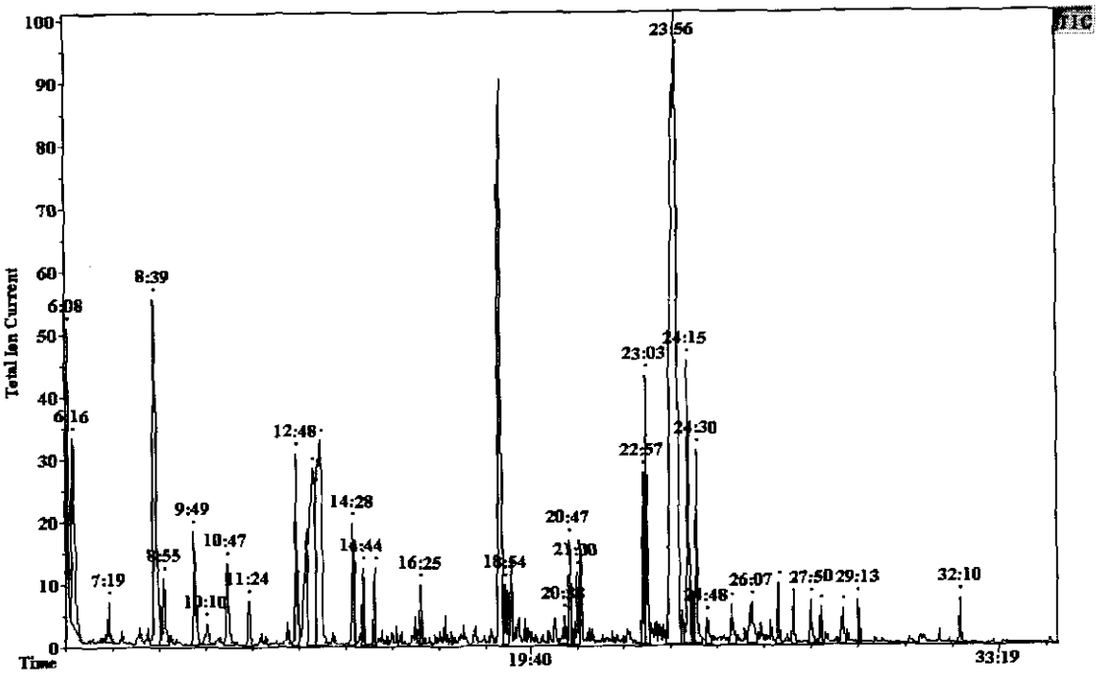

Figure A8: *GC-MS total ion chromatogram of the urine sample on day one after the intake of grapefruit juice of subject AM 32*

Table A8: Interpretation of GC-MS spectrum of urine sample of day one of subject AM 32.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.355	11	301122	161.99
ETHYLENGLYCOL-DITMS	6.879	30	4615	2.48
LACTIC-DITMS	8.698	97	322476	173.48
2-HYDROXYISOBUTYRIC-DITMS	8.909	104	10223	5.50
GLYCOLIC-DITMS	8.963	106	62705	33.73
PYRUVIC-TMS	9.348	120	4891	2.63
1,2-DIHYDROXYBUTANE-DITMS	9.858	139	120555	64.85
OXALIC-DITMS	10.235	153	24170	13.00
p-CRESOL-TMS	10.802	174	80078	43.08
3-HYDROXYPROPIONIC-DITMS	10.868	176	5937	3.19
3-HYDROXYISOBUTYRIC-DITMS	11.438	197	33868	18.22
3-HYDROXY-ISO-VALERIC-DITMS	12.835	248	135548	72.92
UREA-DITMS	13.155	260	79582	42.81

BENZOIC-TMS	13.237	263	8616	4.64
ETHYLMALONIC-DITMS	14.467	308	13679	7.36
PHOSPHORIC-TRITMS	14.489	309	105336	56.67
GLYCERIN-TRITMS	14.786	320	63277	34.04
SUCCINIC-DITMS	15.116	332	84695	45.56
1,2-DIHYDROXYBENZENE-DITMS	15.318	339	24088	12.96
ETHYLMALONIC-DITMS	15.457	344	5249	2.82
4-HYDROXY-2-METHYLVALERIC-DITMS	15.465	345	5169	2.78
URACIL-DITMS	15.621	350	3750	2.02
ITACONIC-DITMS	15.751	355	15958	8.59
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.458	381	57757	31.07
SERINE-TRITMS	16.564	385	3105	1.67
GLUTARIC-DITMS	17.031	402	5484	2.95
2,4-DIHYDROXYBUTYRIC-TRITMS	17.582	422	4092	2.20
3-METHYLGLUTACONIC-DITMS	17.713	427	9825	5.26
3-HYDROXYADIPYLLACTONE-TMS	17.776	429	5489	2.95
CITRAMALIC-TRITMS	18.747	465	5716	3.08
5-PYROLIDON-2-CARBOXYLIC-DITMS	19.108	478	72578	39.04
PIPECOLIC ACID-DITMS	19.108	478	72288	38.89
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	19.219	482	6322	3.40
PIMELIC-DITMS	19.487	492	12663	6.81
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.605	496	9703	5.22
ERYTHRITOL-TMS	19.703	500	7889	4.24
2-HYDROXYGLUTARIC-TRITMS	20.385	525	16584	8.92
ERYTHRONIC-TETRATMS	20.429	527	6975	3.75
3-HYDROXYPHENYLACETIC-DITMS	20.661	535	30316	16.31
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.902	544	2536	1.36
m-HYDROXYBENZOIC-DITMS	21.041	549	27025	14.54
p-HYDROXYPHENYLACETIC-DITMS	21.147	553	20190	10.86
LYXOSE-TETRATMS(i)	22.523	603	10355	5.57
t-ACONITIC-TRITMS	22.982	620	149969	80.68
VANILLIC-DITMS	23.064	623	2474	1.33
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.091	624	260066	139.91
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.119	625	5717	3.08
p-HYDROXYMANDELIC-TRITMS	23.493	639	5441	2.93
AZELAIC-DITMS	23.558	641	7362	3.96
HIPPURIC-TMS	23.957	656	496980	267.36
CITRIC-TETRATMS	24.307	669	83685	45.02
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.539	677	174605	93.93
VANILGLYCOLIC-TRITMS	24.836	688	10627	5.72
VANILGLYCOL-TRITMS	24.847	688	19718	10.61
GALACTOSE-6TMS	25.573	715	11855	6.38
FUCOSE-PENTATMS(iii)	26.697	756	21953	11.81
PALMITIC-TMS	26.918	764	28028	15.08
ARABINOSE-TETRATMS	27.161	773	8314	4.47
m-HYDROXYHIPPURIC-DITMS	27.868	799	15750	8.47
p-HYDROXYHIPPURIC-DITMS	28.798	833	22855	12.30
HEXACOSANE-TMS	30.738	904	2829	1.52
DIOCTYLPHTALATE-TMS	32.208	958	29035	15.62
18.80 min INTERNAL STANDARD	18.799	467	487956	262.5

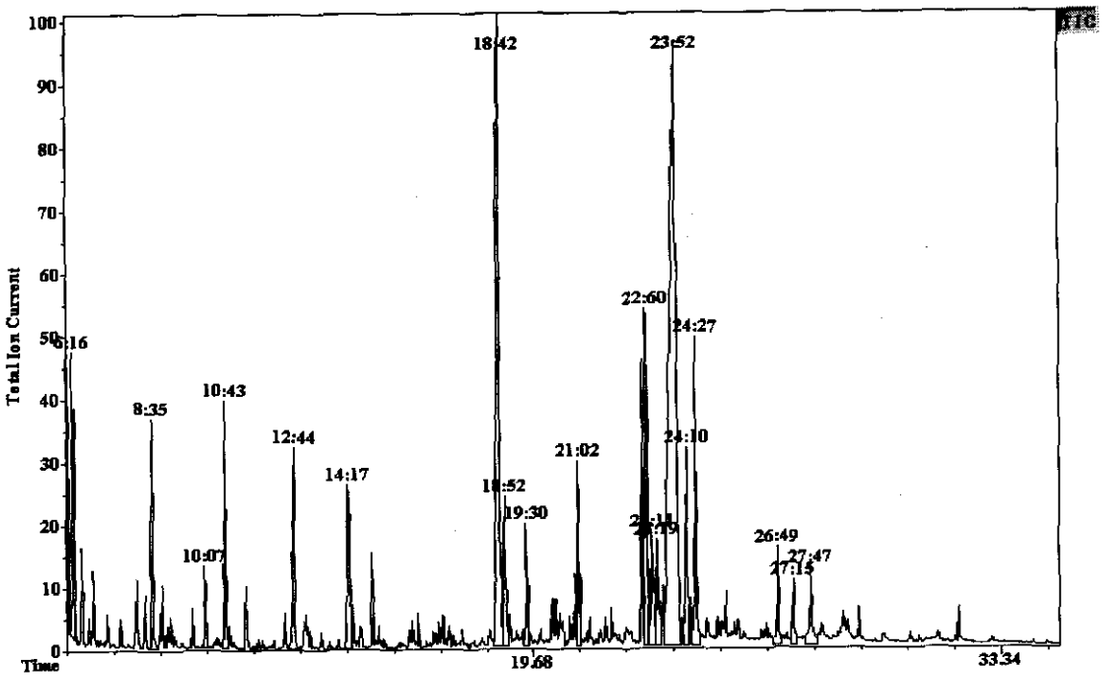

Figure A9: *GC-MS total ion chromatogram of the urine sample on day three after the intake of grapefruit juice of subject AM 32*

Table A9: Interpretation of GC-MS spectrum of urine sample of day three of subject AM 32.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.328	10	474612	186.59
ETHYLENGLYCOL-DITMS	6.835	28	16552	6.51
3-MERCAPTOPROPIONIC-DITMS	7.447	51	1850	0.73
LACTIC-DITMS	8.601	93	210464	82.74
2-HYDROXYISOBUTYRIC-DITMS	8.817	101	18452	7.25
GLYCOLIC-DITMS	8.877	103	64535	25.37
PYRUVIC-TMS	9.259	117	13178	5.18
1,2-DIHYDROXYBUTANE-DITMS	9.772	136	52961	20.82
OXALIC-DITMS	10.138	149	102097	40.14
p-CRESOL-TMS	10.727	171	309005	121.48
DIPROPYLACETIC-TMS	10.97	180	6315	2.48
3-HYDROXYISOBUTYRIC-DITMS	11.33	193	58166	22.87
3-HYDROXY-ISO-VALERIC-DITMS	12.741	245	177997	69.98
UREA-DITMS	13.071	257	32441	12.75
BENZOIC-TMS	13.126	259	16602	6.53
3-ETHYLHYDRACRYLIC-DITMS	13.219	262	14455	5.68
PHOSPHORIC-TRITMS	14.316	302	197140	77.51
ETHYLMALONIC-DITMS	14.348	304	22663	8.91

GLYCERIN-TRITMS	14.679	316	17398	6.84
SUCCINIC-DITMS	15.012	328	142457	56.01
1,2-DIHYDROXYBENZENE-DITMS	15.211	335	40588	15.96
2-METHYLSUCCINIC-DITMS	15.355	340	8739	3.44
URACIL-DITMS	15.522	347	1038	0.41
FUMARIC-DITMS	15.863	359	4408	1.73
NONANOIC-TMS	16.081	367	9255	3.64
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.354	377	32274	12.69
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	16.866	396	8489	3.34
GLUTARIC-DITMS	16.946	399	16942	6.66
HYDROCHINON-DITMS	17.011	401	7638	3.00
3-METHYLGLUTACONIC-DITMS	17.628	424	11354	4.46
CITRAMALIC-TRITMS	18.668	462	11153	4.39
ADIPIC-DITMS	18.859	469	39649	15.59
5-PYROLIDON-2-CARBOXYLIC-DITMS	19.023	475	28012	11.01
3-METHYLADIPIC-DITMS	19.405	489	5682	2.23
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.534	494	70126	27.57
ERYTHRITOL-TMS	19.621	497	3084	1.21
o-HYDROXYPHENYLACETIC-DITMS	19.93	508	10865	4.27
PIMELIC-DITMS	20.509	529	9729	3.83
3-HYDROXYPHENYLACETIC-DITMS	20.574	532	24881	9.78
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.803	540	22059	8.67
m-HYDROXYBENZOIC-DITMS	20.957	546	41158	16.18
p-HYDROXYPHENYLACETIC-DITMS	21.058	549	40817	16.05
ARABINONIC-g-LACTONE-TRITMS	21.29	558	8698	3.42
ISOCITRICLACTON-DITMS	21.839	578	21629	8.50
SUBERIC-DITMS	22.003	584	19645	7.72
c-ACONITIC-TRITMS	22.924	618	201148	79.08
VANILLIC-DITMS	23.005	621	19627	7.72
1,6-ANHYDRO-b-d-GALACTOPYRANOSE-TRITMS	23.016	621	155404	61.10
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.033	622	169711	66.72
p-HYDROXYMANDELIC-TRITMS	23.408	635	17358	6.82
AZELAIC-DITMS	23.479	638	13767	5.41
TECEPHALIC-DITMS	23.503	639	34710	13.65
HIPPURIC-TMS	23.877	653	592782	233.05
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	24.104	661	3169	1.25
3,4-DIHYDROXYPHENYLACETIC-TRITMS	24.104	661	3176	1.25
ISOCITRIC-TETRATMS	24.186	664	110118	43.29
MYRISTIC-TMS	24.266	667	18602	7.31
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.457	674	227672	89.51
VANILGLYCOLIC-TRITMS	24.758	685	24360	9.58
VANILLYLMANDELIC-TRITMS	24.758	685	24356	9.58
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	24.791	686	3177	1.25
GALACTOSE-6TMS(iii)	25.083	697	13519	5.32
p-COUMARIC-DITMS	25.397	708	768	0.31
PENTADECANOIC-TMS	25.583	715	7367	2.90
GLUCOPYRANOSE-HEXATMS(ii)	25.684	719	15776	6.20
GLUCURONIC-PENTATMS	26.356	743	9006	3.54
PALMITOLEIC-TMS	26.487	748	5674	2.23
PALMITIC-TMS	26.84	761	44628	17.55
p-HYDROXYHIPPURIC-DITMS	28.736	830	20233	7.96
STEARIC-TMS	29.186	847	13287	5.22
DEHYDROABIETIC ACID	30.696	902	6010	2.36
DIOCTYLPHTALATE-TMS	32.118	954	30967	12.18
HEXACOSANE-TMS	33.082	990	4380	1.72
18.80 min INTERNAL STANDARD	18.717	464	667692	262.5

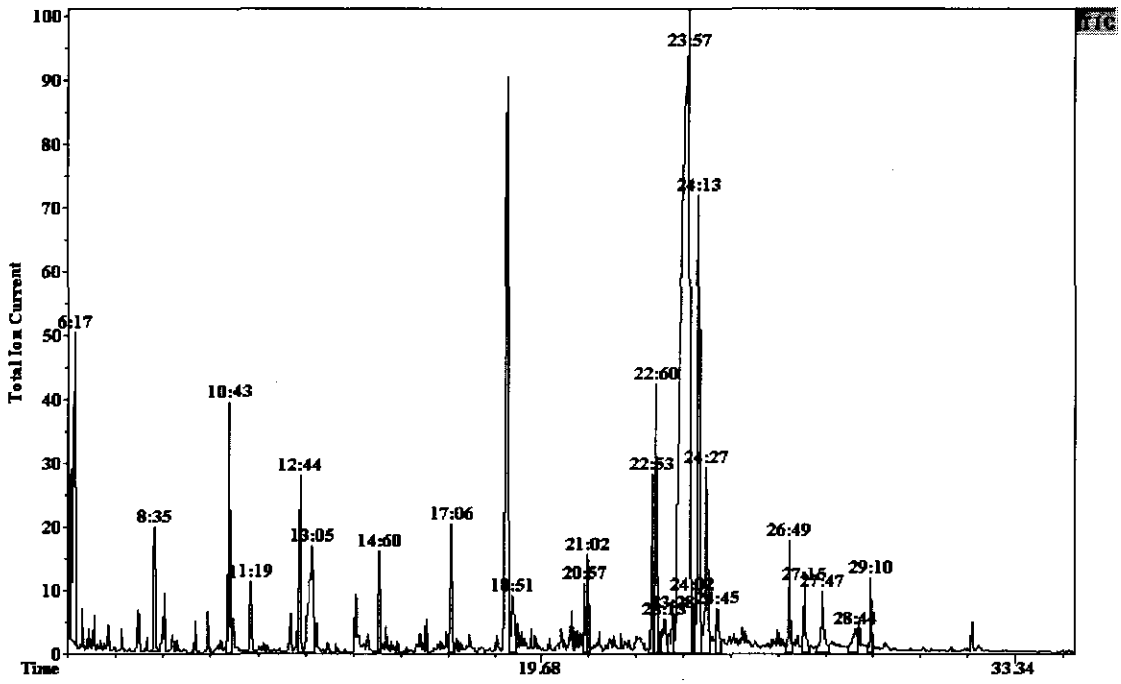

Figure A10: *GC-MS total ion chromatogram of the urine sample on day five after the intake of grapefruit juice of subject AM 32*

Table A10: Interpretation of GC-MS spectrum of urine sample of day five of subject AM 32.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.303	9	743793	302.28
ETHYLENGLYCOL-DITMS	6.806	27	9769	3.98
3-MERCAPTOPROPIONIC-DITMS	7.425	50	3430	1.40
LACTIC-DITMS	8.596	93	131515	53.45
2-HYDROXYISOBUTYRIC-DITMS	8.812	101	14893	6.05
GLYCOLIC-DITMS	8.875	103	60778	24.70
PYRUVIC-TMS	9.256	117	17043	6.93
1,2-DIHYDROXYBUTANE-DITMS	9.772	136	45860	18.64
OXALIC-DITMS	10.133	149	59302	24.10
p-CRESOL-TMS	10.728	171	329038	133.72
3-HYDROXYISOBUTYRIC-DITMS	11.331	193	70521	28.66
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.425	233	3879	1.58
3-HYDROXY-ISO-VALERIC-DITMS	12.744	245	163298	66.36
UREA-DITMS	13.097	258	109812	44.63
BENZOIC-TMS	13.132	259	38049	15.46
3-ETHYLHYDRACRYLIC-DITMS	13.238	263	27206	11.06
OCTANOIC-TMS	13.866	286	1768	0.72
PHOSPHORIC-TRITMS	14.338	303	57891	23.53

GLYCERIN-TRITMS	14.687	316	13657	5.55
SUCCINIC-DITMS	15.017	328	140895	57.26
1,2-DIHYDROXYBENZENE-DITMS	15.228	336	55924	22.73
2-METHYLSUCCINIC-DITMS	15.369	341	11139	4.53
URACIL-DITMS	15.536	347	6949	2.82
GLYCERIC-TRITMS	15.803	357	5098	2.07
FUMARIC-DITMS	15.877	360	2509	1.02
m-HYDROXYBENZALDEHYDE-TMS	15.943	362	1040	0.43
NONANOIC-TMS	16.087	367	3800	1.54
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.374	378	32939	13.39
GLUTARIC-DITMS	16.952	399	12013	4.88
HYDROCHINON-DITMS	17.032	402	5213	2.12
3-METHYLGLUTARIC-DITMS	17.392	415	5401	2.20
3-METHYLGLUTACONIC-DITMS	17.634	424	9329	3.79
ADIPIC-DITMS	18.874	469	30285	12.31
5-PYROLIDON-2-CARBOXYLIC-DITMS	19.027	475	28907	11.75
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	19.139	479	7780	3.16
3-METHYLADIPIC-DITMS	19.409	489	12626	5.13
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.54	494	5822	2.37
MANNONIC-1,4-LACTONE-TMS	19.627	497	3637	1.48
o-HYDROXYPHENYLACETIC-DITMS	19.939	508	11030	4.48
2-HYDROXYGLUTARIC-TRITMS	20.305	522	6689	2.72
PIMELIC-DITMS	20.528	530	4296	1.75
3-HYDROXYPHENYLACETIC-DITMS	20.583	532	53173	21.61
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.823	541	7779	3.16
m-HYDROXYBENZOIC-DITMS	20.962	546	48511	19.72
p-HYDROXYPHENYLACETIC-DITMS	21.064	550	133663	54.32
LAURIC-DITMS	21.375	561	7780	3.16
ISOCITRICLACTON-DITMS	21.814	577	10327	4.20
SUBERIC-DITMS	22.005	584	8669	3.52
t-ACONITIC-TRITMS	22.906	617	159444	64.80
c-ACONITIC-TRITMS	22.909	617	158464	64.40
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.013	621	258458	105.04
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.046	622	9539	3.88
p-HYDROXYMANDELIC-TRITMS	23.42	636	27616	11.22
AZELAIC-DITMS	23.494	639	13724	5.58
TECEPHALIC-DITMS	23.502	639	17176	6.98
HIPPURIC-TMS	23.844	651	406674	165.27
3,4-DIHYDROXYPHENYLACETIC-TRITMS	24.129	662	3157	1.28
CITRIC-TETRATMS	24.238	666	311720	126.68
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.479	675	143474	58.31
ISOVANILGLYCOLIC-TRITMS	24.763	685	43354	17.62
VANILLYLMANDELIC ACID (TRIMETHYLSILYL DERIVATIVE) $	24.771	685	28664	11.65
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	24.812	687	4168	1.69
p-HYDROXYPHENYLLACTIC-TRITMS	25.143	699	7124	2.90
PENTADECANOIC-TMS	25.585	715	5766	2.34
GLUCURONIC-PENTATMS	26.372	744	3776	1.54
FUCOSE-PENTATMS(iii)	26.628	753	7852	3.19
PALMITIC-TMS	26.844	761	51840	21.07
p-HYDROXYHIPPURIC-DITMS	27.792	796	43297	17.60
OLEIC-TMS	28.86	835	10759	4.37
HEXACOSANE-TMS	29.625	863	5661	2.31
DEHYDROABIETIC ACID	30.695	902	2568	1.04
DIOCTYLPHTALATE-TMS	32.128	955	35408	14.39
18.80 min INTERNAL STANDARD	18.718	464	645919	262.5

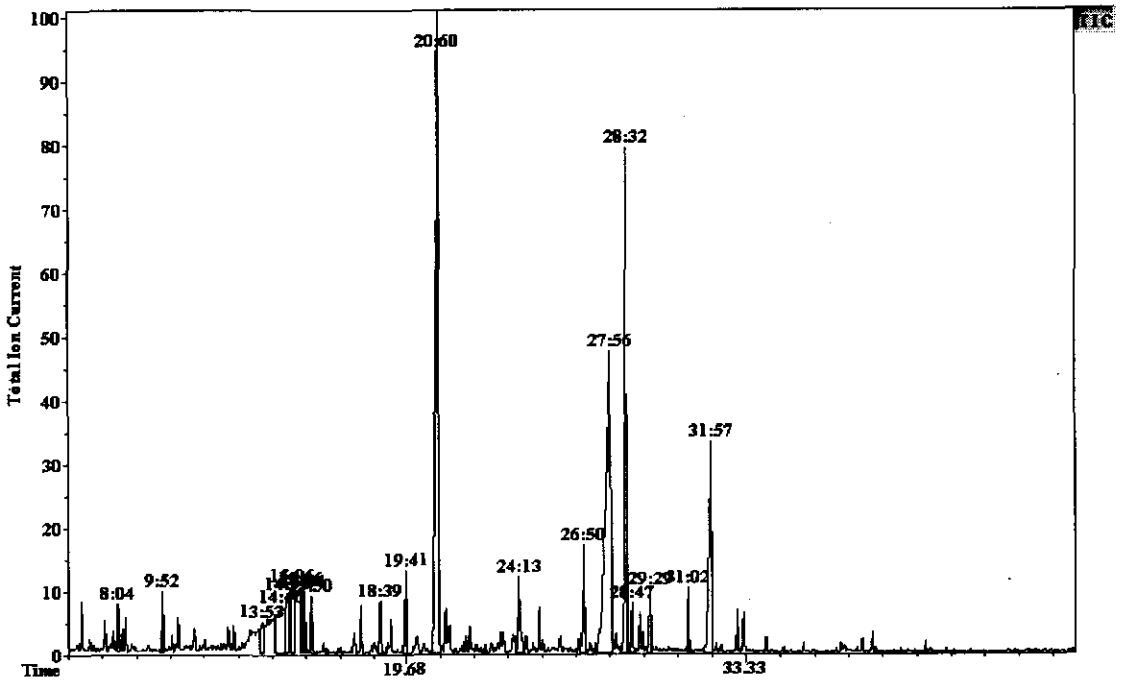

Figure A11: *GC-MS total ion chromatogram of the control sample of subject AM 56*

Table A11: Interpretation of GC-MS spectrum of the control sample of subject AM 56.

Name	R.T.	Scan	Tot.Signal	mg/g creat
2,3-DIHYDROXYBUTANE-DITMS	7.788	63	1889	3.05
LACTIC-DITMS	8.096	75	14512	23.46
2-HYDROXYISOBUTYRIC-DITMS	8.295	82	6041	9.76
GLYCOLIC-DITMS	8.413	86	11303	18.27
1,2-DIHYDROXYBUTANE-DITMS	9.307	119	1711	2.77
OXALIC-DITMS	9.883	140	28334	45.80
p-CRESOL-TMS	10.281	155	3909	6.32
3-HYDROXYISOBUTYRIC-DITMS	11.163	187	5380	8.70
3-HYDROXY-ISO-VALERIC-DITMS	12.751	245	6280	10.15
BENZOIC-TMS	13.084	257	1717	2.78
PHOSPHORIC-TRITMS	15.126	332	7994	12.92
UREA-DITMS	15.453	344	18138	29.32
SUCCINIC-DITMS	15.876	360	17188	27.78
4-HYDROXY-2-METHYLVALERIC-DITMS	16.348	377	2893	4.68
URACIL-DITMS	16.512	383	1256	2.03
4-DEOXYTETRONIC-TRITMS	17.617	423	7742	12.51
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	18.662	462	22752	36.77
SALICYLIC-DITMS	21.36	561	3877	6.27

PYROGLUTAMIC-DITMS	21.445	564	7872	12.72
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.25	593	5593	9.04
MANNONIC-1,4-LACTONE-TMS	22.504	603	3037	4.91
m-HYDROXYBENZOIC-DITMS	24.062	660	1406	2.27
p-HYDROXYPHENYLACETIC-DITMS	24.234	666	38172	61.70
PARACETAMOL-DITMS	24.291	668	2469	3.99
FURAN-2,5-DICARBOXYLIC-DITMS	24.343	670	2840	4.59
TARTARIC-TETRATMS	25.047	696	27942	45.16
m-HYDROXYPHENYLPROPIONIC-DITMS	26.04	732	925	1.50
FUCOSE-PENTATMS	26.802	760	2808	4.54
t-ACONITIC-TRITMS	26.865	762	47491	76.76
O,O,O-EPINEPHRINE-TRITMS	27.317	779	5321	8.60
p-HYDROXYMANDELIC-TRITMS	27.456	784	1871	3.02
HIPPURIC-TMS	27.937	802	88367	142.82
3,4-DIHYDROXYBENZOIC-TRITMS	28.139	809	9454	15.28
CITRIC-TETRATMS	28.543	824	66591	107.63
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.789	833	21125	34.14
VANILGLYCOLIC-TRITMS	29.195	848	9635	15.57
VANILGLYCOL-TRITMS	29.195	848	10052	16.25
o-HYDROXYHIPPURIC-DITMS	31.952	949	52071	84.16
p-HYDROXYHIPPURIC-DITMS	34.191	1031	4815	7.78
DIOCTYLPHTALATE-TMS	38.527	1190	1836	2.97
18.80 min INTERNAL STANDARD	21.006	548	162413	262.5

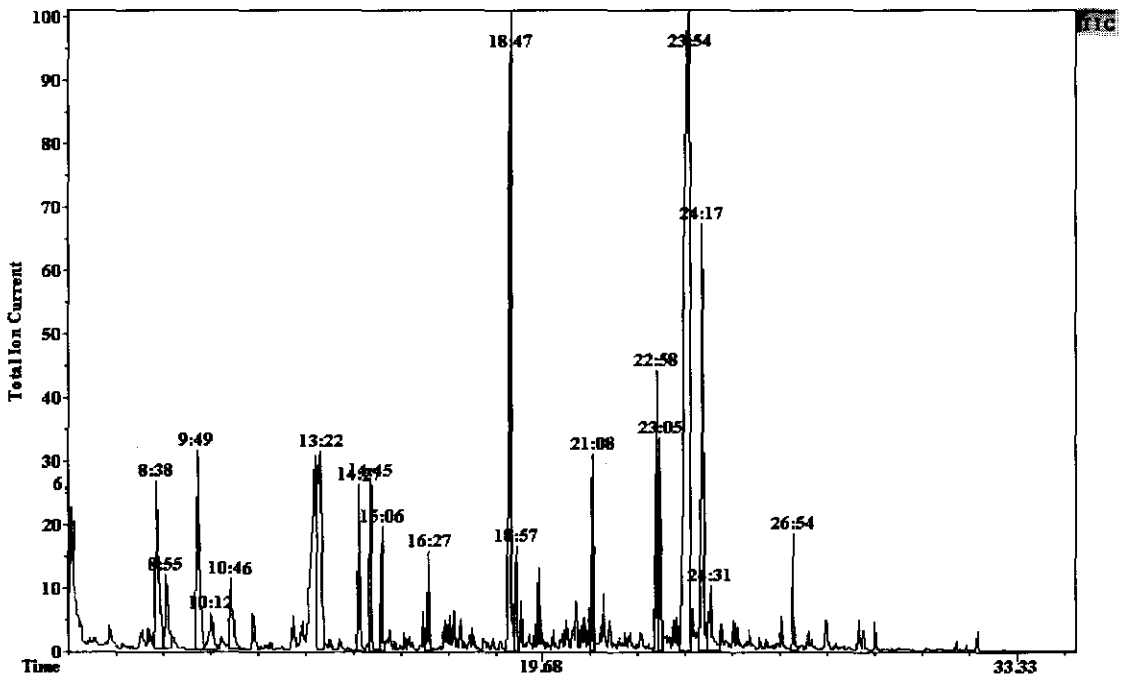

Figure A12: *GC-MS total ion chromatogram of the urine sample on day one after the intake of grapefruit juice of subject AM 56*

Table A12: Interpretation of GC-MS spectrum of urine sample of day one of subject AM 56.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.257	7	134377	53.32
2,3-DIHYDROXYBUTANE-DITMS	8.421	87	16736	6.64
LACTIC-DITMS	8.656	95	194012	76.99
GLYCOLIC-DITMS	8.94	105	74782	29.67
1,2-DIHYDROXYBUTANE-DITMS	9.838	138	255539	101.4
OXALIC-DITMS	10.215	152	28907	11.47
p-CRESOL-TMS	10.785	173	76632	30.41
3-HYDROXYPROPIONIC-DITMS	10.853	176	7051	2.80
2-HYDROXY-ISO-VALERIC-DITMS	10.87	176	5706	2.26
4-HYDROXYBUTANOIC-TRITMS	11.415	196	27738	11.01
3-HYDROXYISOBUTYRIC-DITMS	11.426	197	26738	10.61
3-HYDROXY-ISO-VALERIC-DITMS	12.832	248	19498	7.74
UREA-DITMS	13.211	262	169546	67.28
BENZOIC-TMS	13.225	262	10562	4.19
3-ETHYLHYDRACRYLIC-DITMS	13.331	266	39074	15.51
PHOSPHORIC-TRITMS	14.461	308	160138	63.54
GLYCERIN-TRITMS	14.786	320	182111	72.26
SUCCINIC-DITMS	15.119	332	159348	63.23
1,2-DIHYDROXYBENZENE-DITMS	15.321	339	43267	17.17
ETHYLMALONIC-DITMS	15.462	344	7072	2.81
URACIL-DITMS	15.621	350	2584	1.03
ITACONIC-DITMS	15.754	355	16051	6.37
CITRACONIC-DITMS	15.885	360	13089	5.19
FUMARIC-DITMS	15.967	363	5104	2.03
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.461	381	112447	44.62
GLUTARIC-DITMS	17.035	402	5732	2.28
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	17.065	403	32772	13.00
HYDROCHINON-DITMS	17.116	405	2376	0.94
DECANOIC-TMS	18.126	442	2618	1.04
3-METHYLGLUTACONIC-DITMS	18.505	456	7311	2.90
5-PYROLIDON-2-CARBOXYLIC-DITMS	19.109	478	48830	19.38
3-METHYLADIPIC-DITMS	19.491	492	13369	5.31
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.622	497	59465	23.60
ERYTHRITOL-TMS	19.709	500	13345	5.30
m-HYDROXYBENZOIC-DITMS	20.004	511	3231	1.28
2-HYDROXYGLUTARIC-TRITMS	20.389	525	24096	9.56
ERYTHRONIC-TETRATMS	20.438	527	18429	7.31
TREONIC-TETRATMS	20.447	527	2205	0.88
3-HYDROXYPHENYLACETIC-DITMS	20.665	535	65283	25.91
RIBOSE-PENTATMS(i)	20.826	541	29910	11.87
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.905	544	8578	3.40
p-HYDROXYPHENYLACETIC-DITMS	21.151	553	318048	126.20
TARTARIC-TETRATMS	21.618	570	7043	2.80

ISOCITRICLACTON-DITMS	21.897	580	10445	4.15
m-HYDROXYPHENYLPROPIONIC-DITMS	22.541	604	3756	1.49
t-ACONITIC-TRITMS	22.986	620	278004	110.31
3-HYDROXY-4-METHOXYBENZOIC-DITMS	23.068	623	2963	1.18
VANILLIC-DITMS	23.068	623	3113	1.24
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.093	624	117113	46.47
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.123	625	10850	4.31
p-HYDROXYMANDELIC-TRITMS	23.497	639	11400	4.52
AZELAIC-DITMS	23.565	641	15487	6.15
HIPPURIC-TMS	23.904	654	615537	244.25
a-RESORCYLIC-TRITMS	24.022	658	8328	3.31
3,4-DIHYDROXYPHENYLACETIC-TRITMS	24.183	664	1667	0.66
CITRIC-TETRATMS	24.306	668	202882	80.51
MYRISTIC-TMS	24.349	670	21505	8.53
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.54	677	37791	15.00
VANILLYLMANDELIC-TRITMS	24.841	688	19456	7.72
p-HYDROXYPHENYLLACTIC-TRITMS	25.22	702	19184	7.61
PENTADECANOIC-TMS	25.665	718	8789	3.49
VANILLYLMANDELIC-TRITMS	26.315	742	2610	1.04
ISOVANILHYDRACRYLIC-TRITMS	26.315	742	2077	0.82
PALMITIC-TMS	26.92	764	67390	26.74
GLUCURONIC-PENTATMS	27.16	773	4999	1.98
FERULIC-DITMS	27.36	780	1288	0.51
m-HYDROXYHIPPURIC-DITMS	27.857	798	11118	4.41
HEXACOSANE-TMS	28.776	832	2027	0.80
p-HYDROXYHIPPURIC-DITMS	28.801	833	23021	9.14
OLEIC-TMS	28.938	838	7057	2.80
DIOCTYLPHTALATE-TMS	32.211	958	15687	6.23
18.80 min INTERNAL STANDARD	18.797	467	661531	262.5

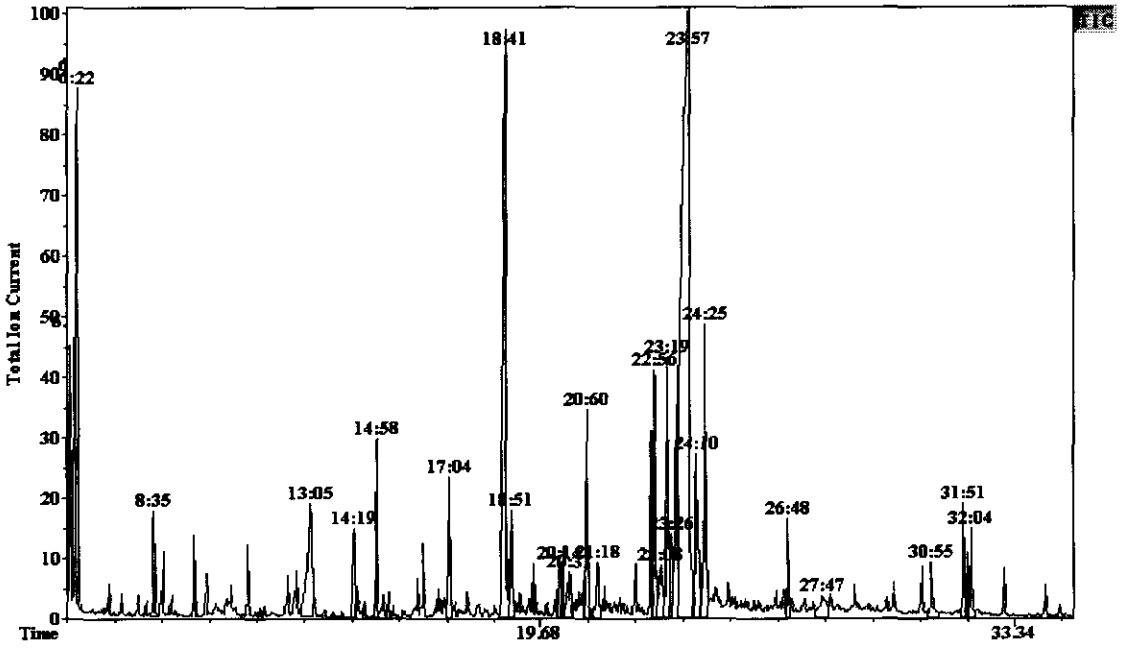

Figure A13: *GC-MS total ion chromatogram of the urine sample on day three after the intake of grapefruit juice of subject AM 56*

Table A13: Interpretation of GC-MS spectrum of urine sample of day three of subject AM 56.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.383	12	1064383	406.18
ETHYLENGLYCOL-DITMS	6.846	29	5621	2.15
LACTIC-DITMS	8.593	93	112583	42.96
2-HYDROXYISOBUTYRIC-DITMS	8.798	100	27284	10.41
GLYCOLIC-DITMS	8.871	103	73716	28.13
PYRUVIC-TMS	9.253	117	4627	1.77
1,2-DIHYDROXYBUTANE-DITMS	9.75	135	100342	38.29
OXALIC-DITMS	10.107	148	59577	22.74
p-CRESOL-TMS	10.68	169	16401	6.26
DIPROPYLACETIC-TMS	10.945	179	2655	1.01
3-HYDROXYISOBUTYRIC-DITMS	11.303	192	71475	27.28
3-HYDROXY-ISO-VALERIC-DITMS	12.694	243	31371	11.97
BENZOIC-TMS	13.093	258	25531	9.74
UREA-DITMS	13.104	258	100398	38.31
3-ETHYLHYDRACRYLIC-DITMS	13.186	261	20039	7.65
ETHYLMALONIC-DITMS	14.309	302	28134	10.74
PHOSPHORIC-TRITMS	14.323	303	92158	35.17
GLYCERIN-TRITMS	14.634	314	14016	5.35
SUCCINIC-DITMS	14.983	327	267302	102.01
1,2-DIHYDROXYBENZENE-DITMS	15.177	334	51727	19.74
2-METHYLSUCCINIC-DITMS	15.319	339	22637	8.64

URACIL-DITMS	15.483	345	5495	2.10
FUMARIC-DITMS	15.832	358	5177	1.98
m-HYDROXYBENZALDEHYDE-TMS	15.906	361	1495	0.57
NONANOIC-TMS	16.053	366	6445	2.46
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.16	370	36343	13.87
GLUTARIC-DITMS	16.915	398	9117	3.48
HYDROCHINON-DITMS	16.984	400	2372	0.91
3-METHYLGLUTACONIC-DITMS	17.595	423	3794	1.45
ISOVALERYLGLYCINE-TMS	18.177	444	3315	1.27
3-METHYLGLUTACONIC-DITMS	18.393	452	7496	2.86
ADIPIC-DITMS	18.81	467	9671	3.69
PIPECOLIC-DITMS	18.98	473	8307	3.17
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	19.092	477	7540	2.88
3-METHYLADIPIC-DITMS	19.371	488	6162	2.35
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.491	492	31420	11.99
ERYTHRITOL	19.598	496	3846	1.47
o-HYDROXYPHENYLACETIC-DITMS	19.897	507	13341	5.09
2-HYDROXYGLUTARIC-TRITMS	20.258	520	27634	10.55
PHENYLLACTIC-DITMS	20.282	521	19046	7.27
PIMELIC-DITMS	20.476	528	11379	4.34
3-HYDROXYPHENYLACETIC-DITMS	20.544	531	61716	23.55
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.774	539	12333	4.71
m-HYDROXYBENZOIC-DITMS	20.913	544	18635	7.11
2-FUROYLGLYCINE-TMS	20.965	546	17238	6.58
p-HYDROXYPHENYLACETIC-DITMS	21.025	548	291828	111.37
FURAN-2,5-DICARBOXYLIC-DITMS	21.064	550	10330	3.94
SUBERIC-DITMS	21.973	583	9204	3.51
PROPANETRICARBOXYLIC-TRITMS	22.628	607	4978	1.90
c-ACONITIC-TRITMS	22.877	616	161165	61.50
VANILLIC-DITMS	22.953	619	5497	2.10
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.009	621	9804	3.74
p-HYDROXYMANDELIC-TRITMS	23.375	634	25982	9.92
4-METHYLSUBERIC-DITMS	23.449	637	18158	6.93
AZELAIC-DITMS	23.449	637	18828	7.19
TECEPHALIC-DITMS	23.46	637	13401	5.11
HIPPURIC-TMS	23.96	656	610939	233.14
CITRIC-TETRATMS	24.184	664	83922	32.03
MYRISTIC-TMS	24.244	666	18654	7.12
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.433	673	228150	87.07
VANILGLYCOLIC-TRITMS	24.734	684	10105	3.86
ISOVANILGLYCOLIC-TRITMS	24.734	684	10244	3.91
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	24.764	685	3315	1.27
p-HYDROXYPHENYLLACTIC-TRITMS	25.095	697	22707	8.67
LYXOSE-TETRATMS(i)	25.447	710	7297	2.79
PENTADECANOIC-TMS	25.554	714	4381	1.67
ISOVANILHYDRACRYLIC-TRITMS	26.206	738	4669	1.78
PALMITOLEIC-TMS	26.46	747	7872	3.00
FUCOSE-PENTATMS(iii)	26.594	752	8279	3.16
1,9-DIMETHYLURIC-DITMS	26.676	755	21242	8.11
PALMITIC-TMS	26.81	760	45933	17.53
p-HYDROXYHIPPURIC-DITMS	27.794	796	9723	3.71
11-OCTADECENOIC-TMS	28.818	833	2240	0.86
HEXACOSANE-TMS	29.85	871	26963	10.29
DEHYDROABIETIC ACID	30.663	901	48755	18.61
DIOCTYLPHTALATE-TMS	32.089	953	83105	31.71
18.80 min INTERNAL STANDARD	18.687	463	687868	262.5

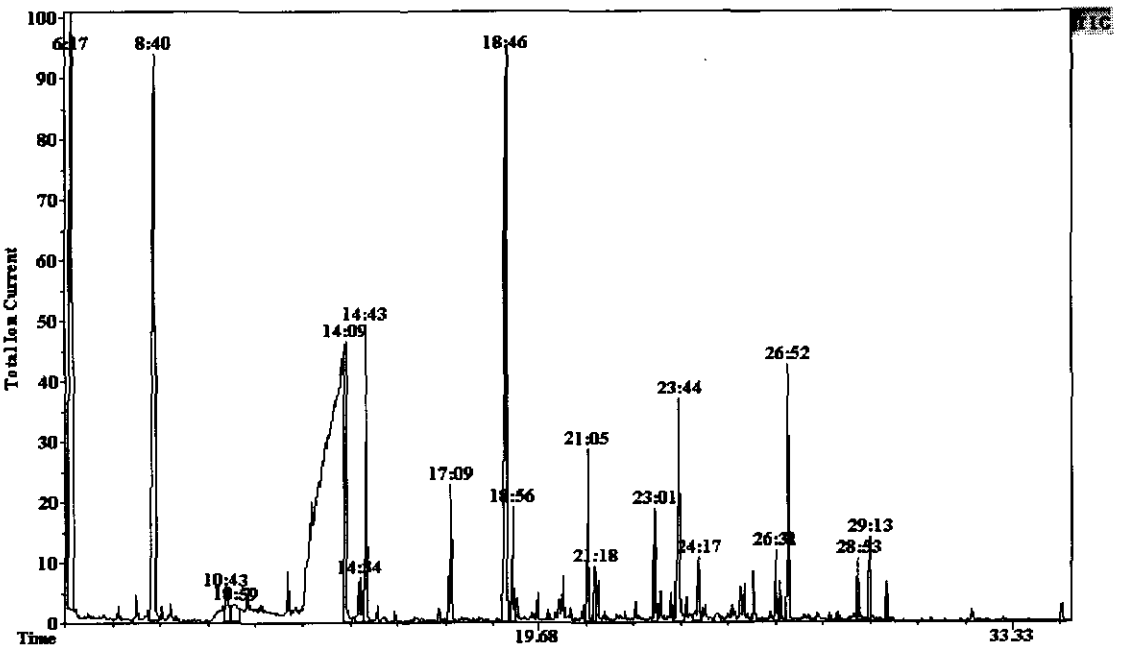

Figure A14: *GC-MS total ion chromatogram of the urine sample on day five after the intake of grapefruit juice of subject AM 56*

Table A14: Interpretation of GC-MS spectrum of urine sample of day five of subject AM 56.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.297	9	1266050	485.68
ETHYLENGLYCOL-DITMS	6.776	26	6680	2.56
1,2-DIHYDROXYPROPANE-DITMS	7.247	43	2780	1.07
3-MERCAPTOPROPIONIC-DITMS	7.422	50	4542	1.74
LACTIC-DITMS	8.675	96	652428	250.28
GLYCOLIC-DITMS	8.904	104	17379	6.67
PYRUVIC-TMS	9.286	118	3812	1.46
1,3-DIHYDROXYBUTANE-DITMS	9.343	120	2522	0.97
p-CRESOL-TMS	10.749	172	26233	10.06
3-HYDROXYPROPIONIC-DITMS	10.809	174	2531	0.97
3-HYDROXYBUTYRIC-DITMS	11.363	194	21244	8.15
3-HYDROXY-ISO-VALERIC-DITMS	12.771	246	7044	2.70
BENZOIC-TMS	13.18	261	23985	9.20
UREA-DITMS	14.158	297	388748	149.13
PHOSPHORIC-TRITMS	14.567	312	57877	22.20
GLYCERIN-TRITMS	14.748	318	260392	99.89
SUCCINIC-DITMS	15.07	330	18592	7.13
1,2-DIHYDROXYBENZENE-DITMS	15.285	338	6881	2.64

URACIL-DITMS	15.587	349	7568	2.90
NONANOIC-TMS	16.133	369	2354	0.90
HYDROCHINON-DITMS	16.63	387	1022	0.39
5-PYROLIDON-2-CARBOXYLIC-DITMS	19.078	477	26374	10.12
ERYTHRITOL-TMS	19.678	499	21504	8.25
o-HYDROXYPHENYLACETIC-DITMS	19.982	510	12437	4.77
ERYTHRONIC-TETRATMS	20.415	526	8726	3.35
3-HYDROXYPHENYLACETIC-DITMS	20.629	534	19118	7.33
m-HYDROXYBENZOIC-DITMS	20.997	547	7255	2.78
p-HYDROXYPHENYLACETIC-DITMS	21.12	552	44393	17.03
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	22.493	602	20790	7.98
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.086	624	3575	1.37
HIPPURIC-TMS	23.744	648	230440	88.40
a-RESORCYLIC-TRITMS	23.965	656	12408	4.76
CITRIC-TETRATMS	24.238	666	7868	3.02
MYRISTIC-TMS	24.303	668	29254	11.22
FRUCTOFURANOSE-TMS	24.402	672	11058	4.24
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.486	675	8510	3.27
PENTADECANOIC-TMS	25.63	717	19483	7.47
FUCOSE-PENTATMS(iii)	25.734	721	2828	1.09
MANNITOL-6TMS	26.383	745	7262	2.79
GALACTOSE-6TMS	26.67	755	35770	13.72
PALMITIC-TMS	26.894	763	133499	51.21
HEPTADECANOIC-TMS	27.383	781	2073	0.80
p-HYDROXYHIPPURIC-DITMS	27.814	797	1788	0.69
MARGARIC-TMS	28.087	807	3300	1.27
OLEIC-TMS	28.908	837	20810	7.98
STEARIC-TMS	29.236	849	43193	16.57
DIOCTYLPHTALATE-TMS	32.173	957	14158	5.43
18.80 min INTERNAL STANDARD	18.77	466	684279	262.5

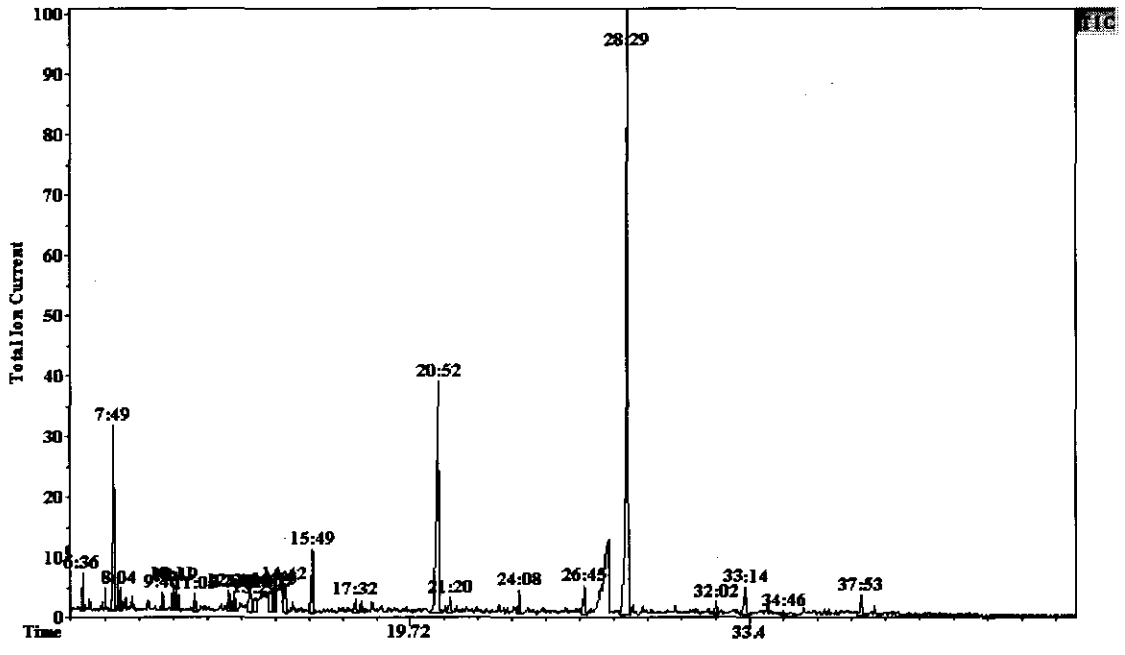

Figure A15: *GC-MS total ion chromatogram of the control sample of subject AF 32*

Table A15: Interpretation of GC-MS spectrum of the control sample of subject AF 32.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.007	71	36501	38.09
GLYCOLIC-DITMS	8.33	83	23092	24.09
OXALIC-DITMS	9.8	137	36460	38.04
p-CRESOL-TMS	10.202	151	41291	43.08
3-HYDROXYISOBUTYRIC-DITMS	11.089	184	27820	29.03
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.322	229	3808	3.97
3-HYDROXY-ISO-VALERIC-DITMS	12.684	242	23690	24.72
UREA-DITMS	14.724	317	45070	47.03
PHOSPHORIC-TRITMS	15.017	327	9104	9.50
SUCCINIC-DITMS	15.846	358	122826	128.16
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.556	420	9710	10.13
4-DEOXYTETRONIC-TRITMS	17.816	430	11305	11.80
PYROGLUTAMIC-DITMS	21.358	559	24934	26.02
2-HYDROXYGLUTARIC-TRITMS	23.322	631	6755	7.05
m-HYDROXYBENZOIC-DITMS	23.985	655	3708	3.87
p-HYDROXYPHENYLACETIC-DITMS	24.148	661	25210	26.30
t-ACONITIC-TRITMS	26.792	758	22355	23.33
p-HYDROXYMANDELIC-TRITMS	27.377	779	3893	4.06
HIPPURIC-TMS	27.746	793	100607	104.97
CITRIC-TETRATMS	28.497	820	502624	524.44
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.716	828	7359	7.68
ISOVANILGLYCOLIC-TRITMS	29.121	843	7131	7.44
p-HYDROXYHIPPURIC-DITMS	34.111	1025	13257	13.83
DIOCTYLPHTALATE-TMS	38.457	1184	14268	14.89
18.80 min INTERNAL STANDARD	20.888	542	251580	262.5

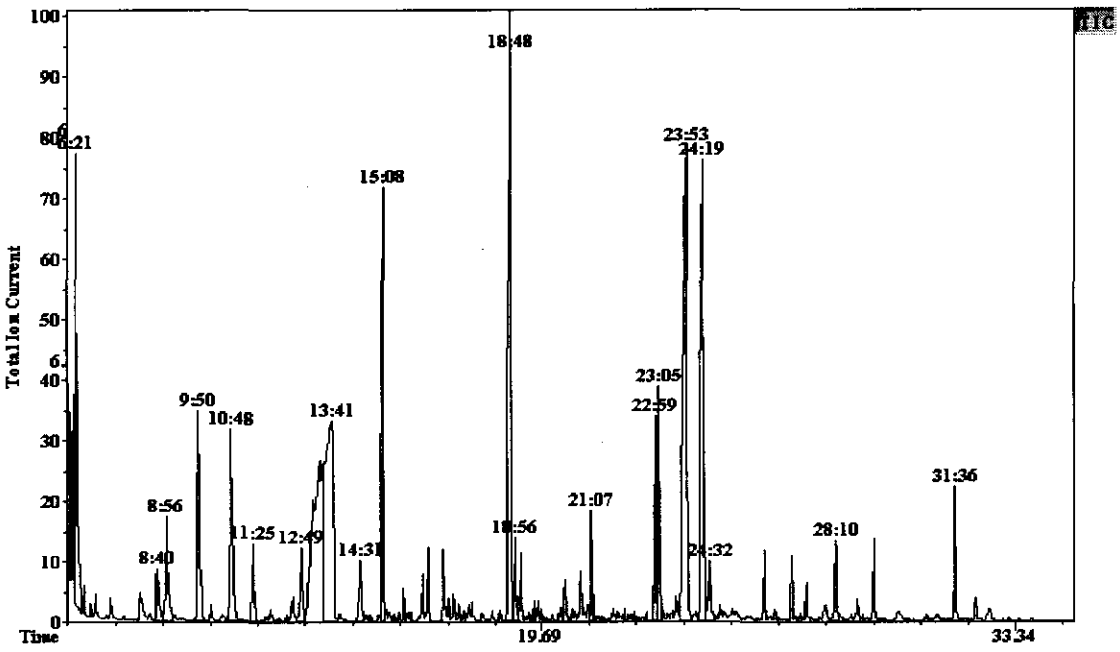

Figure A16: *GC-MS total ion chromatogram of the urine sample on day one after the intake of grapefruit juice of subject AF 32*

Table A16: Interpretation of GC-MS spectrum of urine sample of day one of subject AF 32.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.358	11	552717	323.01
LACTIC-DITMS	8.681	96	47741	27.90
GLYCOLIC-DITMS	8.965	106	88316	51.61
1,2-DIHYDROXYBUTANE-DITMS	9.864	139	190314	111.22
OXALIC-DITMS	10.237	153	19440	11.36
p-CRESOL-TMS	10.814	174	163274	95.42
3-HYDROXYPROPIONIC-DITMS	10.871	176	5133	3.00
3-HYDROXYISOBUTYRIC-DITMS	11.444	197	42630	24.91
3-HYDROXY-ISO-VALERIC-DITMS	12.839	248	47913	28.00
BENZOIC-TMS	13.244	263	6273	3.67
3-ETHYLHYDRACRYLIC-DITMS	13.35	267	45119	26.37
UREA-DITMS	13.7	280	154647	90.38
ETHYLMALONIC-DITMS	14.47	308	17013	9.94
PHOSPHORIC-TRITMS	14.522	310	41694	24.37
GLYCERIN-TRITMS	14.79	320	5884	3.44
SUCCINIC-DITMS	15.15	333	412385	241.00
1,2-DIHYDROXYBENZENE-DITMS	15.336	340	16219	9.48
4-HYDROXY-2-METHYLVALERIC-DITMS	15.478	345	4934	2.88
URACIL-DITMS	15.631	350	3149	1.84

ITACONIC-DITMS	15.762	355	25863	15.11
CITRACONIC-DITMS	15.888	360	6924	4.05
FUMARIC-DITMS	15.973	363	4736	2.77
4-DEOXYTETRONIC-TRITMS	16.303	375	41458	24.23
BUTYRYLGLYCINE-TMS	16.388	378	3151	1.84
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.467	381	63974	37.39
GLUTARIC-DITMS	17.04	402	10190	5.96
HYDROCHINON-DITMS	17.122	405	2029	1.19
3-METHYLGLUTACONIC-DITMS	17.723	427	9365	5.48
CITRAMALIC-TRITMS	18.758	465	6187	3.62
PIPECOLIC ACID-DITMS	19.119	478	50685	29.62
PYROGLUTAMIC-DITMS	19.119	478	52993	30.97
PIMELIC-DITMS	19.498	492	10128	5.92
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.613	496	12504	7.31
2-HYDROXYGLUTARIC-TRITMS	20.394	525	25146	14.70
ERYTHRONIC-TETRATMS	20.452	527	2235	1.31
3-HYDROXYPHENYLACETIC-DITMS	20.67	535	8577	5.01
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.894	543	2802	1.64
m-HYDROXYBENZOIC-DITMS	21.05	549	5566	3.25
ACETYLASPARTIC-DITMS	21.323	559	1063	0.62
RIBONIC-g-LACTONE-TRITMS	21.38	561	4682	2.74
ISOCITRICLACTON-DITMS	21.899	580	2284	1.34
FUCONO-g-LACTONE-PENTATMS	22.203	591	4408	2.58
PROPANETRICARBOXYLIC-TRITMS	22.743	611	5921	3.46
t-ACONITIC-TRITMS	22.992	620	159110	92.98
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.101	624	200912	117.41
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.131	625	4564	2.67
HIPPURIC-TMS	23.888	653	353988	206.87
CITRIC-TETRATMS	24.347	670	265649	155.24
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.549	677	42022	24.56
VANILGLYCOL-TRITMS	24.844	688	16949	9.91
VANILGLYCOLIC-TRITMS	24.846	688	8139	4.76
ISOVANILHYDRACRYLIC-TRITMS	26.323	742	1678	0.98
PALMITIC-TMS	26.929	764	27680	16.18
o-HYDROXYHIPPURIC-DITMS	27.027	768	1455	0.85
ARABINOSE-TETRATMS	27.169	773	5829	3.41
p-HYDROXYHIPPURIC-DITMS	28.808	833	11631	6.80
STEARIC-TMS	29.275	850	31490	18.40
HEXACOSANE-TMS	30.011	877	5337	3.12
DIOCTYLPHTALATE-TMS	32.22	958	11658	6.81
18.80 min INTERNAL STANDARD	18.807	467	449182	262.5

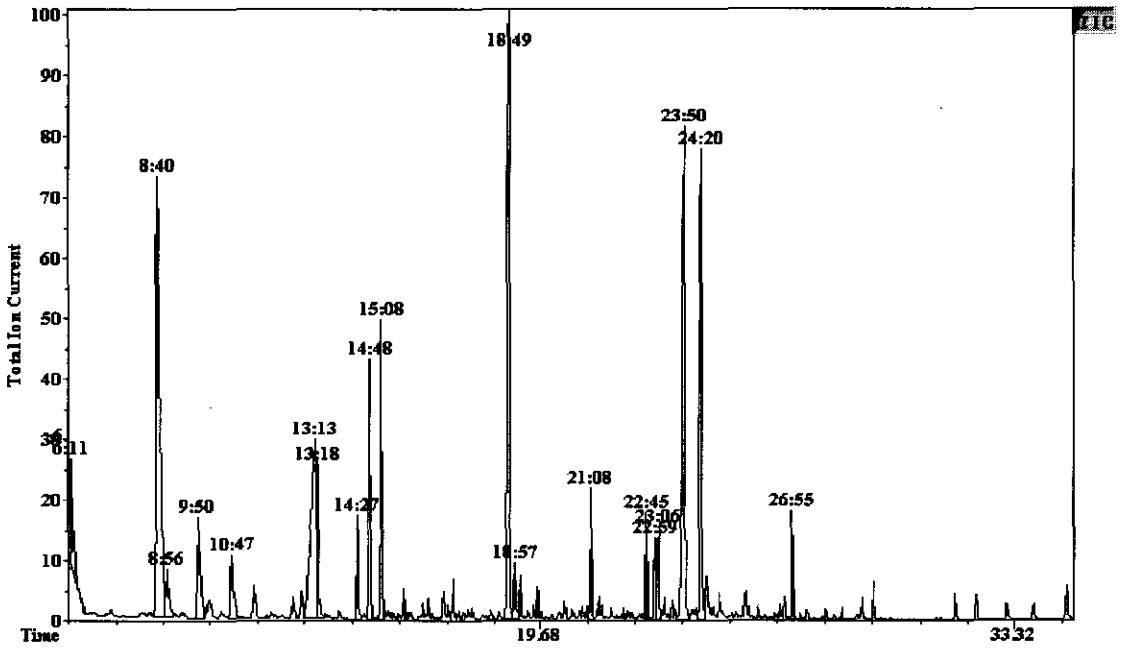

Figure A17: *GC-MS total ion chromatogram of the urine sample on day three after the intake of grapefruit juice of subject AF 32*

Table A17: Interpretation of GC-MS spectrum of urine sample of day three of subject AF 32.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.276	8	63505	31.80
ETHYLENGLYCOL-DITMS	6.824	28	1039	0.52
LACTIC-DITMS	8.69	96	402293	201.47
GLYCOLIC-DITMS	8.963	106	49708	24.90
1,2-DIHYDROXYBUTANE-DITMS	9.863	139	100863	50.51
OXALIC-DITMS	10.24	153	12081	6.05
p-CRESOL-TMS	10.813	174	54777	27.43
3-HYDROXYPROPIONIC-DITMS	10.889	177	3301	1.65
3-HYDROXYISOBUTYRIC-DITMS	11.46	198	22476	11.26
3-HYDROXY-ISO-VALERIC-DITMS	12.859	249	19272	9.65
BENZOIC-TMS	13.249	263	6667	3.34
UREA-DITMS	13.309	266	121309	60.76
PHOSPHORIC-TRITMS	14.458	308	78549	39.34
GLYCERIN-TRITMS	14.815	321	245281	122.84
SUCCINIC-DITMS	15.146	333	302057	151.27
1,2-DIHYDROXYBENZENE-DITMS	15.348	340	13558	6.79
4-HYDROXY-2-METHYLVALERIC-DITMS	15.484	345	3612	1.81

URACIL-DITMS	15.645	351	2507	1.26
ITACONIC-DITMS	15.779	356	27852	13.95
FUMARIC-DITMS	15.992	364	3401	1.70
4-DEOXYTETRONIC-TRITMS	16.32	376	14307	7.17
ERYTHRO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.486	382	20528	10.28
SERINE-TRITMS	16.585	386	5439	2.72
GLUTARIC-DITMS	17.056	403	7241	3.63
3-METHYLGLUTACONIC-DITMS	17.741	428	6698	3.35
MALIC-TRITMS	18.969	473	46393	23.23
PYROGLUTAMIC-DITMS	19.133	479	40463	20.26
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.638	498	21743	10.89
2-HYDROXYGLUTARIC-TRITMS	20.413	526	13318	6.67
ERYTHRONIC-TETRATMS	20.467	528	10634	5.33
PIMELIC-DITMS	20.628	534	1731	0.87
3-HYDROXYPHENYLACETIC-DITMS	20.685	536	7952	3.98
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.925	545	1612	0.81
m-HYDROXYBENZOIC-DITMS	21.068	550	4596	2.30
p-HYDROXYPHENYLACETIC-DITMS	21.174	554	23630	11.83
1,6-ANHYDRO-B-d-GLUCOPYRANOSE-TRITMS	22.549	604	6789	3.40
PROPANETRICARBOXYLIC-TRITMS	22.762	612	76574	38.35
t-ACONITIC-TRITMS	23.005	621	71930	36.02
3-HYDROXY-4-METHOXYBENZOIC-DITMS	23.087	624	807	0.40
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.111	625	100435	50.30
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.142	626	2781	1.39
LYXOSE-TETRATMS(iv)	23.502	639	22889	11.46
HIPPURIC-TMS	23.848	652	440355	220.53
ARABINOSE-TETRATMS	24.088	661	1573	0.79
CITRIC-TETRATMS	24.334	670	346122	173.34
MYRISTIC-TMS	24.367	671	18033	9.03
SORBOFURANOSE-TMS	24.484	675	46319	23.20
PYRUVIC ACID (Q)-TMS	24.492	675	15983	8.00
FUCOSE-PENTATMS(iii)	25.597	716	26349	13.20
PENTADECANOIC-TMS	25.682	719	4952	2.48
LYXOSE-TETRATMS(i)	26.723	757	23428	11.73
GLUCOPYRANOSE-HEKSATMS	26.732	757	9083	4.55
PALMITIC-TMS	26.944	765	50850	25.47
HEXACOSANE-TMS	28.793	833	1892	0.95
p-HYDROXYHIPPURIC-DITMS	28.815	834	3124	1.57
DIOCTYLPHTALATE-TMS	32.233	959	14016	7.02
18.80 min INTERNAL STANDARD	18.822	468	524160	262.5

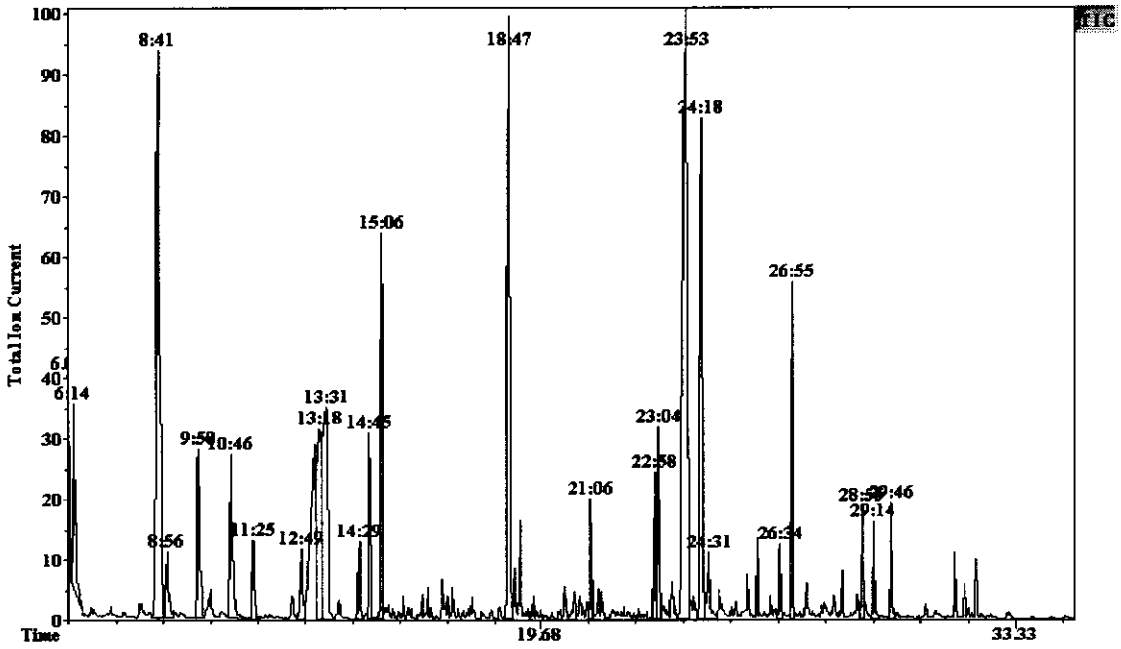

Figure A18: *GC-MS total ion chromatogram of the urine sample on day five after the intake of grapefruit juice of subject AF 32*

Table A18: Interpretation of GC-MS spectrum of urine sample of day five of subject AF 32.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.311	9	149288	77.15
ETHYLENGLYCOL-DITMS	6.83	28	5107	2.64
LACTIC-DITMS	8.701	97	513350	265.30
GLYCOLIC-DITMS	8.956	106	65526	33.86
1,2-DIHYDROXYBUTANE-DITMS	9.853	139	183979	95.08
OXALIC-DITMS	10.216	152	25521	13.19
p-CRESOL-TMS	10.792	173	132329	68.39
3-HYDROXYPROPIONIC-DITMS	10.863	176	5998	3.10
3-HYDROXYISOBUTYRIC-DITMS	11.43	197	61479	31.77
3-HYDROXY-ISO-VALERIC-DITMS	12.831	248	50126	25.91
UREA-DITMS	13.21	262	121226	62.65
BENZOIC-TMS	13.221	262	6519	3.37
3-ETHYLHYDRACRYLIC-DITMS	13.333	266	22542	11.65
PHOSPHORIC-TRITMS	14.485	309	68034	35.16
GLYCERIN-TRITMS	14.78	319	150492	77.77
SUCCINIC-DITMS	15.137	333	363440	187.82
1,2-DIHYDROXYBENZENE-DITMS	15.32	339	24396	12.61
4-HYDROXY-2-METHYLVALERIC-DITMS	15.456	344	6727	3.48
URACIL-DITMS	15.617	350	3276	1.69

ITACONIC-DITMS	15.751	355	19600	10.13
FUMARIC-DITMS	15.964	363	6494	3.36
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.294	375	21178	10.95
SERINE-TRITMS	16.555	384	8151	4.21
PYROLCARBOXYLIC-DITMS	16.698	390	2063	1.07
GLUTARIC-DITMS	17.031	402	12304	6.36
HYDROCHINON-DITMS	17.112	405	1557	0.81
3-METHYLGLUTARIC-DITMS	17.473	418	6134	3.17
3-METHYLGLUTACONIC-DITMS	17.715	427	12639	6.53
3-HYDROXYADIPYLLACTONE-TMS	17.759	429	8376	4.33
P-AMINOBEMZOIC-TMS	18.258	447	1191	0.62
PIPECOLIC ACID-DITMS	19.108	478	90390	46.71
3-METHYLADIPIC-DITMS	19.489	492	11595	5.99
ERYTHRONIC-TETRATMS	20.21	518	7246	3.75
2-HYDROXYGLUTARIC-TRITMS	20.387	525	21880	11.31
3-HYDROXYPHENYLACETIC-DITMS	20.662	535	35297	18.24
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.9	544	1211	0.63
m-HYDROXYBENZOIC-DITMS	21.039	549	6930	3.58
p-HYDROXYPHENYLACETIC-DITMS	21.148	553	12542	6.48
t-ACONITIC-TRITMS	22.979	620	127694	65.99
VANILLIC-DITMS	23.063	623	1306	0.68
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.088	624	185910	96.08
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.118	625	7206	3.72
a-RESORCYLIC-TRITMS	23.443	637	13072	6.76
PYRUVIC ACID (Q)-TMS	23.476	638	5865	3.03
HIPPURIC-TMS	23.899	654	504618	260.78
CITRIC-TETRATMS	24.322	669	327113	169.05
MYRISTIC-TMS	24.347	670	36990	19.12
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.535	677	47320	24.46
VANILLYLMANDELIC-TRITMS	24.836	688	11281	5.83
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	24.874	689	1337	0.69
LYXOSE-TETRATMS(i)	25.576	715	2115	1.09
PENTADECANOIC-TMS	25.66	718	19871	10.27
FUCOSE-PENTATMS(iii)	26.695	756	5455	2.82
PALMITIC-TMS	26.938	765	152126	78.62
GLUCURONIC-PENTATMS	27.159	773	6524	3.37
HEPTADECANOIC-TMS	27.407	782	2042	1.06
p-HYDROXYHIPPURIC-DITMS	28.796	833	13299	6.87
DEHYDROABIETIC ACID	30.784	906	8711	4.50
DIOCTYLPHTALATE-TMS	32.208	958	40080	20.71
HEXACOSANE-TMS	33.166	993	3481	1.80
18.80 min INTERNAL STANDARD	18.796	467	507940	262.5

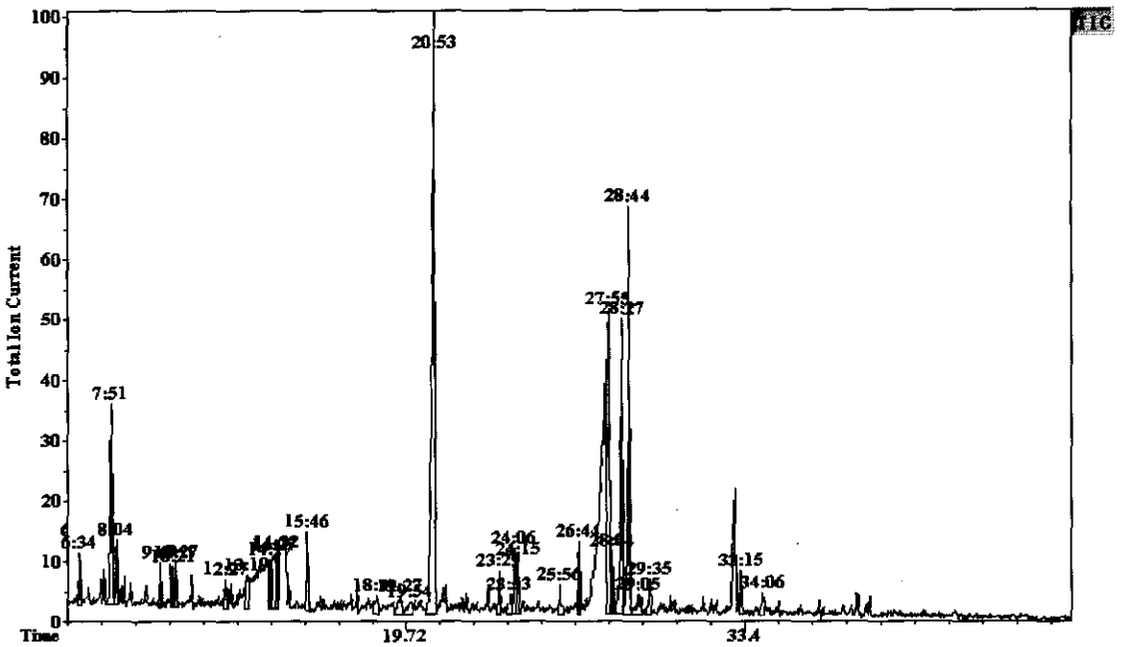

Figure A19: *GC-MS total ion chromatogram of the control sample of subject AF 26*

Table A19: Interpretation of GC-MS spectrum of the control sample of subject AF 26.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.085	74	27749	28.50
GLYCOLIC-DITMS	8.394	85	20906	21.48
OXALIC-DITMS	9.836	138	38032	39.07
p-CRESOL-TMS	10.243	153	35086	36.04
3-HYDROXYISOBUTYRIC-DITMS	11.1	184	19456	19.99
3-HYDROXY-ISO-VALERIC-DITMS	12.674	242	11337	11.65
BENZOIC-TMS	13.016	254	8625	8.86
UREA-DITMS	14.89	323	46024	47.28
PHOSPHORIC-TRITMS	15.035	328	11396	11.71
SUCCINIC-DITMS	15.777	355	66000	67.80
1,2-DIHYDROXYBENZENE-DITMS	15.831	357	15725	16.15
4-HYDROXY-2-METHYLVALERIC-DITMS	16.25	372	8760	8.90
URACIL-DITMS	16.425	379	3780	3.88
ERYTHRO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.777	428	7311	7.51
4-DEOXYTETRONIC-TRITMS	17.78	428	6983	7.17
PYROGLUTAMIC-DITMS	21.337	558	20619	21.18
3-METHYLADIPIC-DITMS	22.005	583	3089	3.17
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.155	588	6775	6.96
3-HYDROXYPHENYLACETIC-DITMS	23.496	637	17337	17.81
m-HYDROXYBENZOIC-DITMS	23.961	654	3625	3.72
p-HYDROXYPHENYLACETIC-DITMS	24.136	660	30100	30.92

FURAN-2,5-DICARBOXYLIC-DITMS	24.259	665	31208	32.06
m-HYDROXYPHENYLPROPIONIC-DITMS	25.952	727	9880	10.15
t-ACONITIC-TRITMS	26.77	757	33160	34.06
p-HYDROXYMANDELIC-TRITMS	27.373	779	6457	6.63
HIPPURIC-TMS	27.923	799	187644	192.75
a-RESORCYLIC-TRITMS	28.092	805	23296	23.93
CITRIC-TETRATMS	28.465	819	108255	111.20
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.769	830	217558	223.47
VANILLYLMANDELIC-TRITMS	29.125	843	11551	11.87
p-HYDROXYHIPPURIC-DITMS	33.012	985	51100	52.49
DEHYDROABIETIC ACID	36.424	1110	9999	10.27
18.80 min INTERNAL STANDARD	20.886	542	255551	262.5

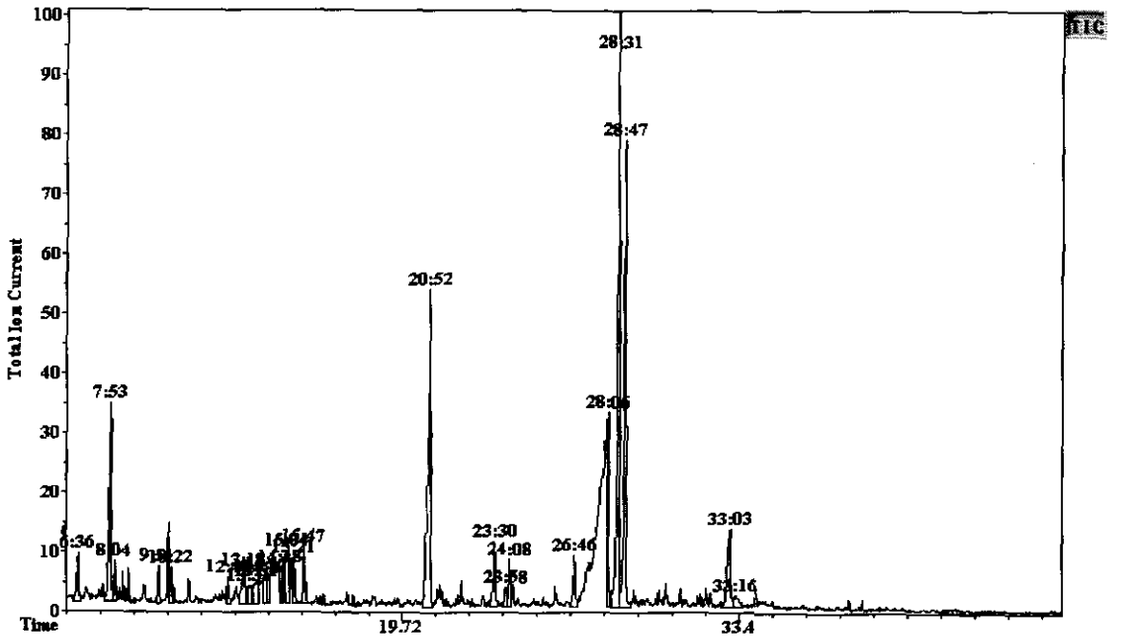

Figure A20: *GC-MS total ion chromatogram of the urine sample on day one after the intake of grapefruit juice of subject AF 26*

Table A20: Interpretation of GC-MS spectrum of urine sample of day one of subject AF 26.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.095	74	29173	30.49
GLYCOLIC-DITMS	8.416	86	39511	41.29
OXALIC-DITMS	9.865	139	53804	56.23
p-CRESOL-TMS	10.273	154	111176	116.18

3-HYDROXYPROPIONIC-DITMS	10.481	161	25183	26.32
3-HYDROXYISOBUTYRIC-DITMS	11.107	184	25850	27.01
1,2-DIHYDROXYBUTANE-DITMS	12.678	242	22884	23.91
3-HYDROXY-ISO-VALERIC-DITMS	12.683	242	24541	25.65
BENZOIC-TMS	13.02	254	9260	9.68
PHOSPHORIC-TRITMS	15.14	331	13451	14.06
UREA-DITMS	15.356	340	56844	59.40
SUCCINIC-DITMS	15.802	356	88708	92.70
ETHYLMALONIC-DITMS	16.267	373	7994	8.35
URACIL-DITMS	16.437	379	5677	5.93
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.788	429	7527	7.87
ERYTHRO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.791	429	8068	8.43
6-METHYLURACIL-DITMS	17.98	435	2167	2.27
1,2-DIHYDROXYBENZENE-DITMS	18.573	457	3486	3.64
CITRAMALIC-TRITMS	20.913	543	25151	26.28
ADIPIC-DITMS	21.283	556	6927	7.24
PYROGLUTAMIC-DITMS	21.37	559	19096	19.96
PIPECOLIC-DITMS	21.37	559	19218	20.08
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.18	589	18269	19.09
m-HYDROXYBENZOIC-DITMS	23.984	655	11328	11.84
p-HYDROXYPHENYLACETIC-DITMS	24.151	661	44291	46.28
FURAN-2,5-DICARBOXYLIC-DITMS	24.268	665	23431	24.49
m-HYDROXYPHENYLPROPIONIC-DITMS	25.959	727	14627	15.29
p-HYDROXYMANDELIC-TRITMS	27.38	779	8303	8.68
HIPPURIC-TMS	28.004	802	83988	87.77
CITRIC-TETRATMS	28.524	821	418881	437.74
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.82	832	398304	416.24
VANILGLYCOLIC-TRITMS	29.151	844	13314	13.91
ISOVANILHYDRACRYLIC-TRITMS	31.024	912	16009	16.73
FERULIC-DITMS	32.252	957	3394	3.55
p-HYDROXYHIPPURIC-DITMS	33.06	987	55533	58.03
CAFFEIC-TRITMS	33.287	995	4482	4.68
18.80 min INTERNAL STANDARD	20.883	542	251192	262.5

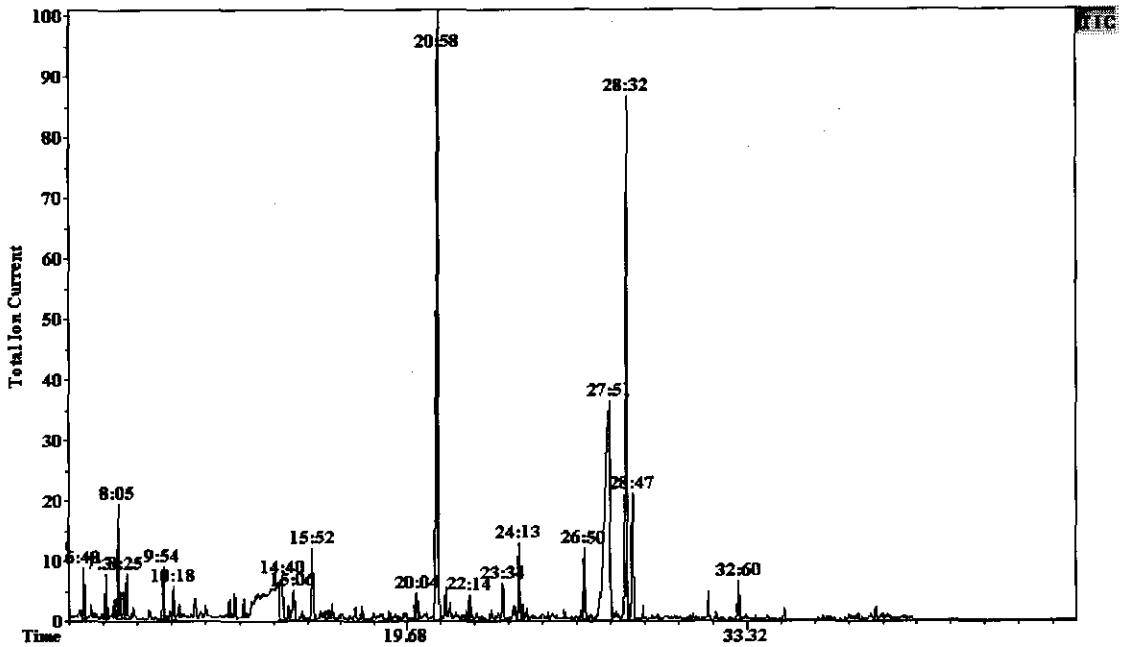

Figure A21: *GC-MS total ion chromatogram of the urine sample on day three after the intake of grapefruit juice of subject AF 26*

Table A21: Interpretation of GC-MS spectrum of urine sample of day three of subject AF 26.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.112	75	27589	53.09
1,2-DIHYDROXYBUTANE-DITMS	8.312	82	5931	11.42
GLYCOLIC-DITMS	8.435	87	14858	28.59
OXALIC-DITMS	9.905	141	20415	39.28
p-CRESOL-TMS	10.315	156	7588	14.60
3-HYDROXYPROPIONIC-DITMS	10.533	164	3293	6.34
3-HYDROXYISOBUTYRIC-DITMS	11.188	188	4662	8.97
1,2-DIHYDROXYPROPANE-DITMS	12.421	233	1468	2.83
3-HYDROXY-ISO-VALERIC-DITMS	12.757	245	5787	11.14
BENZOIC-TMS	13.101	258	3699	7.12
UREA-DITMS	14.674	316	9699	18.66
PHOSPHORIC-TRITMS	15.137	333	12577	24.20
SUCCINIC-DITMS	15.882	360	15253	29.35
4-HYDROXY-2-METHYLVALERIC-DITMS	16.349	377	1813	3.49
URACIL-DITMS	16.531	384	1624	3.13
4-DEOXYTETRONIC-TRITMS	17.877	433	4991	9.60
PYROGLUTAMIC-DITMS	21.432	563	5703	10.97
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.262	594	3098	5.96
3-HYDROXYPHENYLACETIC-DITMS	23.588	642	13137	25.28

p-HYDROXYPHENYLACETIC-DITMS	24.232	666	33437	64.34
FURAN-2,5-DICARBOXYLIC-DITMS	24.344	670	3279	6.31
m-HYDROXYPHENYLPROPIONIC-DITMS	26.033	732	1839	3.54
t-ACONITIC-TRITMS	26.852	762	30492	58.67
HIPPURIC-TMS	27.881	800	42133	81.07
3,4-DIHYDROXYBENZOIC-TRITMS	28.132	809	3790	7.29
CITRIC-TETRATMS	28.544	824	56171	108.08
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.811	834	11856	22.81
PALMITIC-TMS	31.823	944	4637	8.92
p-HYDROXYHIPPURIC-DITMS	33.021	988	10793	20.77
DIOCTYLPHTALATE-TMS	38.534	1190	3077	5.92
18.80 min INTERNAL STANDARD	20.982	547	136426	262.5

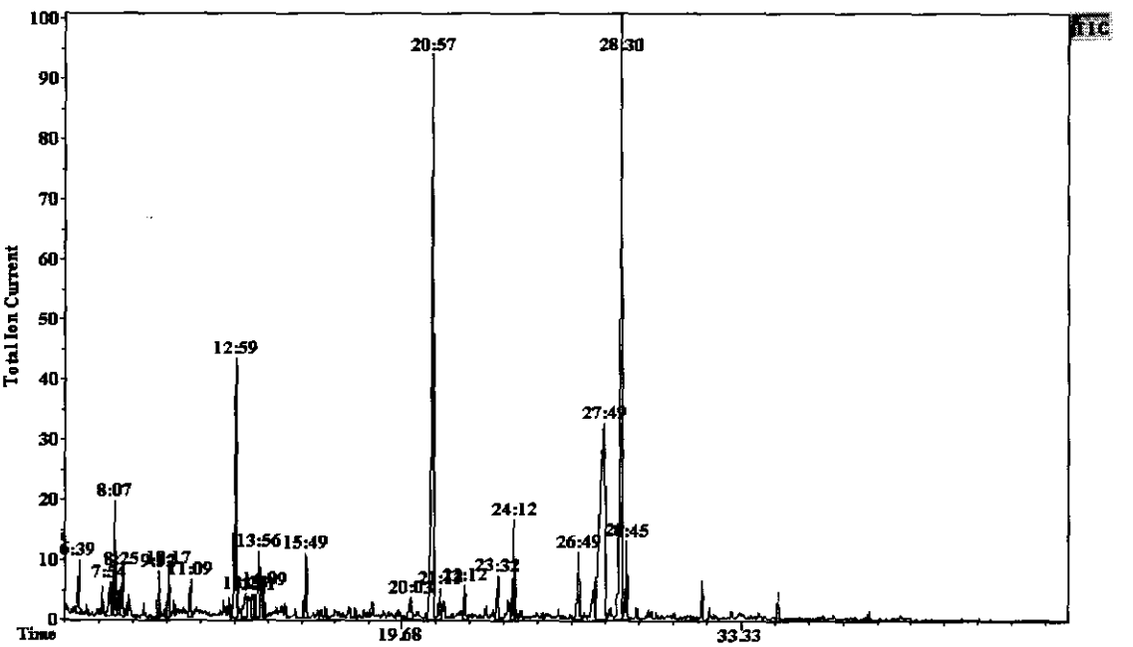

Figure A22: *GC-MS total ion chromatogram of the urine sample on day five after the intake of grapefruit juice of subject AF 26*

Table A22: Interpretation of GC-MS spectrum of urine sample of day five of subject AF 26.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.132	76	34558	66.16
GLYCOLIC-DITMS	8.44	87	13840	26.50
OXALIC-DITMS	9.882	140	19371	37.09
p-CRESOL-TMS	10.294	155	9832	18.83

3-HYDROXYPROPIONIC-DITMS	10.518	163	3653	7.00
3-HYDROXYISOBUTYRIC-DITMS	11.165	187	8878	17.00
3-HYDROXY-ISO-VALERIC-DITMS	12.745	245	4856	9.30
BENZOIC-TMS	13.101	258	2145	4.11
UREA-DITMS	14.157	297	6558	12.56
MALONIC-DITMS	14.872	323	2007	3.84
PHOSPHORIC-TRITMS	15.022	328	5315	10.18
METHYLMALONIC-DITMS	15.849	359	15377	29.44
4-HYDROXY-2-METHYLVALERIC-DITMS	16.321	376	2248	4.30
URACIL-DITMS	16.483	382	1359	2.60
SUCCINIC-DITMS	16.635	387	2069	3.96
4-DEOXYTETRONIC-TRITMS	17.591	422	4909	9.40
PHENOXYACETATE	18.532	457	4101	7.85
PYROGLUTAMIC-DITMS	21.402	562	5493	10.52
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.223	592	6656	12.74
3-HYDROXYPHENYLACETIC-DITMS	23.56	641	17331	33.18
p-HYDROXYPHENYLACETIC-DITMS	24.209	665	46779	89.56
FURAN-2,5-DICARBOXYLIC-DITMS	24.313	669	2784	5.33
m-HYDROXYPHENYLPROPIONIC-DITMS	26.016	731	1358	2.60
t-ACONITIC-TRITMS	26.824	761	30471	58.34
HIPPURIC-TMS	27.859	799	42741	81.83
3,4-DIHYDROXYBENZOIC-TRITMS	28.107	808	4129	7.91
CITRIC-TETRATMS	28.519	823	77079	147.57
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.765	832	33545	64.22
HEXACOSANE-TMS	29.969	876	1186	2.27
PALMITIC-TMS	31.801	943	6359	12.17
p-HYDROXYHIPPURIC-DITMS	32.994	987	1877	3.60
STEARIC-TMS	34.843	1055	4302	8.24
DIOCTYLPHTALATE-TMS	38.495	1188	2115	4.05
18.80 min INTERNAL STANDARD	20.955	546	137110	262.5

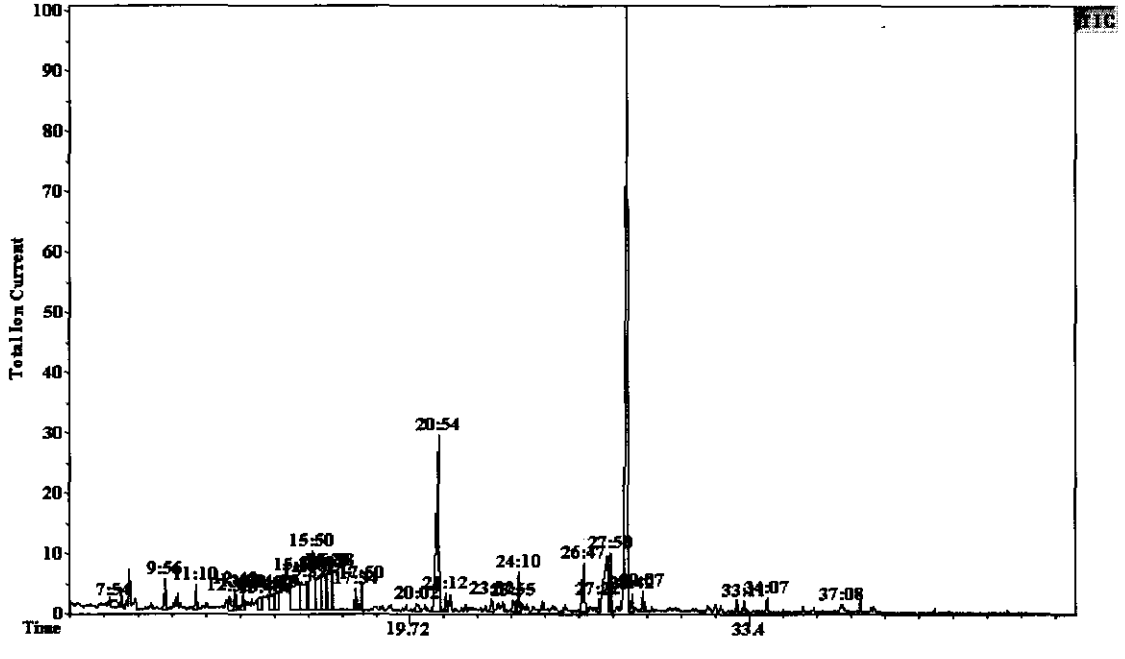

Figure A23: *GC-MS total ion chromatogram of the control sample of subject CM 10*

Table A23: Interpretation of GC-MS spectrum of the control sample of subject CM 10.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.2	78	36725	38.01
2-HYDROXYISOBUTYRIC-DITMS	8.405	86	7950	8.23
GLYCOLIC-DITMS	8.528	90	93758	97.04
OXALIC-DITMS	9.948	142	88707	91.82
p-CRESOL-TMS	10.33	156	16566	17.15
3-HYDROXYPROPIONIC-DITMS	10.557	164	15995	16.56
3-HYDROXYISOBUTYRIC-DITMS	11.184	187	53423	55.30
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.393	231	11836	12.25
3-HYDROXY-ISO-VALERIC-DITMS	12.741	244	30178	31.24
BENZOIC-TMS	13.08	256	17653	18.27
PHOSPHORIC-TRITMS	15.212	334	24901	25.77
SUCCINIC-DITMS	15.861	358	83937	86.88
UREA-DITMS	16.643	387	93578	96.86
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.579	421	34578	35.79
PYROGLUTAMIC-DITMS	21.405	561	51919	53.74
PIPECOLIC-DITMS	21.408	561	53135	55.00
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.183	589	4928	5.10
2-HYDROXYGLUTARIC-TRITMS	23.324	631	7396	7.66
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.931	653	14237	14.74
m-HYDROXYBENZOIC-DITMS	23.986	655	9127	9.45
p-HYDROXYPHENYLACETIC-DITMS	24.169	662	69105	71.53
FURAN-2,5-DICARBOXYLIC-DITMS	24.262	665	14127	14.62
ISOCITRICLACTON-DITMS	25.116	696	8827	9.14
1,6-ANHYDRO-B-d-GLUCOPYRANOSE-TRITMS	26.013	729	6361	6.58
p-HYDROXYMANDELIC-TRITMS	27.379	779	18448	19.10
HIPPURIC-TMS	27.858	797	114559	118.57
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	28.159	808	6915	7.16
CITRIC-TETRATMS	28.52	821	864406	894.70
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.723	828	20525	21.24
VANILLYLMANDELIC-TRITMS	29.128	843	25171	26.05
p-HYDROXYPHENYLLACTIC-TRITMS	29.511	857	10130	10.49
p-HYDROXYHIPPURIC-DITMS	34.129	1026	31889	33.01
DIOCTYLPHTALATE-TMS	38.455	1184	18911	19.57
18.80 min INTERNAL STANDARD	20.916	543	253611	262.5

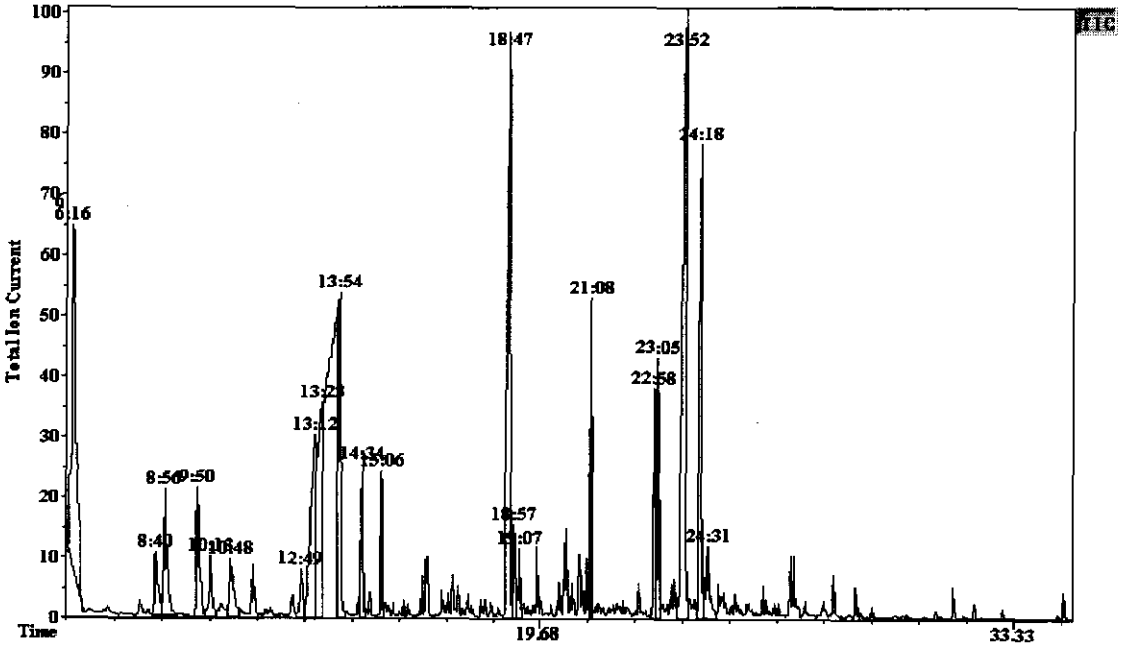

Figure A24: *GC-MS total ion chromatogram of the urine sample on day one after the intake of grapefruit juice of subject CM 10*

Table A24: Interpretation of GC-MS spectrum of urine sample of day one of subject CM 10.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.3	9	606623	286.85
ETHYLENGLYCOL-DITMS	6.83	28	1969	0.93
1,2-DIHYDROXYPROPANE-DITMS	7.305	46	8468	4.00
LACTIC-DITMS	8.675	96	77757	36.77
2-HYDROXYISOBUTYRIC-DITMS	8.886	103	13951	6.60
GLYCOLIC-DITMS	8.962	106	157298	74.38
1,2-DIHYDROXYBUTANE-DITMS	9.863	139	170893	80.81
OXALIC-DITMS	10.24	153	80882	38.25
p-CRESOL-TMS	10.81	174	55237	26.12
3-HYDROXYPROPIONIC-DITMS	10.876	176	12878	6.09
3-HYDROXYISOBUTYRIC-DITMS	11.449	197	39991	18.91
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.587	239	27111	12.82
3-HYDROXY-ISO-VALERIC-DITMS	12.851	249	42888	20.28
UREA-DITMS	13.212	262	160442	75.87
BENZOIC-TMS	13.248	263	13309	6.29
3-ETHYLHYDRACRYLIC-DITMS	13.357	267	47285	22.36
ETHYLMALONIC-DITMS	14.473	308	17130	8.10
PHOSPHORIC-TRITMS	14.577	312	164664	77.86
GLYCERIN-TRITMS	14.801	320	25032	11.84

SUCCINIC-DITMS	15.142	333	187620	88.72
1,2-DIHYDROXYBENZENE-DITMS	15.344	340	23659	11.19
4-HYDROXY-2-METHYLVALERIC-DITMS	15.483	345	11837	5.60
URACIL-DITMS	15.645	351	2919	1.38
ITACONIC-DITMS	15.781	356	16641	7.87
GLYCERIC-TRITMS	15.917	361	15052	7.12
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.318	376	50236	23.76
METHYLMALONIC-DITMS	16.414	379	32999	15.60
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	17.085	404	27980	13.23
HYDROCHINON-DITMS	17.137	406	1273	0.60
3-METHYLGLUTACONIC-DITMS	17.74	428	7339	3.47
3,4-DIHYDROXYBUTYRIC-TRITMS	18.019	438	24617	11.64
SALICYLIC-DITMS	19.083	477	8944	4.23
5-PYROLIDON-2-CARBOXYLIC-DITMS	19.135	479	85729	40.54
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	19.244	483	6070	2.87
3-METHYLADIPIC-DITMS	19.511	493	3097	1.47
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.629	497	54457	25.75
ERYTHRITOL-TMS	19.73	501	3431	1.62
o-HYDROXYPHENYLACETIC-DITMS	20.038	512	16705	7.90
ERYTHRONIC-TETRATMS	20.229	519	11972	5.66
3-HYDROXYPHENYLACETIC-DITMS	20.671	535	25793	12.20
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.911	544	42484	20.09
m-HYDROXYBENZOIC-DITMS	21.05	549	45005	21.28
p-HYDROXYPHENYLACETIC-DITMS	21.16	553	513077	242.61
ACETYLASPARTIC-DITMS	21.324	559	1736	0.82
ARABINONIC-g-LACTONE-TRITMS	21.389	562	12694	6.00
LAURIC-DITMS	21.468	564	4142	1.96
ISOCITRICLACTON-DITMS	21.918	581	13230	6.26
SUBERIC-DITMS	22.104	588	11302	5.34
1,6-ANHYDRO-B-d-GLUCOPYRANOSE-TRITMS	22.547	604	37503	17.73
t-ACONITIC-TRITMS	23	621	252262	119.29
c-ACONITIC-TRITMS	23.008	621	56949	26.93
VANILLIC-DITMS	23.079	623	1964	0.93
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.104	624	305998	144.70
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.145	626	13112	6.20
p-HYDROXYMANDELIC-TRITMS	23.505	639	27951	13.22
AZELAIC-DITMS	23.585	642	17516	8.28
HIPPURIC-TMS	23.876	653	618655	292.54
a-RESORCYLIC-TRITMS	24.022	658	2226	1.05
3,4-DIHYDROXYPHENYLACETIC-TRITMS	24.183	664	6871	3.25
CITRIC-TETRATMS	24.316	669	474849	224.54
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.543	677	55746	26.36
METHYLCITRIC-TETRATMS	24.702	683	2014	0.95
VANILLYLMANDELIC-TRITMS	24.844	688	32088	15.17
HYDANTOINPROPIONIC-TRITMS	24.926	691	6591	3.12
VANILGLYCOL-TRITMS	26.321	742	4208	1.99
PALMITIC-TMS	26.943	765	43280	20.47
o-HYDROXYHIPPURIC-DITMS	27.031	768	28513	13.48
GLUCURONIC-PENTATMS	27.184	774	1578	0.75
FERULIC-DITMS	27.377	781	1524	0.72
p-HYDROXYHIPPURIC-DITMS	28.807	833	26374	12.47
6-OCTADECENOIC-TMS	28.946	838	1957	0.93
DIOCTYLPHTALATE-TMS	32.218	958	10227	4.84
18.80 min INTERNAL STANDARD	18.81	467	555131	262.5

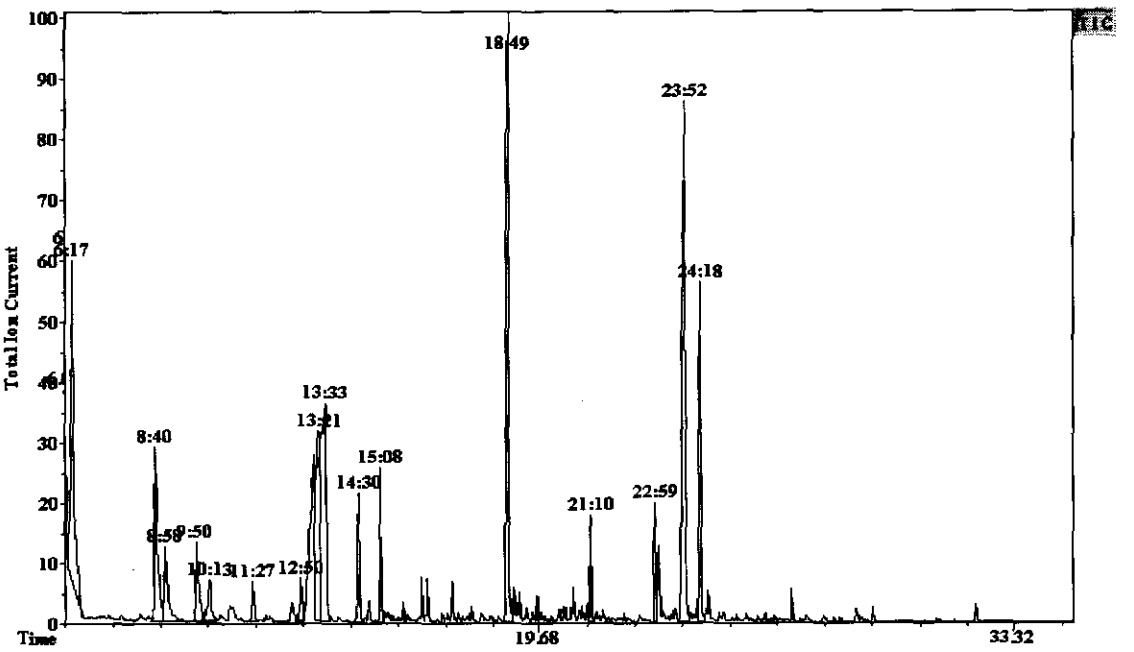

Figure A25: *GC-MS total ion chromatogram of the urine sample on day three after the intake of grapefruit juice of subject CM 10*

Table A25: Interpretation of GC-MS spectrum of urine sample of day three of subject CM 10.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.309	9	480846	241.27
LACTIC-DITMS	8.686	96	161253	80.91
2-HYDROXYISOBUTYRIC-DITMS	8.907	104	8720	4.38
GLYCOLIC-DITMS	8.975	107	82470	41.38
1,2-DIHYDROXYBUTANE-DITMS	9.875	140	85061	42.68
OXALIC-DITMS	10.246	153	29244	14.67
2,3-DIHYDROXYBUTANE-DITMS	10.255	154	36822	18.48
p-CRESOL-TMS	10.816	174	13481	6.76
3-HYDROXYPROPIONIC-DITMS	10.893	177	6587	3.30
3-HYDROXYISOBUTYRIC-DITMS	11.466	198	27392	13.74
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.598	240	17162	8.61
3-HYDROXY-ISO-VALERIC-DITMS	12.86	249	30167	15.14
UREA-DITMS	13.237	263	112084	56.24
BENZOIC-TMS	13.261	264	7018	3.52
ETHYLMALONIC-DITMS	14.495	309	13405	6.73
PHOSPHORIC-TRITMS	14.517	310	115641	58.02
GLYCERIN-TRITMS	14.809	321	18117	9.09
SUCCINIC-DITMS	15.147	333	176647	88.63
1,2-DIHYDROXYBENZENE-DITMS	15.346	340	16728	8.39
4-HYDROXY-2-METHYLVALERIC-DITMS	15.494	346	4823	2.42
URACIL-DITMS	15.654	352	1345	0.68

ITACONIC-DITMS	15.78	356	17652	8.86
CITRACONIC-DITMS	15.913	361	5907	2.96
FUMARIC-DITMS	15.99	364	1869	0.94
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.322	376	46318	23.24
GLUTARIC-DITMS	17.059	403	3834	1.92
3-METHYLGLUTACONIC-DITMS	17.741	428	10420	5.23
PYROGLUTAMIC-DITMS	19.132	479	23588	11.84
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.64	498	18167	9.12
ERYTHRITOL-TMS	19.73	501	6394	3.21
MANNONIC-1,4-LACTONE	19.732	501	6367	3.20
2-HYDROXYGLUTARIC-TRITMS	20.412	526	10698	5.37
ERYTHRONIC-TETRATMS	20.467	528	12129	6.09
3-HYDROXYPHENYLACETIC-DITMS	20.688	536	46317	23.24
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.928	545	2267	1.14
m-HYDROXYBENZOIC-DITMS	21.065	550	11411	5.73
p-HYDROXYPHENYLACETIC-DITMS	21.174	554	19428	9.75
LYXOSE-TETRATMS(i)	22.546	604	7883	3.96
t-ACONITIC-TRITMS	23.008	621	110650	55.52
3-HYDROXY-4-METHOXYBENZOIC-DITMS	23.09	624	1433	0.72
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.111	625	70286	35.27
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.142	626	10340	5.19
p-HYDROXYMANDELIC-TRITMS	23.521	640	8114	4.07
AZELAIC-DITMS	23.584	642	4584	2.30
HIPPURIC-TMS	23.87	653	461951	231.79
CITRIC-TETRATMS	24.315	669	292230	146.63
LYXOSE-TETRATMS(iv)	24.482	675	13475	6.76
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.555	678	9609	4.82
VANILLYLMANDELIC-TRITMS	24.852	689	5444	2.73
VANILGLYCOLIC-TRITMS	24.858	689	6911	3.47
PALMITIC-TMS	26.939	765	15953	8.01
p-HYDROXYHIPPURIC-DITMS	28.819	834	7525	3.78
HEXACOSANE-TMS	28.868	836	3968	1.99
DIOCTYLPHTALATE-TMS	32.234	959	9524	4.78
18.80 min INTERNAL STANDARD	18.821	468	523165	262.5

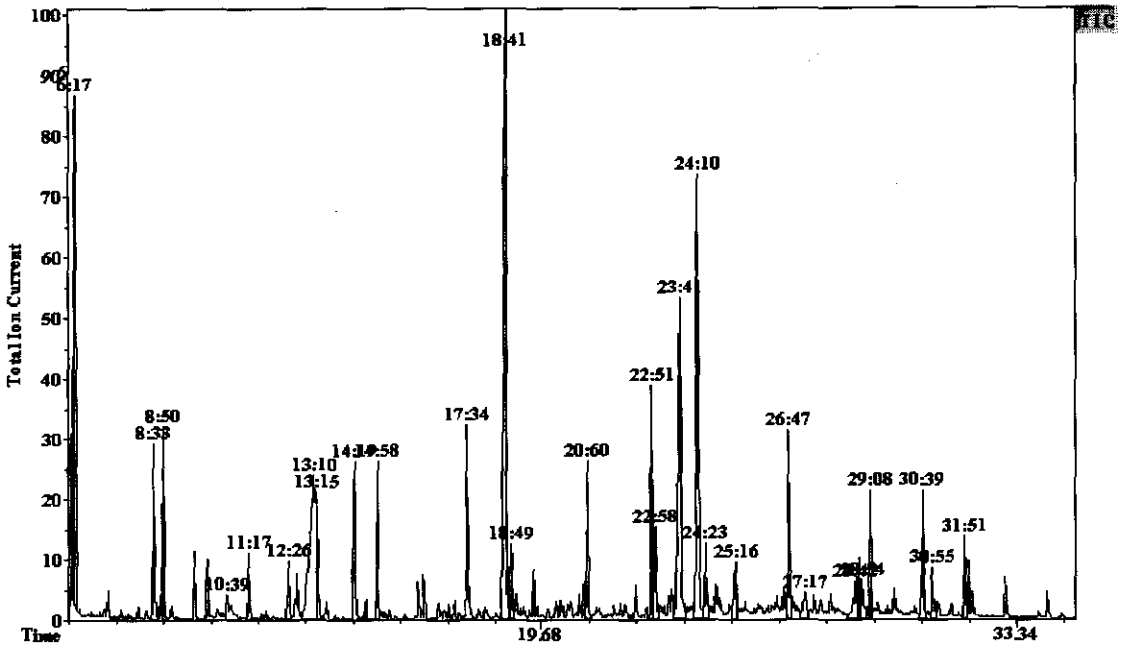

Figure A26: *GC-MS total ion chromatogram of the urine sample on day five after the intake of grapefruit juice of subject CM 10*

Table A26: Interpretation of GC-MS spectrum of urine sample of day five of subject CM 10.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.298	9	1086988	437.30
ETHYLENGLYCOL-DITMS	6.773	26	4871	1.96
LACTIC-DITMS	8.569	92	170587	68.63
2-HYDROXYISOBUTYRIC-DITMS	8.774	99	24459	9.84
GLYCOLIC-DITMS	8.85	102	197368	79.40
1,2-DIHYDROXYBUTANE-DITMS	9.74	135	99893	40.19
OXALIC-DITMS	10.103	148	82173	33.06
p-CRESOL-TMS	10.676	169	26776	10.77
3-HYDROXYPROPIONIC-DITMS	10.731	171	10749	4.32
3-HYDROXYISOBUTYRIC-DITMS	11.301	192	64506	25.95
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.395	232	7627	3.07
3-HYDROXY-ISO-VALERIC-DITMS	12.696	243	33019	13.28
BENZOIC-TMS	13.089	257	7221	2.91
UREA-DITMS	13.184	261	138310	55.64
PHOSPHORIC-TRITMS	14.325	303	202877	81.62
GLYCERIN-TRITMS	14.656	315	16835	6.77
SUCCINIC-DITMS	14.989	327	205240	82.57
1,2-DIHYDROXYBENZENE-DITMS	15.185	334	13769	5.54
2-METHYLSUCCINIC-DITMS	15.338	340	10441	4.20

URACIL-DITMS	15.502	346	1842	0.74
GLYCERIC-TRITMS	15.777	356	1825	0.73
1,2-DIHYDROXYPROPANE-DITMS	16.331	376	47544	19.13
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.336	376	39084	15.72
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	16.838	395	5553	2.23
GLUTARIC-DITMS	16.918	398	4266	1.72
HYDROCHINON-DITMS	16.986	400	1356	0.55
3-METHYLGLUTARIC-DITMS	17.357	414	3100	1.25
3-METHYLGLUTACONIC-DITMS	17.605	423	3021	1.22
P-AMINOBEMZOIC-TMS	18.154	443	1522	0.61
PYROGLUTAMIC-DITMS	18.995	474	26835	10.80
5-PYROLIDON-2-CARBOXYLIC-DITMS	18.998	474	27110	10.91
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	19.11	478	2246	0.90
3-METHYLADIPIC-DITMS	19.38	488	3620	1.46
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.497	492	28760	11.57
TIGLYLGLYCINE-TMS	19.577	495	2947	1.19
ERYTHRITOL-TMS	19.601	496	7838	3.15
o-HYDROXYPHENYLACETIC-DITMS	19.904	507	8053	3.24
2-HYDROXYGLUTARIC-TRITMS	20.281	521	11988	4.82
ERYTHRONIC-TETRATMS	20.333	523	9172	3.69
3-HYDROXYPHENYLACETIC-DITMS	20.554	531	17862	7.19
p-HYDROXYPHENYLACETIC-DITMS	20.554	531	4562	1.84
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.794	540	5894	2.37
m-HYDROXYBENZOIC-DITMS	20.93	545	25036	10.07
LAURIC-DITMS	21.345	560	3073	1.24
ISOCITRICLACTON-DITMS	21.785	576	10175	4.09
SUBERIC-DITMS	21.976	583	6979	2.81
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	22.413	599	31848	12.81
1,6-ANHYDRO-B-d-GLUCOPYRANOSE-TRITMS	22.416	599	31170	12.54
c-ACONITIC-TRITMS	22.877	616	183463	73.81
VANILLIC-DITMS	22.956	619	3104	1.25
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	22.978	620	64900	26.11
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.011	621	17709	7.12
p-HYDROXYMANDELIC-TRITMS	23.388	635	23450	9.43
TECEPHALIC-DITMS	23.464	637	8637	3.48
HIPPURIC-TMS	23.694	646	288661	116.13
CITRIC-TETRATMS	24.185	664	290534	116.88
MYRISTIC-TMS	24.237	666	15378	6.19
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.426	673	57329	23.06
VANILLYLMANDELIC-TRITMS	24.713	683	28770	11.57
VANILGLYCOLIC-TRITMS	24.729	684	31332	12.61
p-HYDROXYPHENYLLACTIC-TRITMS	25.092	697	6723	2.71
FUCOSE-PENTATMS(iii)	25.45	710	2470	0.99
PENTADECANOIC-TMS	25.554	714	5212	2.10
ISOVANILHYDRACRYLIC-TRITMS	26.204	738	6476	2.61
1,9-DIMETHYLURIC-DITMS	26.688	756	18342	7.38
PALMITIC-TMS	26.813	760	82715	33.28
GLUCURONIC-PENTATMS	27.054	769	6152	2.48
FERULIC-DITMS	27.248	776	2399	0.97
p-HYDROXYHIPPURIC-DITMS	28.69	829	12978	5.22
HEXACOSANE-TMS	28.726	830	23640	9.51
OLEIC-TMS	28.911	837	9984	4.02
STEARIC-TMS	29.16	846	60470	24.33
DEHYDROABIETIC ACID	30.667	901	119612	48.12
DIOCTYLPHTALATE-TMS	32.087	953	22392	9.01
18.80 min INTERNAL STANDARD	18.689	463	652499	262.5

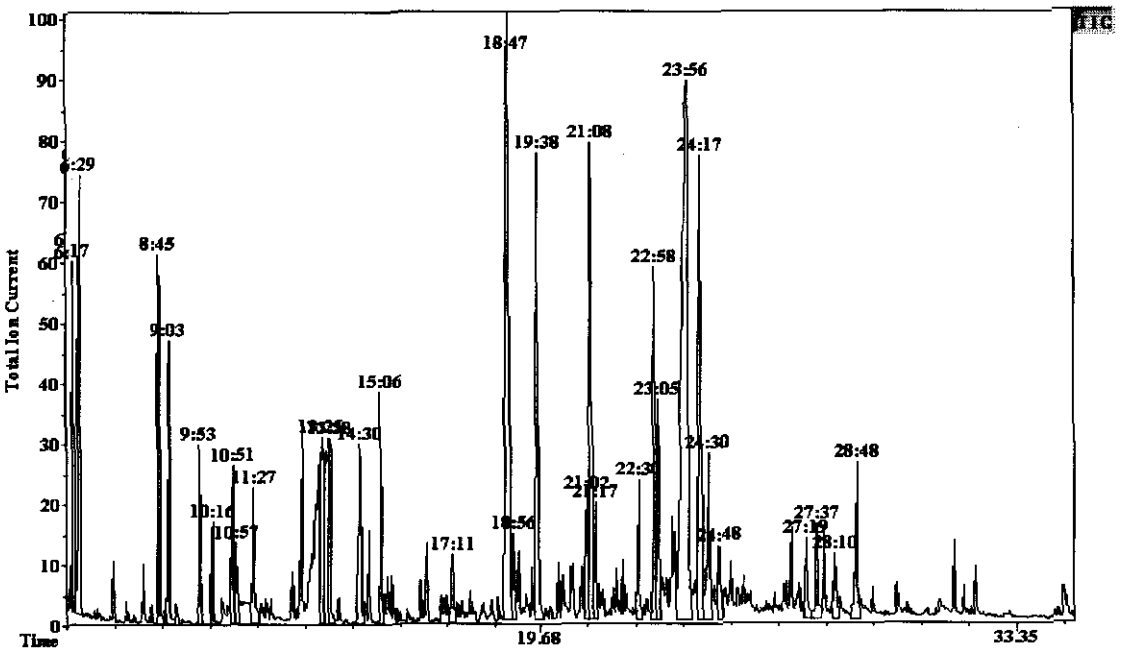

Figure A27: *GC-MS total ion chromatogram of the control sample of subject CF 9*

Table A27: Interpretation of GC-MS spectrum of the control sample of subject CF 9.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.494	16	1003654	345.86
ETHYLENGLYCOL-DITMS	6.988	34	16091	5.55
LACTIC-DITMS	8.773	99	404174	139.28
2,3-DIHYDROXYBUTANE-DITMS	8.773	99	413474	142.48
GLYCOLIC-DITMS	9.057	110	369147	127.21
PYRUVIC-TMS	9.414	123	5786	1.99
1,2-DIHYDROXYBUTANE-DITMS	9.916	141	254818	87.81
2-FURANCARBOXYLIC -TMS	10.098	148	1494	0.52
OXALIC-DITMS	10.284	155	183180	63.12
p-CRESOL-TMS	10.86	176	234879	80.94
3-HYDROXYISOBUTYRIC-DITMS	11.466	198	134077	46.20
2-HYDROXY-3-METHYLBUTYRIC-DITMS	11.687	206	9943	3.43
2-HYDROXY-ISO-VALERIC-DITMS	11.689	206	10244	3.53
MALONIC-DITMS	12.404	232	4141	1.43
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.54	237	16463	5.67
3-HYDROXY-ISO-VALERIC-DITMS	12.854	249	180048	62.04
UREA-DITMS	13.127	259	64829	22.34
BENZOIC-TMS	13.242	263	30320	10.45
ETHYLMALONIC-DITMS	14.462	308	47071	16.22
PHOSPHORIC-TRITMS	14.528	310	264581	91.17
GLYCERIN-TRITMS	14.774	319	90378	31.14

SUCCINIC-DITMS	15.123	332	356882	122.98
1,2-DIHYDROXYBENZENE-DITMS	15.317	339	107163	36.93
2-METHYLSUCCINIC-DITMS	15.454	344	48400	16.68
URACIL-DITMS	15.618	350	7932	2.73
GLYCERIC-TRITMS	15.885	360	12063	4.16
FUMARIC-DITMS	15.942	362	5017	1.73
1,2-DIHYDROXYPROPANE-DITMS	16.278	374	50217	17.31
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.456	381	90525	31.20
GLUTARIC-DITMS	17.029	402	18090	6.23
HYDROCHINON-DITMS	17.106	405	2876	0.99
3-METHYLGLUTARIC-DITMS	17.471	418	12061	4.16
2,4-DIHYDROXYBUTYRIC-TRITMS	17.58	422	7527	2.59
3-METHYLGLUTACONIC-DITMS	17.714	427	19570	6.74
3-HYDROXYADIPYLLACTONE-TMS	17.799	430	16184	5.58
P-AMINOBEMZOIC-TMS	18.266	447	1933	0.67
ISOVALERYLGLYCINE-TMS	18.315	449	5190	1.79
ADIPIC-DITMS	18.943	472	53929	18.58
5-PYROLIDON-2-CARBOXYLIC-DITMS	19.11	478	88358	30.45
ACETYLTRHEONINE-DITMS	19.353	487	7283	2.51
3-METHYLADIPIC-DITMS	19.492	492	32439	11.18
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.648	498	324548	111.84
THREITOL-TETRATMS	19.708	500	5685	1.96
o-HYDROXYPHENYLACETIC-DITMS	20.019	511	13295	4.58
ERYTHRONIC-TETRATMS	20.202	518	2231	0.77
2-HYDROXYGLUTARIC-TRITMS	20.385	525	34492	11.89
3-HYDROXYPHENYLACETIC-DITMS	20.661	535	70317	24.23
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.893	543	42999	14.82
m-HYDROXYBENZOIC-DITMS	21.041	549	52366	18.05
2-FUROYLGLYCINE-TMS	21.071	550	82287	28.36
p-HYDROXYPHENYLACETIC-DITMS	21.151	553	482090	166.13
FURAN-2,5-DICARBOXYLIC-DITMS	21.181	554	387582	133.56
OCTENEDIOIC-DITMS	21.79	576	20585	7.09
ISOCITRICLACTON-DITMS	21.899	580	42406	14.61
SUBERIC-DITMS	22.085	587	32092	11.06
1,6-ANHYDRO-B-d-GLUCOPYRANOSE-TRITMS	22.525	603	145977	50.31
PROPANETRICARBOXYLIC-TRITMS	22.741	611	6447	2.22
c-ACONITIC-TRITMS	22.99	620	322643	111.18
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.094	624	197429	68.03
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.124	625	51668	17.80
p-HYDROXYMANDELIC-TRITMS	23.485	638	120497	41.52
AZELAIC-DITMS	23.562	641	24622	8.49
4-METHYLSUBERIC-DITMS	23.562	641	27560	9.50
TECEPHALIC-DITMS	23.573	641	12599	4.34
HIPPURIC-TMS	23.936	655	608132	209.56
3,4-DIHYDROXYPHENYLACETIC-TRITMS	24.166	663	11528	3.97
CITRIC-TETRATMS	24.297	668	343779	118.47
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.53	676	125970	43.41
VANILLYLMANDELIC-TRITMS	24.831	687	78409	27.02
HYDANTOINPROPIONIC-TRITMS	24.905	690	10372	3.57
FERULIC-DITMS	25.176	700	9964	3.43
p-HYDROXYPHENYLLACTIC-TRITMS	25.206	701	44256	15.25
GLUCOPYRANOSE-HEXATMS(ii)	25.559	714	28342	9.77
GALACTOSE-6TMS(iii)	25.561	714	26878	9.26
3,4-DIHYDROXYMANDELIC-TETRATMS	25.61	716	1649	0.57
ISOVANILHYDRACRYLIC-TRITMS	26.302	741	7257	2.50
GLUCURONIC-PENTATMS	26.436	746	11119	3.83
PALMITIC-TMS	26.925	764	54199	18.68

o-HYDROXYHIPPURIC-DITMS	27.015	767	2610	0.90
ACETYLTYROSINE-DITMS	27.721	793	3621	1.25
p-HYDROXYHIPPURIC-DITMS	28.812	833	154293	53.17
DIOCTYLPHTALATE-TMS	32.203	957	56175	19.36
HEXACOSANE-TMS	33.161	992	4679	1.61
18.80 min INTERNAL STANDARD	18.809	467	761756	262.5

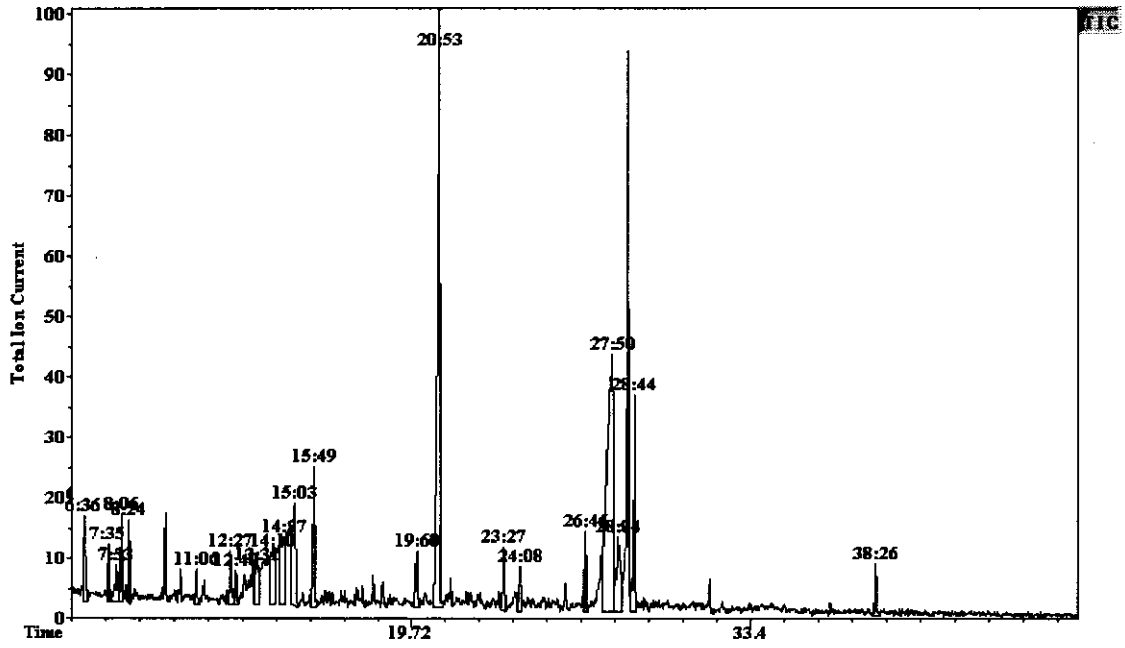

Figure A28: *GC-MS total ion chromatogram of the urine sample on day one after the intake of grapefruit juice of subject CF 9*

Table A28: Interpretation of GC-MS spectrum of urine sample of day one of subject CF 9.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.108	75	32862	36.56
2-HYDROXYISOBUTYRIC-DITMS	8.294	82	4036	4.49
GLYCOLIC-DITMS	8.423	86	43709	48.63
OXALIC-DITMS	9.862	139	63847	71.03
3-HYDROXYISOBUTYRIC-DITMS	11.121	185	19238	21.40
a-RESORCYLIC-TRITMS	11.403	195	7550	8.40
3-HYDROXY-ISO-VALERIC-DITMS	12.692	242	12564	13.98
BENZOIC-TMS	13.029	255	10897	12.12
3-ETHYLHYDRACRYLIC-DITMS	13.368	267	21965	24.44

SUCCINIC-DITMS	15.828	357	95371	106.10
URACIL-DITMS	16.435	379	3717	4.14
4-DEOXYTETRONIC-TRITMS	17.782	429	5012	5.58
ERYTHRO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.787	428	3698	4.11
MALONIC-DITMS	18.603	458	5835	6.49
GLUTARIC-DITMS	18.608	459	6232	6.93
PYROGLUTAMIC-DITMS	21.353	559	17389	19.35
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.173	588	3339	3.72
2-HYDROXYGLUTARIC-TRITMS	23.303	630	4578	5.09
m-HYDROXYBENZOIC-DITMS	23.985	655	2665	2.97
p-HYDROXYPHENYLACETIC-DITMS	24.143	661	18318	20.38
m-HYDROXYPHENYLPROPIONIC-DITMS	25.96	727	7613	8.47
t-ACONITIC-TRITMS	26.778	757	25937	28.86
HIPPURIC-TMS	27.836	796	113840	126.65
CITRIC-TETRATMS	28.468	819	160915	179.03
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.741	829	72212	80.34
ISOVANILGLYCOLIC-TRITMS	29.125	843	3953	4.40
PALMITIC-TMS	31.749	939	11204	12.47
DIOCTYLPHTALATE-TMS	38.457	1184	32434	36.09
18.80 min INTERNAL STANDARD	20.887	542	235942	262.5

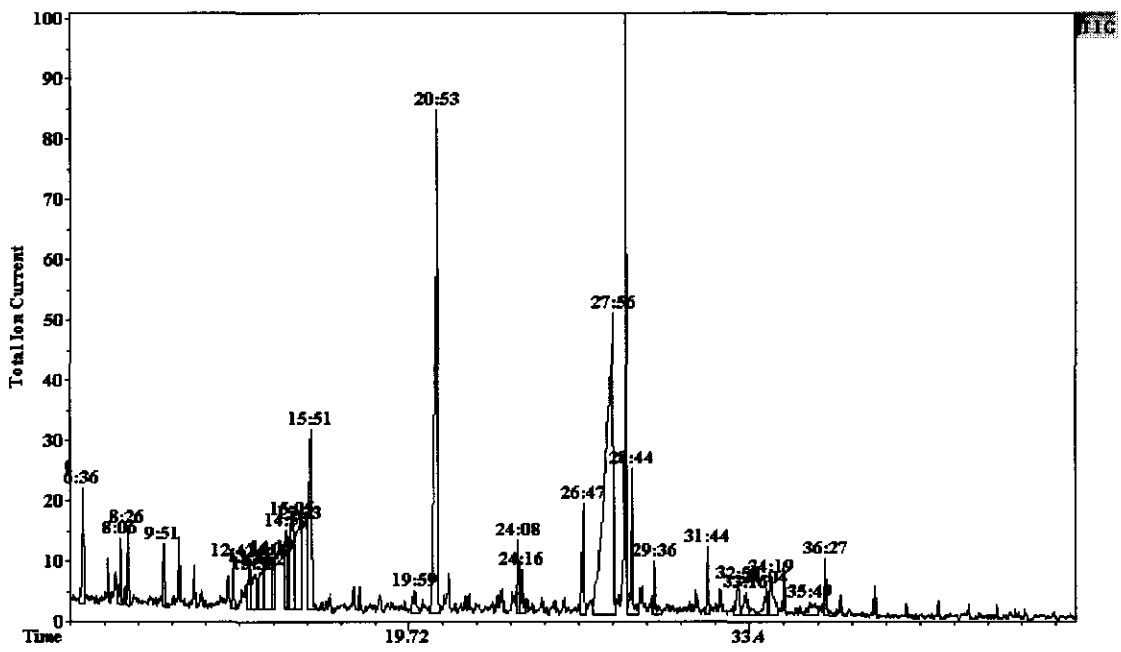

Figure A29: *GC-MS total ion chromatogram of the urine sample on day three after the intake of grapefruit juice of subject CF 9*

Table A29: Interpretation of GC-MS spectrum of urine sample of day three of subject CF 9.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.125	75	22377	25.65
1,2-DIHYDROXYBUTANE-DITMS	8.314	82	4443	5.09
GLYCOLIC-DITMS	8.449	87	48883	56.03
OXALIC-DITMS	9.885	140	53019	60.77
p-CRESOL-TMS	10.274	154	7998	9.17
3-HYDROXYISOBUTYRIC-DITMS	11.133	185	24494	28.08
3-HYDROXY-ISO-VALERIC-DITMS	12.713	243	24395	27.96
3-ETHYLHYDRACRYLIC-DITMS	13.386	268	13649	15.64
ETHYLMALONIC-DITMS	14.847	321	12865	14.75
PHOSPHORIC-TRITMS	15.047	328	9890	11.34
SUCCINIC-DITMS	15.858	358	138630	158.90
4-HYDROXY-2-METHYLVALERIC-DITMS	16.293	374	6916	7.93
URACIL-DITMS	16.465	380	2794	3.20
ERYTHRO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.557	420	9181	10.52
4-DEOXYTETRONIC-TRITMS	17.56	420	9899	11.35
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.811	429	7553	8.66
PYROGLUTAMIC-DITMS	21.373	559	26979	30.92
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.178	589	6328	7.25
2-HYDROXYGLUTARIC-TRITMS	23.308	630	5853	6.71
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.926	653	6731	7.72
m-HYDROXYBENZOIC-DITMS	23.984	655	4221	4.84
p-HYDROXYPHENYLACETIC-DITMS	24.153	661	38133	43.71
FURAN-2,5-DICARBOXYLIC-DITMS	24.285	666	22066	25.29
m-HYDROXYPHENYLPROPIONIC-DITMS	25.961	727	3696	4.24
ARABINOSE-TETRATMS	26.016	729	5248	6.02
t-ACONITIC-TRITMS	26.801	758	43663	50.05
p-HYDROXYMANDELIC-TRITMS	27.384	779	5556	6.37
HIPPURIC-TMS	27.945	800	169135	193.86
CITRIC-TETRATMS	28.473	819	249152	285.58
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.741	829	59399	68.08
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	29.048	840	5559	6.37
ISOVANILGLYCOLIC-TRITMS	29.133	843	9612	11.02
p-HYDROXYPHENYLLACTIC-TRITMS	29.527	857	4168	4.78
ISOVANILHYDRACRYLIC-TRITMS	31.034	913	3594	4.12
FERULIC-DITMS	32.257	957	4222	4.84
p-HYDROXYHIPPURIC-DITMS	32.982	984	10530	12.07
DEHYDROABIETIC ACID	36.462	1111	35109	40.24
DIOCTYLPHTALATE-TMS	38.461	1184	21243	24.35
18.80 min INTERNAL STANDARD	20.892	542	229020	262.5

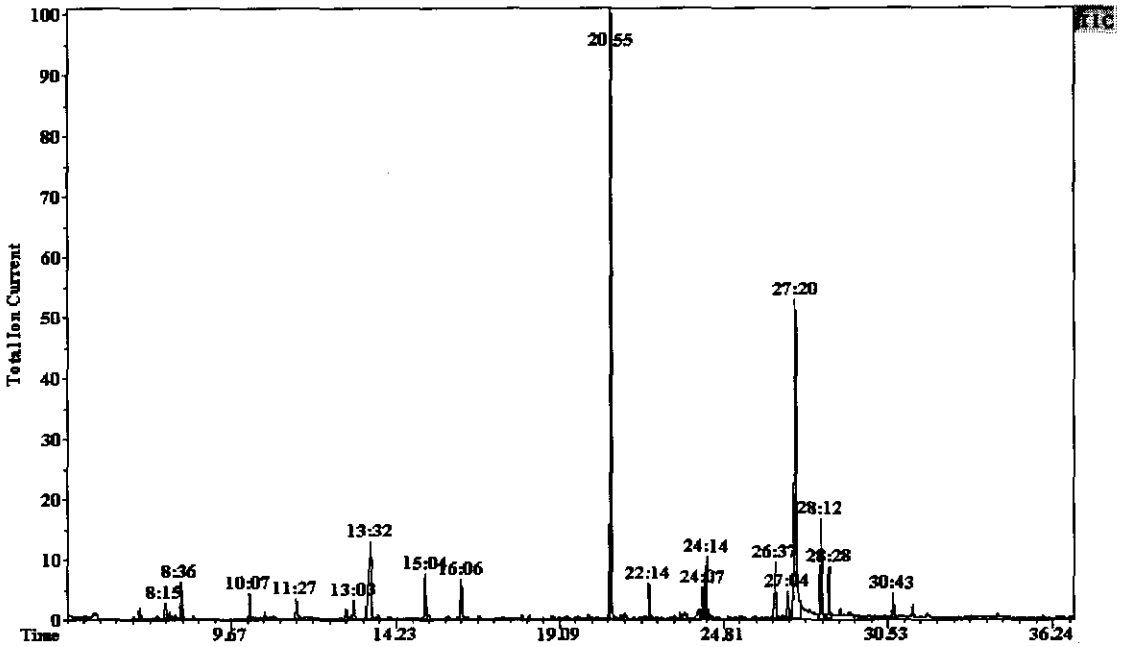

Figure A30: *GC-MS total ion chromatogram of the urine sample on day five after the intake of grapefruit juice of subject CF 9*

Table A30: Interpretation of GC-MS spectrum of urine sample of day five of subject CF 9.

Name	R.T.	Scan	Tot.Signal	mg/g creat
ETHYLENGLYCOL-DITMS	6.122	8	21941	0.77
2,3-DIHYDROXYBUTANE-DITMS	7.971	524	22540	0.79
LACTIC-DITMS	8.249	602	221148	7.71
2-HYDROXYISOBUTYRIC-DITMS	8.463	661	48016	1.68
GLYCOLIC-DITMS	8.591	697	698274	24.36
OXALIC-DITMS	10.12	1114	509116	17.76
p-CRESOL-TMS	10.547	1206	137756	4.81
3-HYDROXYPROPIONIC-DITMS	10.791	1259	28294	0.99
4-HYDROXYBUTANOIC-TRITMS	11.439	1398	316682	11.05
3-HYDROXYISOBUTYRIC-DITMS	11.44	1398	318558	11.11
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.693	1668	19793	0.69
3-HYDROXY-ISO-VALERIC-DITMS	13.054	1745	212910	7.43
UREA-DITMS	13.528	1847	1347152	46.99
PHOSPHORIC-TRITMS	15.07	2179	1056400	36.85
4-HYDROXY-2-METHYLVALERIC-DITMS	15.168	2200	66741	2.33
GLYCERIN-TRITMS	15.717	2318	30160	1.05
SUCCINIC-DITMS	16.093	2399	810227	28.26
1,2-DIHYDROXYBENZENE-DITMS	16.168	2416	54651	1.91
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.829	2773	39953	1.39

4-DEOXYTETRONIC-TRITMS	17.831	2773	35065	1.22
GLUTARIC-DITMS	18.843	2955	42217	1.47
2,4-DIHYDROXYBUTYRIC-TRITMS	19.655	3097	18231	0.64
ADIPIC-DITMS	21.392	3401	33258	1.16
PIPECOLIC-DITMS	21.439	3409	66212	2.31
PYROGLUTAMIC-DITMS	21.439	3409	66212	2.31
3-METHYLADIPIC-DITMS	22.099	3525	21653	0.76
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.239	3549	378855	13.22
ERYTHRITOL-TMS	22.502	3595	13682	0.48
ERYTHRONIC-TETRATMS	23.118	3703	22494	0.79
2-HYDROXYGLUTARIC-TRITMS	23.328	3740	37730	1.32
3-HYDROXYPHENYLACETIC-DITMS	23.521	3774	66643	2.33
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.913	3842	47523	1.66
m-HYDROXYBENZOIC-DITMS	23.965	3852	49386	1.72
p-HYDROXYPHENYLACETIC-DITMS	24.121	3879	432106	15.07
FURAN-2,5-DICARBOXYLIC-DITMS	24.235	3898	956126	33.35
ISOCITRICLACTON-DITMS	25	4032	29936	1.04
m-HYDROXYPHENYLPROPIONIC-DITMS	25.861	4183	19077	0.67
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	25.916	4193	30288	1.06
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	26.576	4308	211009	7.36
c-ACONITIC-TRITMS	26.621	4316	489196	17.06
p-HYDROXYMANDELIC-TRITMS	27.184	4414	153581	5.36
HIPPURIC-TMS	27.33	4440	4106019	143.23
CITRIC-TETRATMS	28.194	4591	843996	29.44
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.465	4639	642190	22.40
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	28.564	4656	18031	0.63
VANILGLYCOLIC-TRITMS	28.868	4709	152138	5.31
p-HYDROXYPHENYLLACTIC-TRITMS	29.251	4776	48073	1.68
VANYLHYDRACRYLIC-TRITMS	30.718	5033	553658	19.31
o-HYDROXYHIPPURIC-DITMS	31.383	5149	101405	3.54
PALMITIC-TMS	31.405	5153	69804	2.44
FERULIC-DITMS	31.656	5197	16574	0.58
ISOFERULIC-DITMS	31.876	5235	32352	1.13
GLUCURONIC-PENTATMS	31.964	5251	16117	0.56
STEARIC-TMS	34.332	5665	35312	1.23
18.80 min INTERNAL STANDARD	20.915	3318	7525408	262.5

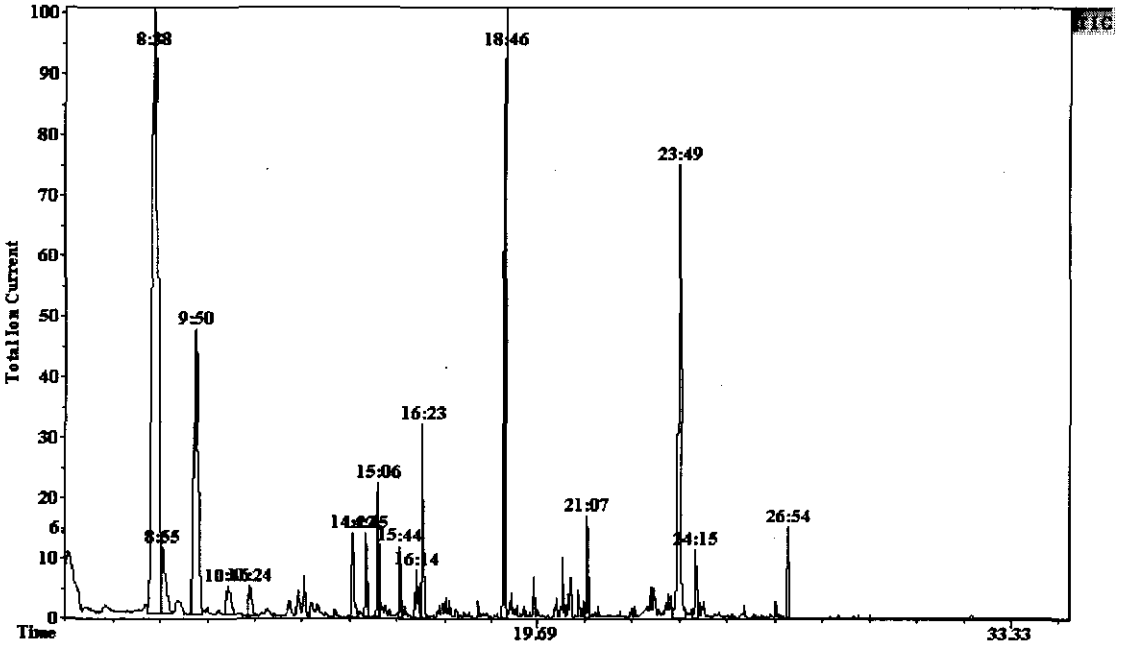

Figure A31: *GC-MS total ion chromatogram of the control sample of subject CF 7*

Table A31: Interpretation of GC-MS spectrum of the control sample of subject CF 7.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.205	5	32022	22.64
2,3-DIHYDROXYBUTANE-DITMS	7.248	43	4743	3.35
LACTIC-DITMS	8.662	95	338888	239.59
GLYCOLIC-DITMS	8.94	105	41315	29.21
PYRUVIC-TMS	9.366	121	9252	6.54
1,2-DIHYDROXYBUTANE-DITMS	9.852	139	219098	154.90
OXALIC-DITMS	10.204	152	6695	4.73
p-CRESOL-TMS	10.788	173	18063	12.77
3-HYDROXYPROPIONIC-DITMS	10.851	175	3965	2.80
3-HYDROXYISOBUTYRIC-DITMS	11.433	197	12329	8.72
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.533	237	2694	1.91
3-HYDROXY-ISO-VALERIC-DITMS	12.836	248	13524	9.56
UREA-DITMS	13.019	255	17126	12.11
BENZOIC-TMS	13.232	263	5885	4.16
3-ETHYLHYDRACRYLIC-DITMS	13.379	268	5287	3.74
PHOSPHORIC-TRITMS	14.392	305	41912	29.63
GLYCERIN-TRITMS	14.782	319	48904	34.58
SUCCINIC-DITMS	15.118	332	94476	66.80
1,2-DIHYDROXYBENZENE-DITMS	15.325	339	13386	9.46
4-HYDROXY-2-METHYLVALERIC-DITMS	15.464	344	3118	2.20
ITACONIC-DITMS	15.759	355	41854	29.59

FUMARIC-DITMS	15.961	363	1148	0.81
4-DEOXYTETRONIC-TRITMS	16.3	375	17841	12.61
METHYLMALONIC-DITMS	16.409	379	101431	71.71
ERYTHRO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.463	381	11152	7.89
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.463	381	10891	7.70
LACTYLLACTATE-TMS	16.895	397	7525	5.32
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	17.064	403	10300	7.28
HYDROCHINON-DITMS	17.119	405	914	0.65
2,4-DIHYDROXYBUTYRIC-TRITMS	17.586	422	2903	2.05
3-METHYLGLUTACONIC-DITMS	17.719	427	2526	1.79
DECANOIC-TMS	18.121	442	1035	0.73
PYROGLUTAMIC-DITMS	19.109	478	7264	5.14
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.617	497	18974	13.42
MANNONIC-1,4-LACTONE-TMS	19.712	500	4438	3.14
2-HYDROXYGLUTARIC-TRITMS	20.392	525	2195	1.55
ERYTHRONIC-TETRATMS	20.45	527	40469	28.61
3-HYDROXYPHENYLACETIC-DITMS	20.668	535	38769	27.41
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.905	544	2333	1.65
m-HYDROXYBENZOIC-DITMS	21.047	549	4407	3.12
p-HYDROXYPHENYLACETIC-DITMS	21.153	553	12548	8.87
LAURIC-DITMS	21.459	564	3810	2.69
PROSTAGLANDIN F-2 .BETA.-TETRATMS	22.027	585	2252	1.59
LYXOSE-TETRATMS(i)	22.527	603	7887	5.58
t-ACONITIC-TRITMS	22.985	620	15954	11.28
3-HYDROXY-4-METHOXYBENZOIC-DITMS	23.067	623	936	0.66
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.092	624	5623	3.98
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.122	625	2064	1.46
p-HYDROXYMANDELIC-TRITMS	23.499	639	7862	5.56
HIPPURIC-TMS	23.826	651	267081	188.83
CITRIC-TETRATMS	24.291	668	9875	6.98
MYRISTIC-TMS	24.348	670	8084	5.72
LYXOSE-TETRATMS(iv)	24.465	674	6479	4.58
VANILGLYCOL-TRITMS	24.837	688	2832	2.00
HEXACOSANE-TMS	25.615	716	1337	0.95
PENTADECANOIC-TMS	25.664	718	3959	2.80
PALMITIC-TMS	26.923	764	30369	21.48
DIOCTYLPHTALATE-TMS	32.215	958	1682	1.19
18.80 min INTERNAL STANDARD	18.776	466	371287	262.5

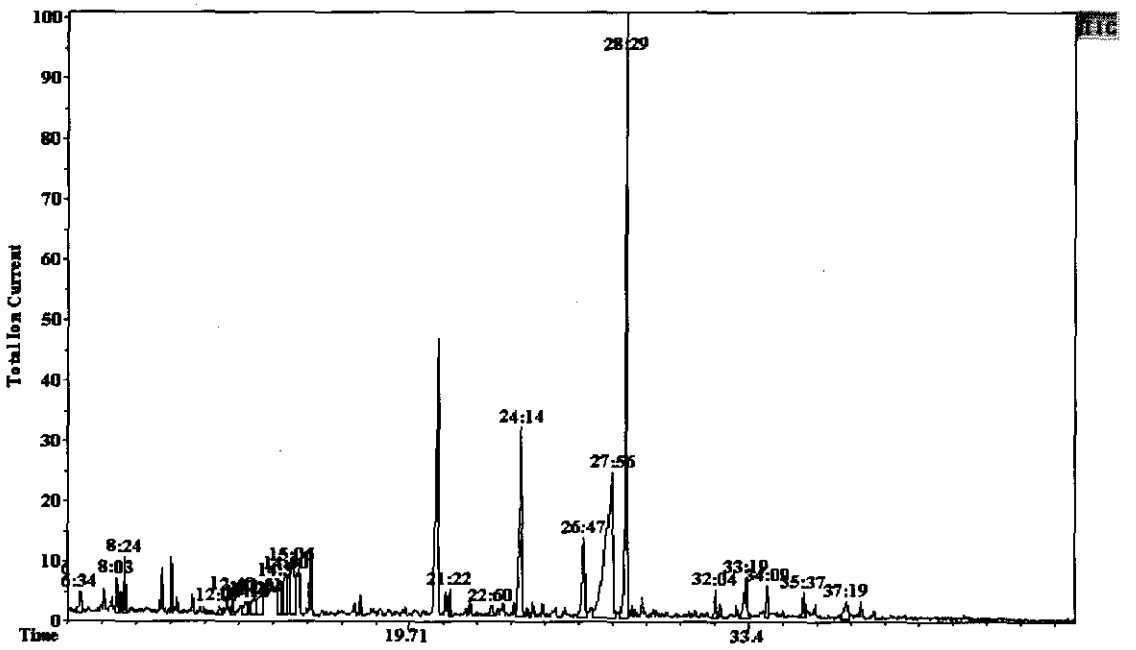

Figure A32: *GC-MS total ion chromatogram of the urine sample on day one after the intake of grapefruit juice of subject CF 7*

Table A32: Interpretation of GC-MS spectrum of urine sample of day one of subject CF 7.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.065	73	29602	33.66
2-HYDROXYISOBUTYRIC-DITMS	8.265	80	16540	18.81
GLYCOLIC-DITMS	8.41	86	88711	100.88
OXALIC-DITMS	9.862	139	72938	82.94
p-CRESOL-TMS	10.262	153	74888	85.16
3-HYDROXYISOBUTYRIC-DITMS	11.107	184	22166	25.21
3-HYDROXY-ISO-VALERIC-DITMS	12.691	242	16748	19.05
BENZOIC-TMS	13.022	254	4785	5.44
PHOSPHORIC-TRITMS	15.115	331	14794	16.82
UREA-DITMS	15.38	341	57551	65.45
SUCCINIC-DITMS	15.848	358	101935	115.92
MALONIC-DITMS	16.285	374	8624	9.81
4-DEOXYTETRONIC-TRITMS	17.56	420	8624	9.81
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.806	429	14951	17.00
PYROGLUTAMIC-DITMS	21.383	560	43121	49.04
PIPECOLIC ACID-DITMS	21.383	560	43121	49.04
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.196	590	15439	17.56
2-HYDROXYGLUTARIC-TRITMS	23.329	631	4812	5.47
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.975	655	9177	10.44

Table A33: Interpretation of GC-MS spectrum of urine sample of day three of subject CF 7.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.062	73	34377	27.73
2-HYDROXYISOBUTYRIC-DITMS	8.257	80	14361	11.59
GLYCOLIC-DITMS	8.408	86	79321	63.99
OXALIC-DITMS	9.858	139	53234	42.95
p-CRESOL-TMS	10.271	154	128247	103.46
3-HYDROXYISOBUTYRIC-DITMS	11.113	184	22589	18.22
a-RESORCYLIC-TRITMS	11.392	195	9147	7.38
UREA-TRITMS	12.17	223	25849	20.85
3-HYDROXY-ISO-VALERIC-DITMS	12.701	243	24580	19.83
BENZOIC-TMS	13.026	255	3841	3.10
PHOSPHORIC-TRITMS	15.061	329	14498	11.70
SUCCINIC-DITMS	15.855	358	148224	119.58
UREA-DITMS	15.904	360	43292	34.93
4-HYDROXY-2-METHYLVALERIC-DITMS	16.29	374	7215	5.82
4-DEOXYTETRONIC-TRITMS	17.553	420	12459	10.05
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.807	429	14059	11.34
PIPECOLIC ACID-DITMS	21.388	560	31848	25.69
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.201	590	19117	15.42
2-HYDROXYGLUTARIC-TRITMS	23.33	631	3546	2.86
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.987	655	9168	7.40
m-HYDROXYBENZOIC-DITMS	24.012	656	5366	4.33
p-HYDROXYPHENYLACETIC-DITMS	24.283	666	301875	243.54
FURAN-2,5-DICARBOXYLIC-DITMS	24.313	667	28236	22.78
ISOCITRICLACTON-DITMS	25.134	697	6466	5.22
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	26.801	758	5321	4.29
t-ACONITIC-TRITMS	26.825	759	63708	51.40
HIPPURIC-TMS	28.219	810	200592	161.83
CITRIC-TETRATMS	28.572	823	377214	304.32
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.78	830	7068	5.70
VANILGLYCOLIC-TRITMS	29.176	845	13603	10.97
INDOL-3-ACETIC-DITMS	29.981	874	12709	10.25
PALMITIC-TMS	31.756	939	8125	6.56
GLUCURONIC-PENTATMS	32.303	959	6274	5.06
p-HYDROXYHIPPURIC-DITMS	34.172	1027	29536	23.83
STEARIC-TMS	34.793	1050	8173	6.59
DIOCTYLPHTALATE-TMS	38.465	1184	11640	9.39
18.80 min INTERNAL STANDARD	20.942	544	325380	262.5

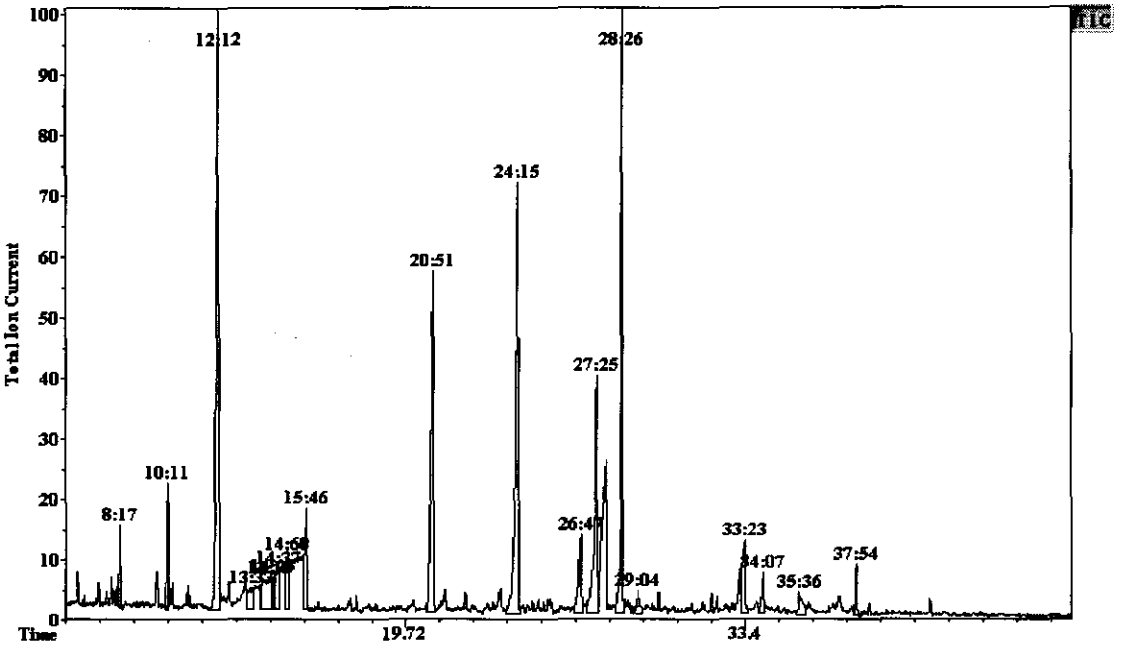

Figure A34: *GC-MS total ion chromatogram of the urine sample on day five after the intake of grapefruit juice of subject CF 7*

Table A34: Interpretation of GC-MS spectrum of urine sample of day five of subject CF 7.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	7.954	69	20150	23.85
2-HYDROXYISOBUTYRIC-DITMS	8.157	77	16444	19.44
GLYCOLIC-DITMS	8.305	82	83881	99.18
OXALIC-DITMS	9.78	136	50750	60.01
p-CRESOL-TMS	10.193	151	137454	162.52
3-HYDROXYISOBUTYRIC-DITMS	11.036	182	22290	26.36
UREA-TRITMS	12.213	225	293793	347.37
3-HYDROXY-ISO-VALERIC-DITMS	12.651	241	20905	24.71
BENZOIC-TMS	12.979	253	2835	3.35
PHOSPHORIC-TRITMS	14.996	327	10415	12.31
UREA-DITMS	15.77	355	117268	138.65
4-HYDROXY-2-METHYLVALERIC-DITMS	16.238	372	8149	9.64
4-DEOXYTETRONIC-TRITMS	17.519	419	7407	8.76
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.773	428	10281	12.16
PYROGLUTAMIC-DITMS	21.344	558	22359	26.44
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.162	588	9721	11.50
p-HYDROXYPHENYLACETIC-DITMS	24.258	665	308809	365.12
t-ACONITIC-TRITMS	26.801	758	50754	60.01
HIPPURIC-TMS	27.811	795	134639	159.19
CITRIC-TETRATMS	28.446	818	250888	296.64
VANILGLYCOL-TRITMS	29.098	842	13138	15.53

INDOLE 3 ACETIC ACID DITMS	29.916	872	20919	24.73
p-HYDROXYHIPPURIC-DITMS	34.138	1026	29647	35.05
18.80 min INTERNAL STANDARD	20.862	541	222014	262.5

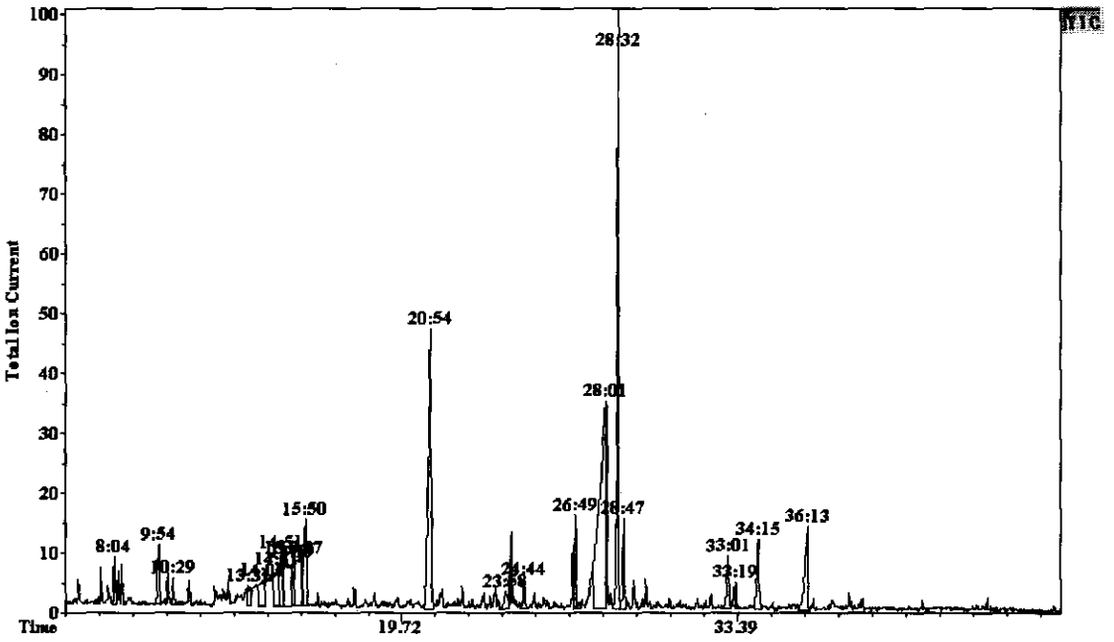

Figure A35: *GC-MS total ion chromatogram of the control sample of subject CF 5*

Table A35: Interpretation of GC-MS spectrum of the control sample of subject CF 5.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.083	74	41323	46.90
2-HYDROXYISOBUTYRIC-DITMS	8.28	81	30832	34.99
GLYCOLIC-DITMS	8.409	86	59335	67.35
OXALIC-DITMS	9.91	141	109980	124.83
p-CRESOL-TMS	10.274	154	40940	46.47
3-HYDROXYISOBUTYRIC-DITMS	11.142	186	28016	31.80
UREA-TRITMS	12.168	223	11094	12.59
3-HYDROXY-ISO-VALERIC-DITMS	12.726	244	16896	19.18
ETHYLMALONIC-DITMS	14.883	322	30068	34.13
PHOSPHORIC-TRITMS	15.094	330	9639	10.94
UREA-DITMS	15.846	358	40136	45.55
METHYLMALONIC-DITMS	15.862	358	112670	127.88
SUCCINIC-DITMS	15.882	359	107118	121.58

2-METHYLSUCCINIC-DITMS	16.325	375	16314	18.52
URACIL-DITMS	16.494	381	4007	4.55
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.842	431	16239	18.43
MALONIC-DITMS	18.646	460	7813	8.87
GLUTARIC-DITMS	18.654	460	8232	9.34
3-HYDROXYADIPYLLACTONE-TMS	19.587	494	5528	6.27
ADIPIC-DITMS	21.325	558	6286	7.14
PYROGLUTAMIC-DITMS	21.396	560	22213	25.21
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.209	590	15435	17.52
o-HYDROXYPHENYLACETIC-DITMS	22.636	606	5175	5.87
2-HYDROXYGLUTARIC-TRITMS	23.355	632	6267	7.11
3-HYDROXYPHENYLACETIC-DITMS	23.552	639	16951	19.24
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.976	655	12110	13.75
m-HYDROXYBENZOIC-DITMS	24.02	656	6471	7.35
2-FUROYLGLYCINE-TMS	24.083	659	4948	5.62
p-HYDROXYPHENYLACETIC-DITMS	24.22	664	72256	82.01
m-HYDROXYPHENYLPROPIONIC-DITMS	25.998	729	4001	4.54
VANILLIC-DITMS	26.742	756	18376	20.86
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	26.825	759	47118	53.48
t-ACONITIC-TRITMS	26.833	759	57233	64.96
c-ACONITIC-TRITMS	26.847	760	38429	43.62
p-HYDROXYMANDELIC-TRITMS	27.421	781	6932	7.87
HIPPURIC-TMS	28.105	806	189215	214.76
3,4-DIHYDROXYPHENYLACETIC-TRITMS	28.25	811	11158	12.66
CITRIC-TETRATMS	28.549	822	320235	363.46
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.795	831	61462	69.76
VANILGLYCOLIC-TRITMS	29.183	845	23385	26.54
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	29.547	858	6253	7.10
ISOVANILHYDRACRYLIC-TRITMS	31.064	914	10749	12.20
p-HYDROXYHIPPURIC-DITMS	34.262	1031	84439	95.84
STEARIC-TMS	34.817	1051	3778	4.29
DIOCTYLPHTALATE-TMS	38.484	1185	13098	14.87
18.80 min INTERNAL STANDARD	20.934	544	231280	262.5

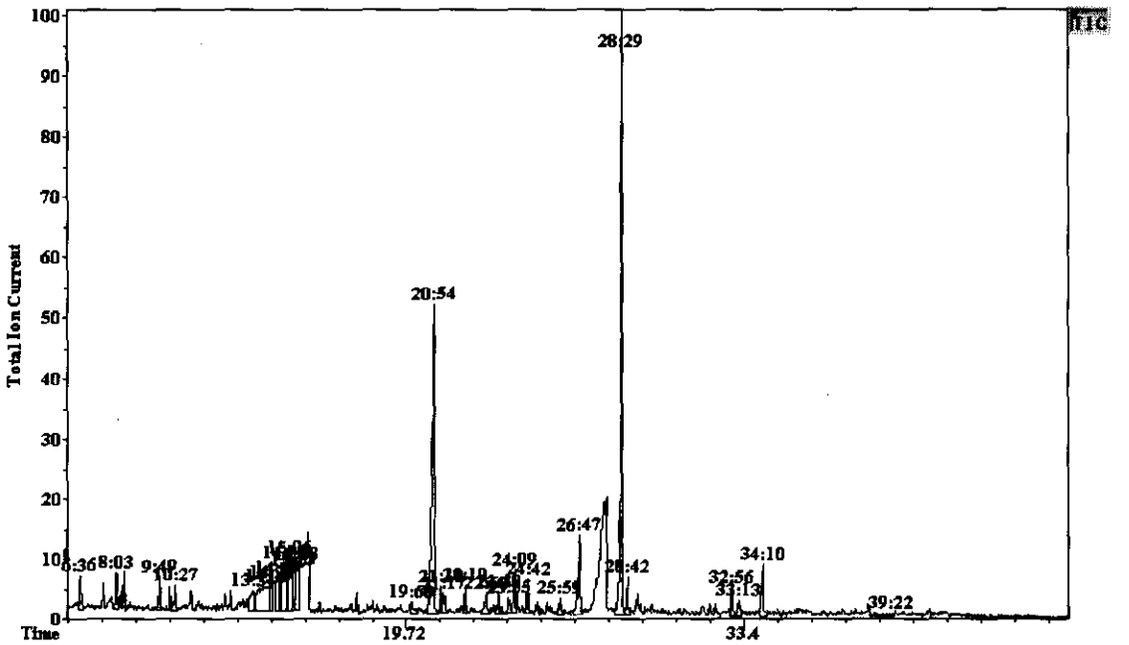

Figure A36 *GC-MS total ion chromatogram of the urine sample on day one after the intake of grapefruit juice of subject CF 5*

Table A36: Interpretation of GC-MS spectrum of urine sample of day one of subject CF 5.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.079	74	29615	35.34
2-HYDROXYISOBUTYRIC-DITMS	8.275	81	16898	20.17
GLYCOLIC-DITMS	8.407	86	50606	60.40
OXALIC-DITMS	9.838	138	51701	61.70
p-CRESOL-TMS	10.248	153	33958	40.53
3-HYDROXYISOBUTYRIC-DITMS	11.105	184	19594	23.38
3-HYDROXY-ISO-VALERIC-DITMS	12.689	242	14625	17.45
ETHYLMALONIC-DITMS	14.841	321	22994	27.44
PHOSPHORIC-TRITMS	15.112	331	16816	20.07
UREA-DITMS	15.807	356	54347	64.86
SUCCINIC-DITMS	15.848	358	95244	113.67
4-HYDROXY-2-METHYLVALERIC-DITMS	16.286	374	11384	13.59
4-DEOXYTETRONIC-TRITMS	17.555	420	6443	7.69
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.809	429	14287	17.05
LACTYLLACTATE-TMS	18.433	452	10579	12.63
PIPECOLIC-DITMS	21.374	560	24367	29.08
PYROGLUTAMIC-DITMS	21.374	560	24818	29.62
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.181	589	15165	18.10
3-HYDROXYPHENYLACETIC-DITMS	23.515	638	13241	15.80
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.928	653	8944	10.67
m-HYDROXYBENZOIC-DITMS	23.988	655	4152	4.96

p-HYDROXYPHENYLACETIC-DITMS	24.166	662	38908	46.43
FURAN-2,5-DICARBOXYLIC-DITMS	24.284	666	17366	20.73
1,6-ANHYDRO-B-d-GLUCOPYRANOSE-TRITMS	26.025	730	11198	13.36
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	26.03	730	10879	12.98
t-ACONITIC-TRITMS	26.799	758	54400	64.92
p-HYDROXYMANDELIC-TRITMS	27.387	779	5362	6.40
HIPPURIC-TMS	27.858	797	121265	144.72
CITRIC-TETRATMS	28.493	820	340177	405.98
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.72	828	22343	26.67
VANILGLYCOLIC-TRITMS	29.128	843	15424	18.41
ISOVANILGLYCOLIC-TRITMS	29.128	843	15839	18.90
ISOVANILHYDRACRYLIC-TRITMS	31.032	913	4454	5.32
PALMITIC-TMS	31.763	939	4543	5.42
ISOFERULIC-DITMS	32.269	958	2716	3.24
p-HYDROXYHIPPURIC-DITMS	32.95	983	16399	19.57
18.80 min INTERNAL STANDARD	20.909	543	219955	262.5

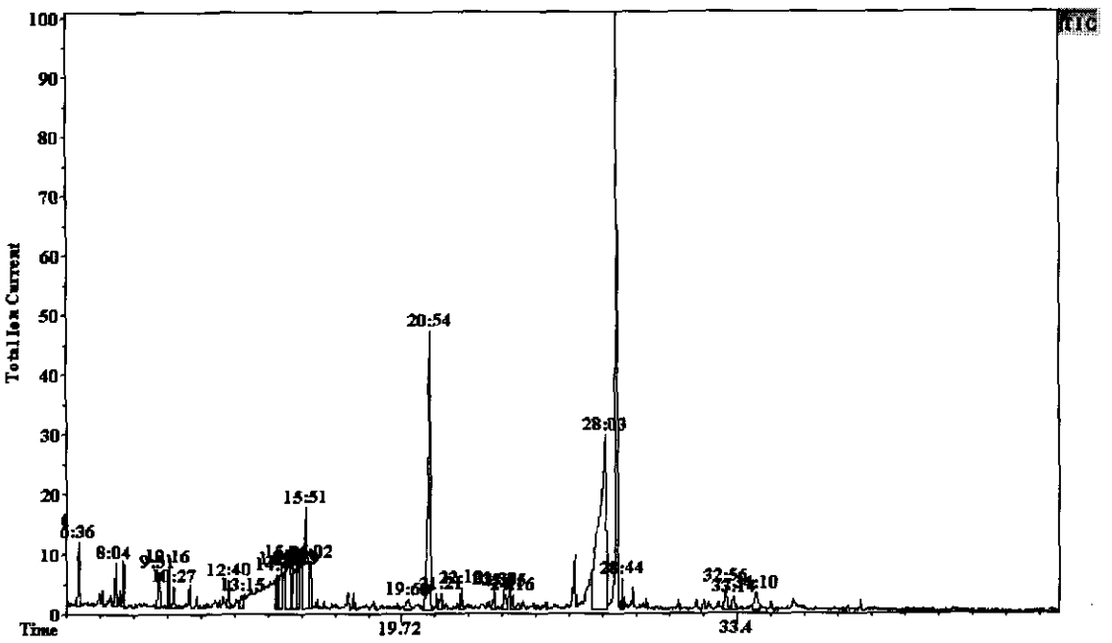

Figure A37: *GC-MS total ion chromatogram of the urine sample on day three after the intake of grapefruit juice of subject CF 5*

Table A37: Interpretation of GC-MS spectrum of urine sample of day three of subject CF 5.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.094	74	39379	38.01
2-HYDROXYISOBUTYRIC-DITMS	8.285	81	17318	16.72
GLYCOLIC-DITMS	8.428	86	77419	74.72
OXALIC-DITMS	9.886	140	76141	73.49
p-CRESOL-TMS	10.272	154	69866	67.43
3-HYDROXYISOBUTYRIC-DITMS	11.123	185	32710	31.57
a-RESORCYLIC-TRITMS	11.394	195	6084	5.87
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.338	229	6510	6.28
3-HYDROXY-ISO-VALERIC-DITMS	12.704	243	31633	30.53
BENZOIC-TMS	13.033	254	4289	4.14
ETHYLMALONIC-DITMS	14.841	321	20207	19.50
PHOSPHORIC-TRITMS	15.099	331	16240	15.68
SUCCINIC-DITMS	15.859	358	95688	92.36
UREA-DITMS	16.04	365	70490	68.04
4-HYDROXY-2-METHYLVALERIC-DITMS	16.294	374	11734	11.33
4-DEOXYTETRONIC-TRITMS	17.556	420	15779	15.23
PYROGLUTAMIC-DITMS	21.378	560	23472	22.66
PIPECOLIC-DITMS	21.38	560	25096	24.22
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.184	589	15969	15.41
3-HYDROXYPHENYLACETIC-DITMS	23.519	638	15912	15.36
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.929	653	14707	14.20
m-HYDROXYBENZOIC-DITMS	23.987	655	6483	6.26
p-HYDROXYPHENYLACETIC-DITMS	24.17	662	36946	35.66
FURAN-2,5-DICARBOXYLIC-DITMS	24.282	666	16679	16.10
t-ACONITIC-TRITMS	26.804	758	42444	40.97
p-HYDROXYMANDELIC-TRITMS	27.398	780	8351	8.06
HIPPURIC-TMS	28.06	804	199476	192.53
CITRIC-TETRATMS	28.526	821	525981	507.67
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.75	829	26414	25.49
VANILGLYCOLIC-TRITMS	29.155	844	16651	16.07
ISOVANILHYDRACRYLIC-TRITMS	31.036	913	11820	11.41
VANYLHYDRACRYLIC-TRITMS	31.039	913	13362	12.90
PALMITIC-TMS	31.75	939	6864	6.63
FERULIC-DITMS	32.27	958	2241	2.16
p-HYDROXYHIPPURIC-DITMS	34.182	1028	20964	20.23
STEARIC-TMS	34.792	1050	4884	4.71
DIOCTYLPHTALATE-TMS	38.453	1184	17404	16.80
18.80 min INTERNAL STANDARD	20.915	543	271970	262.5

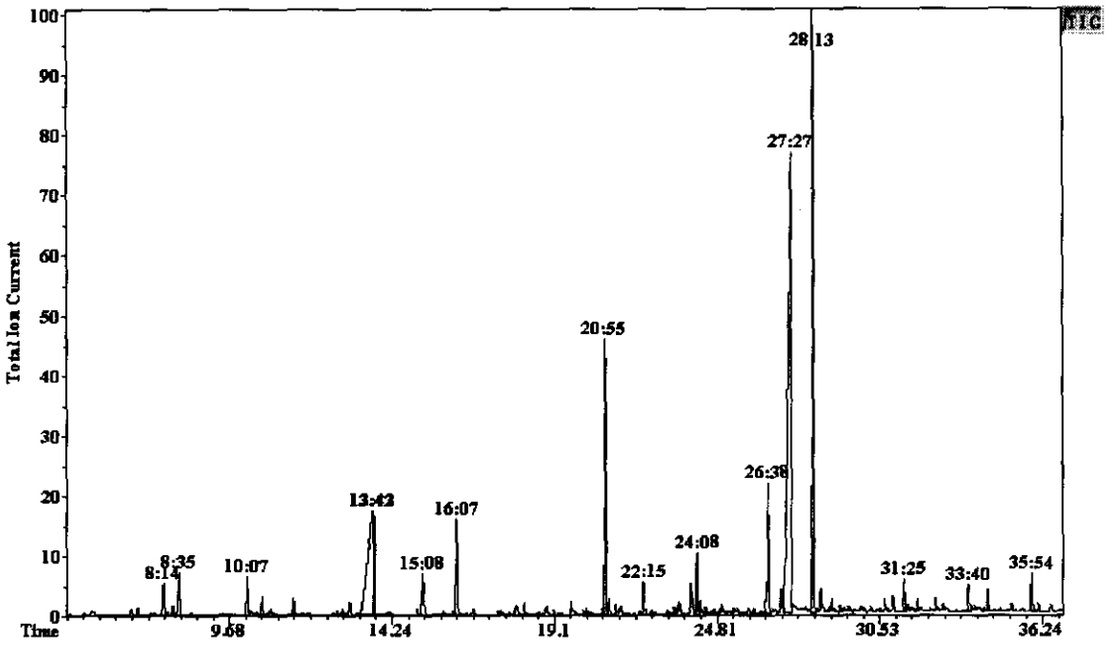

Figure A38: *GC-MS total ion chromatogram of the urine sample on day five after the intake of grapefruit juice of subject CF 5*

Table A38: Interpretation of GC-MS spectrum of urine sample of day five of subject CF 5.

Name	R.T.	Scan	Tot.Signal	mg/g creat
1,2-DIHYDROXYPROPANE-DITMS	6.665	159	56603	2.31
2,3-DIHYDROXYBUTANE-DITMS	7.963	521	67574	2.76
LACTIC-DITMS	8.236	597	787159	32.10
2-HYDROXYISOBUTYRIC-DITMS	8.447	657	204442	8.34
GLYCOLIC-DITMS	8.584	694	1449924	59.12
1,2-DIHYDROXYBUTANE-DITMS	9.54	961	55416	2.26
OXALIC-DITMS	10.116	1113	1453406	59.27
2-HYDROXYBUTYRIC-DITMS	10.398	1173	39908	1.63
p-CRESOL-TMS	10.543	1204	666933	27.20
2-HYDROXY-3-METHYLBUTYRIC-DITMS	10.722	1243	42341	1.73
3-HYDROXYPROPIONIC-DITMS	10.787	1257	196550	8.05
3-HYDROXYISOBUTYRIC-DITMS	11.444	1398	481043	19.62
4-HYDROXYBUTANOIC-TRITMS	11.447	1399	513958	20.96
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.694	1667	73819	3.01
3-HYDROXY-ISO-VALERIC-DITMS	13.055	1745	257318	10.49
BENZOIC-TMS	13.392	1817	29511	1.20
UREA-DITMS	13.695	1882	643716	26.25
PHOSPHORIC-TRITMS	15.13	2191	1687676	68.82
ETHYLMALONIC-DITMS	15.176	2201	313528	12.79
GLYCERIN-TRITMS	15.732	2321	62431	2.55
SUCCINIC-DITMS	16.107	2401	3526643	143.81

4-HYDROXY-2-METHYLVALERIC-DITMS	16.592	2506	176482	7.20
GLYCERIC-TRITMS	17.288	2655	79494	3.24
FUMARIC-DITMS	17.369	2673	169754	6.92
NONANOIC-TMS	17.589	2720	24104	0.98
METHYLMALONIC-DITMS	17.79	2763	148240	6.05
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	18.068	2819	243358	9.92
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	18.655	2921	23003	0.94
HYDROCHINON-DITMS	18.837	2953	75910	3.10
GLUTARIC-DITMS	18.847	2955	113678	4.64
CITRAMALIC-TRITMS	21.033	3338	288360	11.76
ADIPIC-DITMS	21.395	3401	68091	2.78
PIPECOLIC-DITMS	21.448	3410	228498	9.32
MALIC-TRITMS	21.467	3414	145495	5.93
ACETYLTRHEONINE-DITMS	21.915	3492	28821	1.18
3-METHYLADIPIC-DITMS	22.102	3525	44129	1.80
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.247	3550	627536	25.59
o-HYDROXYPHENYLACETIC-DITMS	22.658	3622	39268	1.60
2-HYDROXYGLUTARIC-TRITMS	23.334	3740	89643	3.66
ERYTHRONIC-TETRATMS	23.457	3762	158767	6.47
3-HYDROXYPHENYLACETIC-DITMS	23.524	3773	260006	10.60
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.913	3841	425032	17.33
m-HYDROXYBENZOIC-DITMS	23.972	3852	252793	10.31
p-HYDROXYPHENYLACETIC-DITMS	24.127	3879	1525514	62.21
FURAN-2,5-DICARBOXYLIC-DITMS	24.24	3899	384539	15.68
ACETYLASPARTIC-DITMS	24.432	3932	97711	3.98
LAURIC-DITMS	24.579	3958	30066	1.23
TARTARIC-TETRATMS	24.917	4017	84670	3.45
ISOCITRICLACTON-DITMS	25.003	4032	154263	6.29
3-METHYLPIMELIC-DITMS	25.418	4105	60432	2.46
QUINOLINIC-DITMS	25.547	4127	66225	2.70
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	25.926	4194	75285	3.07
PROPANETRICARBOXYLIC-TRITMS	26.309	4261	19829	0.81
VANILLIC-DITMS	26.547	4303	117691	4.80
3-HYDROXY-4-METHOXYBENZOIC-DITMS	26.547	4303	120623	4.92
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	26.625	4316	237401	9.68
c-ACONITIC-TRITMS	26.633	4317	2174585	88.67
p-HYDROXYMANDELIC-TRITMS	27.208	4418	854297	34.84
HIPPURIC-TMS	27.446	4460	7897800	322.05
3,4-DIHYDROXYPHENYLACETIC-TRITMS	27.938	4546	60636	2.47
CITRIC-TETRATMS	28.221	4595	7742424	315.71
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.475	4640	504868	20.59
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	28.776	4692	70476	2.87
VANILGLYCOLIC-TRITMS	28.877	4710	491513	20.04
p-HYDROXYPHENYLLACTIC-TRITMS	29.251	4775	52223	2.13
PENTADECANOIC-TMS	29.843	4879	30584	1.25
VANYLHYDRACRYLIC-TRITMS	30.723	5033	517792	21.11
1,9-DIMETHYLURIC-DITMS	31.027	5086	630068	25.69
PALMITIC-TMS	31.412	5154	590821	24.09
ISOFERULIC-DITMS	31.656	5196	23459	0.96
FERULIC-DITMS	31.882	5236	192792	7.86
CAFFEIC-TRITMS	32.872	5409	39791	1.62
p-HYDROXYHIPPURIC-DITMS	33.662	5547	706955	28.83
T-9-OCTADECENOIC-TMS	34.009	5608	36468	1.49
OCTADECENOIC-TMS	34.009	5608	38791	1.58
STEARIC-TMS	34.325	5664	414510	16.90
HEXACOSANE-TMS	35.185	5813	100644	4.10
DEHYDROABIETIC ACID	35.891	5937	1339283	54.61

18.80 min INTERNAL STANDARD	20.913	3317	6437487	262.5

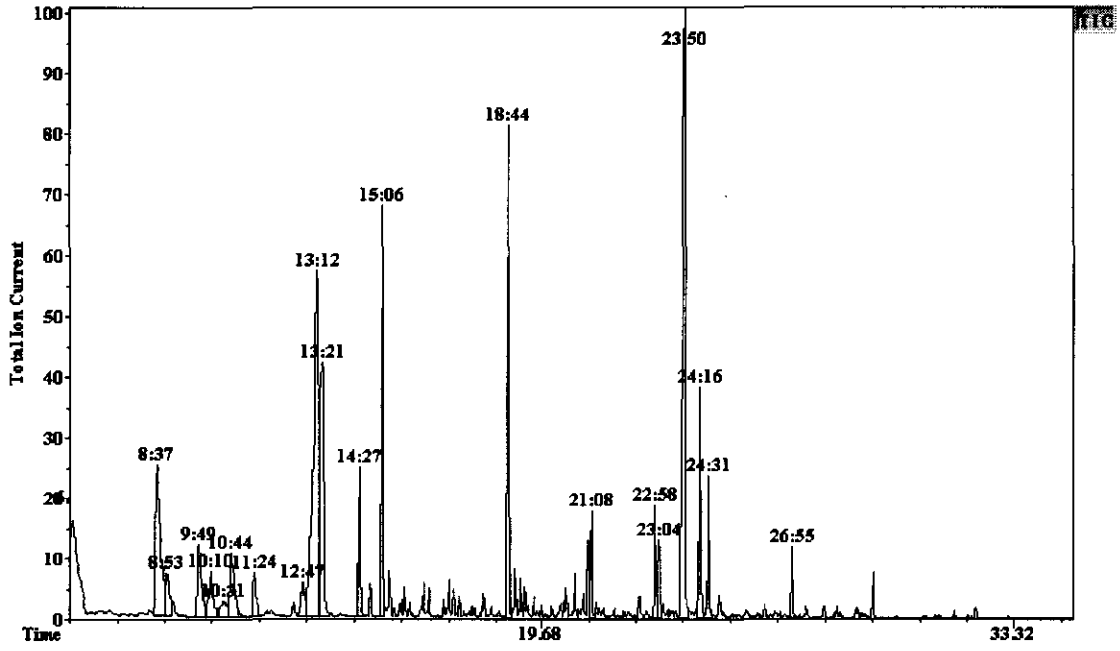

Figure A39: *GC-MS total ion chromatogram of the control sample of subject CM 7*

Table A39: Interpretation of GC-MS spectrum of the control sample of subject CM 7.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.183	4	49337	40.75
LACTIC-DITMS	8.631	94	111438	92.04
2,3-DIHYDROXYBUTANE-DITMS	8.631	94	93200	76.98
GLYCOLIC-DITMS	8.909	104	29399	24.28
1,2-DIHYDROXYBUTANE-DITMS	9.823	138	57669	47.63
p-CRESOL-TMS	10.764	172	38435	31.74
3-HYDROXYPROPIONIC-DITMS	10.844	175	4733	3.91
2-HYDROXY-ISO-VALERIC-DITMS	10.844	175	4723	3.90
3-HYDROXYISOBUTYRIC-DITMS	11.416	196	22634	18.69
3-HYDROXY-ISO-VALERIC-DITMS	12.827	248	22259	18.38
BENZOIC-TMS	13.22	262	66940	55.29
UREA-DITMS	13.233	263	146127	120.69
PHOSPHORIC-TRITMS	14.464	308	113958	94.12
GLYCERIN-TRITMS	14.775	319	23013	19.01
SUCCINIC-DITMS	15.118	332	302630	249.95
1,2-DIHYDROXYBENZENE-DITMS	15.321	339	64832	53.55
4-HYDROXY-2-METHYLVALERIC-DITMS	15.462	345	4030	3.33
URACIL-DITMS	15.615	350	3124	2.58
ITACONIC-DITMS	15.752	355	18693	15.44
GLYCERIC-TRITMS	15.891	360	11754	9.71
4-DEOXYTETRONIC-TRITMS	16.294	375	25392	20.97

THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.458	381	21607	17.85
GLUTARIC-DITMS	17.031	402	15594	12.88
HYDROCHINON-DITMS	17.112	405	1385	1.14
3-METHYLGLUTACONIC-DITMS	17.713	427	2613	2.16
3-HYDROXYADIPYLLACTONE-TMS	17.762	429	8100	6.69
P-AMINOBEMZOIC-TMS	18.258	447	1910	1.58
PYROGLUTAMIC-DITMS	19.104	478	25549	21.10
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	19.227	483	6390	5.28
3-METHYLADIPIC-DITMS	19.486	492	8907	7.36
MANNONIC-1,4-LACTONE-TMS	19.704	500	7811	6.45
TREONIC-TETRATMS	20.217	519	720	0.60
ERYTHRONIC-TETRATMS	20.217	519	742	0.61
2-HYDROXYGLUTARIC-TRITMS	20.386	525	14743	12.18
3-HYDROXYPHENYLACETIC-DITMS	20.662	535	43090	35.59
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.902	544	13801	11.40
m-HYDROXYBENZOIC-DITMS	21.041	549	35608	29.41
p-HYDROXYPHENYLACETIC-DITMS	21.144	553	119968	99.08
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	22.533	604	16909	13.97
t-ACONITIC-TRITMS	22.978	620	74339	61.40
VANILLIC-DITMS	23.062	623	1382	1.14
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.087	624	21830	18.03
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.114	625	7511	6.20
p-HYDROXYMANDELIC-TRITMS	23.494	639	3858	3.19
HIPPURIC-TMS	23.84	652	396157	327.19
CITRIC-TETRATMS	24.282	668	154311	127.45
MYRISTIC-TMS	24.343	670	5342	4.41
RIBOSE-PENTATMS(i)	24.471	675	4877	4.03
PYRUVIC ACID (Q)-TMS	24.471	675	4090	3.38
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	24.531	677	51297	42.37
VANILGLYCOLIC-TRITMS	24.831	688	9310	7.69
VANILLYLMANDELIC-TRITMS	24.85	689	18239	15.06
FUCOSE-PENTATMS(iii)	25.57	715	1165	0.96
PALMITIC-TMS	26.929	765	26708	22.06
p-HYDROXYHIPPURIC-DITMS	27.856	799	6741	5.57
HEXACOSANE-TMS	28.841	835	4584	3.79
DIOCTYLPHTALATE	-TMS32.22	959	4929	4.07
18.80 min INTERNAL STANDARD	18.749	465	317830	262.5

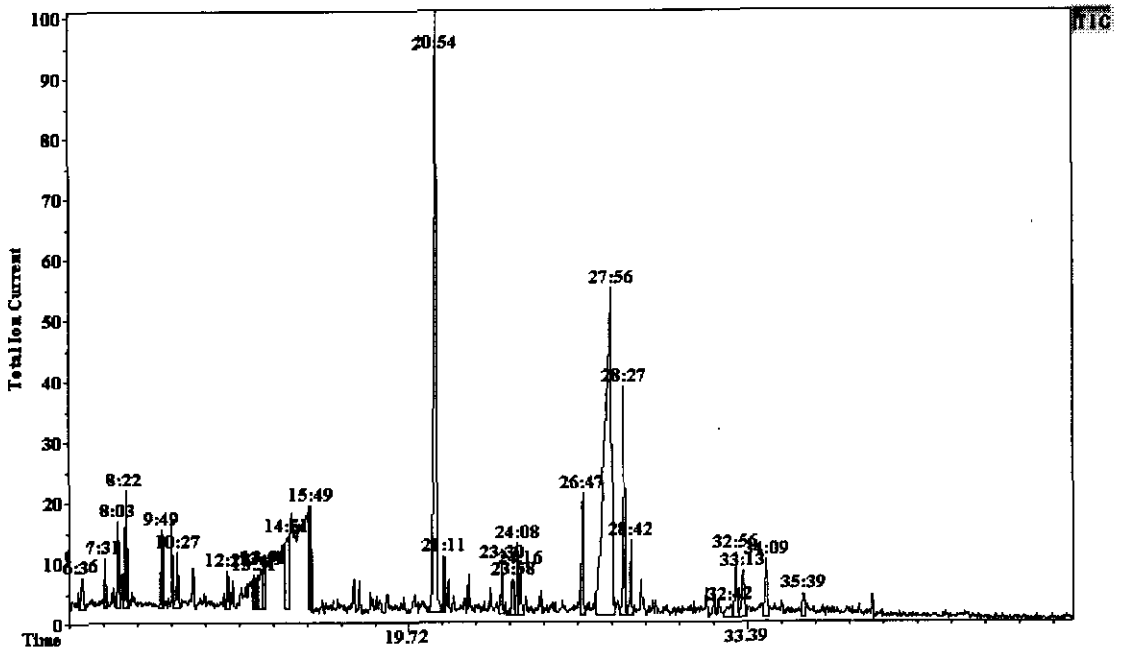

Figure A40: *GC-MS total ion chromatogram of the urine sample on day one after the intake of grapefruit juice of subject CM 7*

Table A40: Interpretation of GC-MS spectrum of urine sample of day one of subject CM 7.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS2	8.065	73	40929	43.13
2-HYDROXYISOBUTYRIC-DITMS	8.26	80	12801	13.49
GLYCOLIC-DITMS	8.408	86	93268	98.29
OXALIC-DITMS	9.855	139	63858	67.30
p-CRESOL-TMS	10.249	153	58390	61.54
3-HYDROXYISOBUTYRIC-DITMS	11.102	184	22494	23.71
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.331	229	5631	5.93
3-HYDROXY-ISO-VALERIC-DITMS	12.69	242	15714	16.56
BENZOIC-TMS	13.018	254	7818	8.24
PHOSPHORIC-TRITMS	15.087	330	14191	14.96
UREA-DITMS	15.744	354	41903	44.16
SUCCINIC-DITMS	15.828	357	80638	84.98
ETHYLMALONIC-DITMS	16.276	373	6802	7.17
4-DEOXYTETRONIC-TRITMS	17.554	420	12185	12.84
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.805	429	10805	11.39
PHENOXYACETATE	18.486	454	3925	4.14
PYROGLUTAMIC-DITMS	21.378	560	28110	29.62
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	21.556	566	3315	3.49
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.182	589	12665	13.35
3-HYDROXYPHENYLACETIC-DITMS	23.52	638	21144	22.28
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.93	653	9114	9.61

m-HYDROXYBENZOIC-DITMS	23.991	655	8758	9.23
p-HYDROXYPHENYLACETIC-DITMS	24.169	662	12070	12.72
FURAN-2,5-DICARBOXYLIC-DITMS	24.281	666	26350	27.77
ISOCITRICLACTON-DITMS	25.099	696	6315	6.66
m-HYDROXYPHENYLPROPIONIC-DITMS	25.961	727	3869	4.08
t-ACONITIC-TRITMS	26.798	758	45894	48.37
p-HYDROXYMANDELIC-TRITMS	27.386	779	5805	6.12
HIPPURIC-TMS	27.941	800	168228	177.29
CITRIC-TETRATMS	28.464	819	69469	73.21
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.721	828	24808	26.15
VANILGLYCOLIC-TRITMS	29.134	843	12649	13.33
ISOFERULIC-DITMS	32.254	957	2917	3.07
m-HYDROXYHIPPURIC-DITMS	32.957	983	17786	18.74
p-HYDROXYHIPPURIC-DITMS	34.155	1027	30846	32.51
DIOCTYLPHTALATE	38.454	1184	13078	13.78
18.80 min INTERNAL STANDARD	20.91	543	249081	262.5

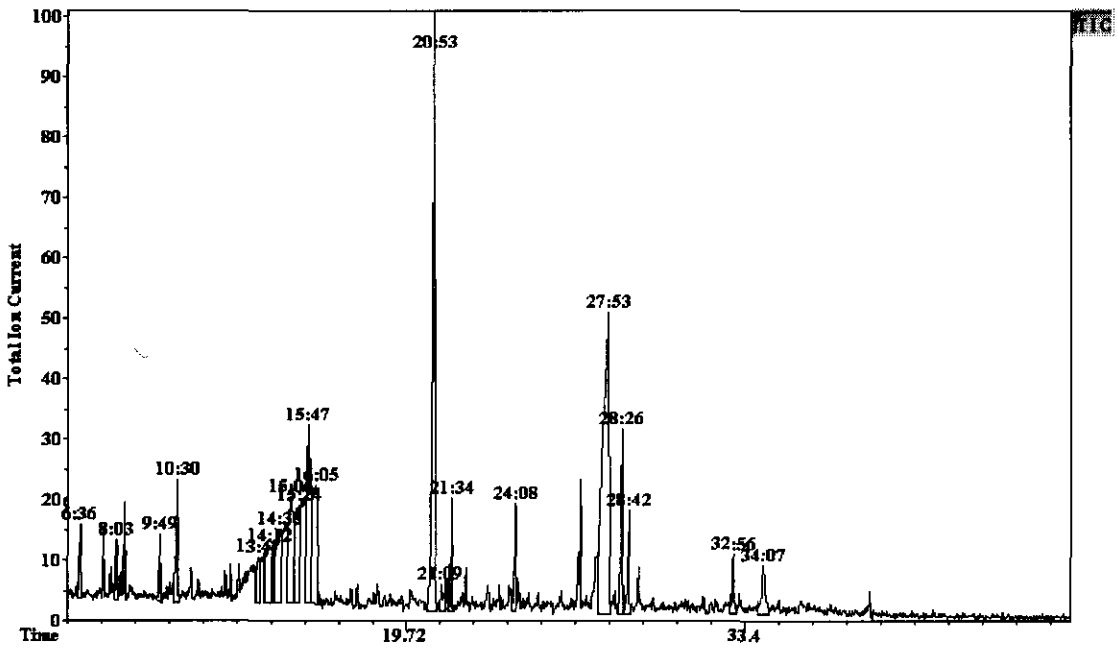

Figure A41: *GC-MS total ion chromatogram of the urine sample on day three after the intake of grapefruit juice of subject CM 7*

Table A41: Interpretation of GC-MS spectrum of urine sample of day three of subject CM 7.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.068	73	19984	24.02
2-HYDROXYISOBUTYRIC-DITMS	8.273	81	9636	11.58
GLYCOLIC-DITMS	8.41	86	63803	76.70
OXALIC-DITMS	9.837	138	47639	57.27
3-HYDROXYISOBUTYRIC-DITMS	11.106	184	14488	17.42
3-HYDROXY-ISO-VALERIC-DITMS	12.693	242	13877	16.68
BENZOIC-TMS	13.025	254	15092	18.14
PHOSPHORIC-TRITMS	15.116	331	10872	13.07
SUCCINIC-DITMS	15.824	357	65317	78.52
UREA-DITMS	16.152	369	73139	87.93
NORDAZEPAM-TMS	17.154	405	3100	3.73
ERYTHRO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.55	420	6732	8.09
4-DEOXYTETRONIC-TRITMS	17.802	429	7852	9.44
GLUTARIC-DITMS	18.622	459	6222	7.48
PYROGLUTAMIC-DITMS	21.374	560	20628	24.80
PIPECOLIC-DITMS	21.374	560	20246	24.34
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	21.577	567	19638	23.61
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.178	589	12375	14.88
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.929	653	5586	6.72
m-HYDROXYBENZOIC-DITMS	23.984	655	4042	4.86
p-HYDROXYPHENYLACETIC-DITMS	24.165	662	42894	51.57
FURAN-2,5-DICARBOXYLIC-DITMS	24.263	665	12355	14.85
HIPPURIC-TMS	27.888	798	151447	182.07
CITRIC-TETRATMS	28.44	818	52621	63.26
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.714	828	32747	39.37
VANILLYLMANDELIC ACID-TRITMS	29.127	843	13072	15.72
VANILGLYCOLIC-TRITMS	29.127	843	12630	15.18
VANILGLYCOL-TRITMS	31.023	912	5862	7.05
PALMITIC-TMS	31.745	939	3230	3.88
p-HYDROXYHIPPURIC-DITMS	34.149	1027	23726	28.52
DIOCTYLPHTALATE-TMS	38.451	1184	15335	18.44
18.80 min INTERNAL STANDARD	20.887	542	218355	262.5

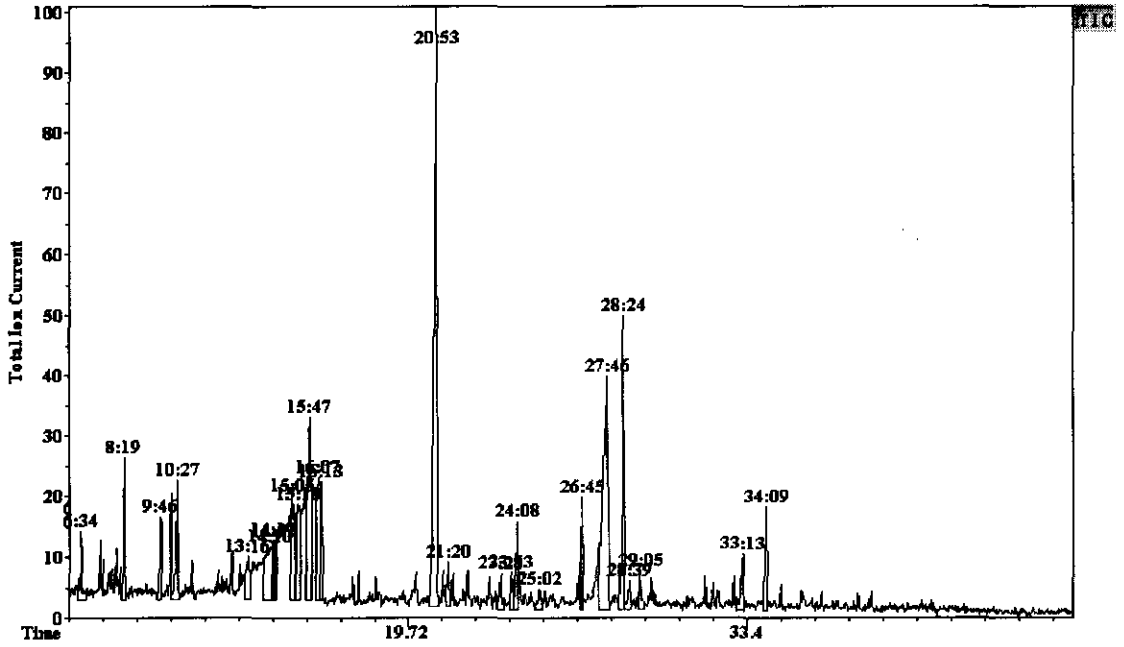

Figure A42: *GC-MS total ion chromatogram of the urine sample on day five after the intake of grapefruit juice of subject CM 7*

Table A42: Interpretation of GC-MS spectrum of urine sample of day five of subject CM 7.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	7.986	70	18721	19.98
2-HYDROXYISOBUTYRIC-DITMS	8.186	78	10633	11.35
GLYCOLIC-DITMS	8.334	83	87280	93.13
OXALIC-DITMS	9.802	137	69422	74.07
p-CRESOL-TMS	10.194	151	69185	73.82
3-HYDROXYISOBUTYRIC-DITMS	11.058	183	16762	17.89
UREA-TRITMS	12.093	220	5616	5.99
1,2-DIHYDROXYPROPANE-DITMS	12.29	228	4701	5.02
3-HYDROXY-ISO-VALERIC-DITMS	12.651	241	22078	23.56
BENZOIC-TMS	12.985	253	14409	15.38
PHOSPHORIC-TRITMS	15.092	330	11013	11.75
SUCCINIC-DITMS	15.801	356	60228	64.26
UREA-DITMS	16.257	373	73770	78.71
4-DEOXYTETRONIC-TRITMS	17.527	419	11423	12.19
PHENOXYACETATE	18.468	453	7437	7.94
PIPECOLIC-DITMS	21.357	559	28456	30.36
PYROGLUTAMIC-DITMS	21.357	559	28303	30.20
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	21.549	566	5002	5.34
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.162	588	10141	10.82
2-HYDROXYGLUTARIC-TRITMS	23.302	630	3388	3.62
3-HYDROXYPHENYLACETIC-DITMS	23.494	637	12838	13.70

3-HYDROXY-3-METHYLGLUTARIC-TRITMS	23.907	652	10639	11.35
m-HYDROXYBENZOIC-DITMS	23.967	654	6299	6.72
p-HYDROXYPHENYLACETIC-DITMS	24.142	661	42324	45.16
FURAN-2,5-DICARBOXYLIC-DITMS	24.254	665	14539	15.51
t-ACONITIC-TRITMS	26.771	757	38992	41.61
p-HYDROXYMANDELIC-TRITMS	27.365	779	4987	5.32
HIPPURIC-TMS	27.778	794	128469	137.08
CITRIC-TETRATMS	28.415	817	106167	113.28
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.667	826	6282	6.70
VANILLYLMANDELIC-TRITMS	29.1	842	12045	12.85
VANILGLYCOL-TRITMS	29.102	842	11245	12.00
PALMITIC-TMS	31.725	938	8218	8.77
ISOFERULIC-DITMS	32.242	957	2506	2.67
p-HYDROXYHIPPURIC-DITMS	34.154	1027	56082	59.84
STEARIC-TMS	34.767	1049	6512	6.95
DEHYDROABIETIC ACID	36.424	1110	7240	7.73
DIOCTYLPHTALATE-TMS	38.431	1183	11493	12.26
18.80 min INTERNAL STANDARD	20.887	542	246016	262.5

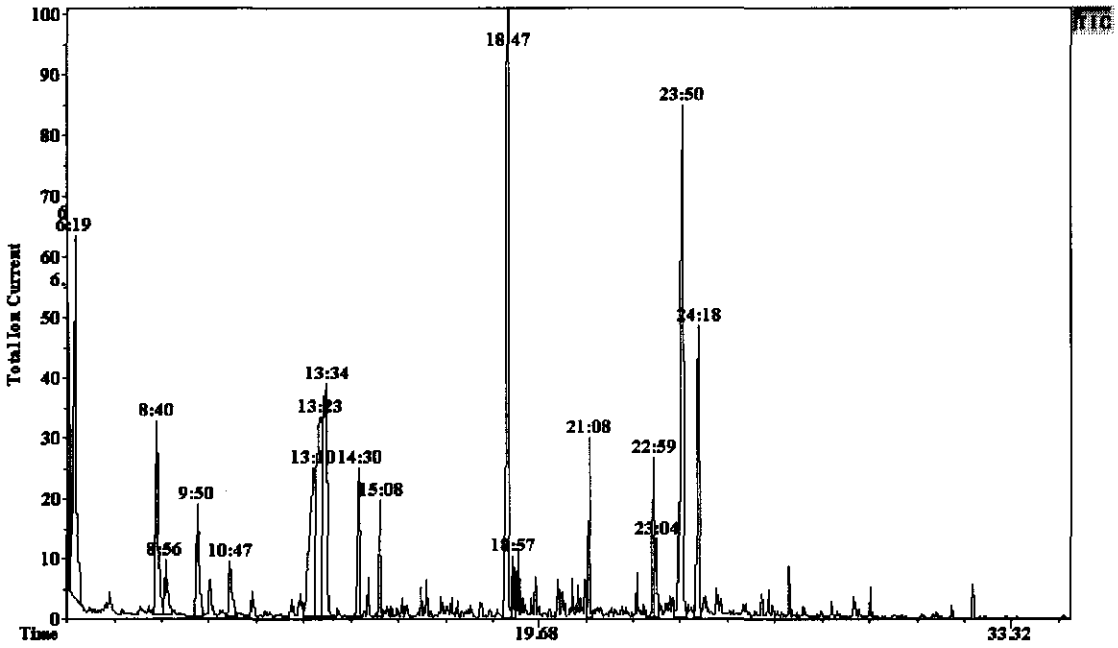

Figure A43: *GC-MS total ion chromatogram of the control sample of subject CM 11*

Table A43: Interpretation of GC-MS spectrum of the control sample of subject CM 11.

Name	R.T.	Scan	Tot.Signal	mg/g creat
BORIC ACID	6.334	10	486745	261.24
LACTIC-DITMS	8.684	96	171881	92.25
2-HYDROXYISOBUTYRIC-DITMS	8.903	104	9388	5.04
GLYCOLIC-DITMS	8.968	107	46483	24.95
1,2-DIHYDROXYBUTANE-DITMS	9.874	140	110660	59.39
OXALIC-DITMS	10.239	153	40738	21.86
p-CRESOL-tms	10.812	174	39318	21.10
2-HYDROXY-ISO-VALERIC-DITMS	10.889	177	3411	1.83
3-HYDROXYISOBUTYRIC-DITMS	11.454	198	14499	7.78
3-HYDROXY-ISO-VALERIC-DITMS	12.85	249	16329	8.76
UREA-DITMS	13.191	261	100168	53.76
BENZOIC-TMS	13.246	263	5770	3.10
ETHYLMALONIC-DITMS	14.48	308	9610	5.16
PHOSPHORIC-TRITMS	14.513	310	121359	65.13
GLYCERIN-TRITMS	14.799	320	32834	17.62
SUCCINIC-DITMS	15.14	333	124930	67.05
1,2-DIHYDROXYBENZENE-DITMS	15.342	340	21629	11.61
4-HYDROXY-2-METHYLVALERIC-DITMS	15.478	345	6380	3.42
URACIL-DITMS	15.642	351	3336	1.79
ITACONIC-DITMS	15.776	356	16476	8.84
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	16.483	382	36963	19.84
SERINE-TRITMS	16.587	386	3024	1.62
LACTYLLACTATE-TMS	16.851	395	2659	1.43
GLUTARIC-DITMS	17.061	403	4146	2.23
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	17.086	404	4683	2.51
HYDROCHINON-DITMS	17.135	406	972	0.52
3-METHYLGLUTACONIC-DITMS	17.738	428	4657	2.50
3-HYDROXYADIPYLLACTONE-TMS	17.787	430	8431	4.53
PYROGLUTAMIC-DITMS	19.132	479	57342	30.78
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	19.241	483	7962	4.27
3-METHYLADIPIC-DITMS	19.511	493	9391	5.04
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	19.628	497	25630	13.76
ERYTHRITOL-TMS	19.732	501	6911	3.71
2-HYDROXYGLUTARIC-TRITMS	20.411	526	16998	9.12
ERYTHRONIC-TETRATMS	20.466	528	9004	4.83
3-HYDROXYPHENYLACETIC-DITMS	20.684	536	48835	26.21
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	20.908	544	4301	2.31
m-HYDROXYBENZOIC-DITMS	21.064	550	12087	6.49
ISOCITRICLACTON-DITMS	21.917	581	5015	2.69
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	22.545	604	44034	23.63
t-ACONITIC-TRITMS	23.003	621	132552	71.14
VANILLIC-DITMS	23.085	624	2643	1.42
3-HYDROXY-4-METHOXYBENZOIC-DITMS	23.085	624	2477	1.33
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	23.109	625	83461	44.79
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	23.14	626	7457	4.00
p-HYDROXYMANDELIC-TRITMS	23.516	640	8929	4.79
AZELAIC-DITMS	23.587	642	5930	3.18
HIPPURIC-TMS	23.865	653	401644	215.56
a-RESORCYLIC-TRITMS	24.016	658	5502	2.95
CITRIC-TETRATMS	24.308	669	218149	117.08
LYXOSE-TETRATMS(iv)	24.48	675	14039	7.54

VANILGLYCOL-TRITMS	24.853	689	27224	14.61
VANILLYLMANDELIC-TRITMS	24.853	689	12787	6.86
MANNONIC-1,4-LACTONE	25.435	710	3126	1.68
VANYLHYDRACRYLIC-TRITMS	26.332	743	23384	12.55
FUCOSE-PENTATMS(iii)	26.719	757	4900	2.63
PALMITIC-TMS	26.938	765	24591	13.20
p-HYDROXYHIPPURIC-DITMS	28.818	834	11888	6.38
HEXACOSANE-TMS	31.082	917	3911	2.10
DIOCTYLPHTALATE-TMS	32.231	959	18525	9.94
18.80 min INTERNAL STANDARD	18.818	468	489100	262.5

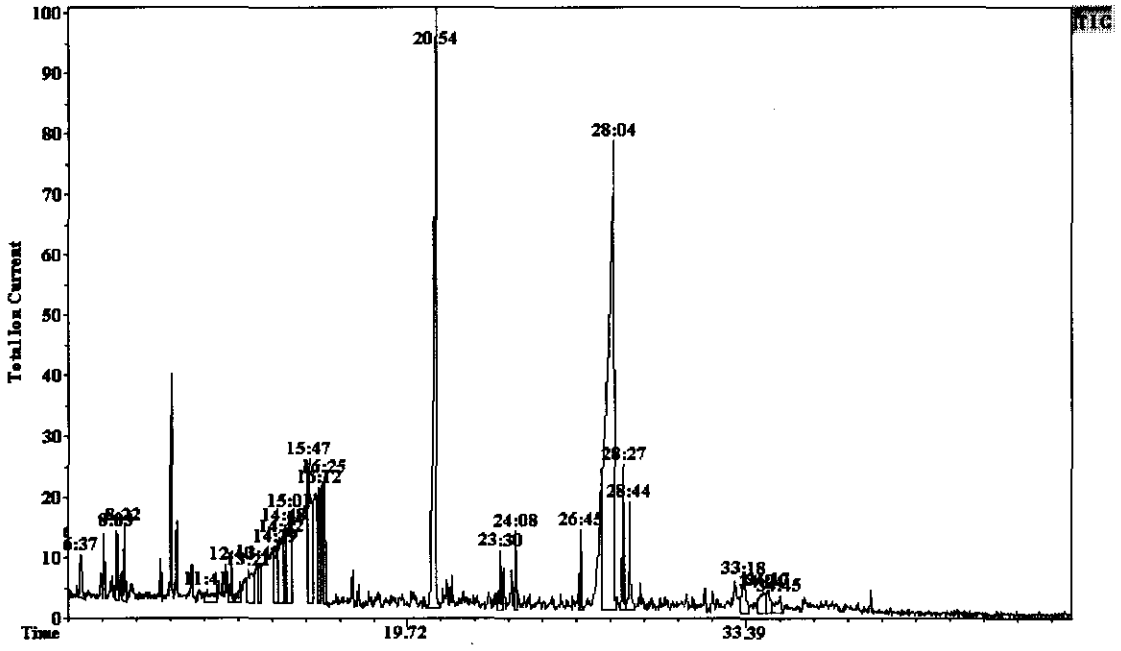

Figure A44: *GC-MS total ion chromatogram of the urine sample on day one after the intake of grapefruit juice of subject CM 11*

Table A44: Interpretation of GC-MS spectrum of urine sample of day one of subject CM 11.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.071	73	28313	30.15
2-HYDROXYISOBUTYRIC-DITMS	8.273	81	12032	12.81
GLYCOLIC-DITMS	8.394	85	44624	47.52
OXALIC-DITMS	9.839	138	29087	30.98
p-CRESOL-TMS	10.274	154	156484	166.64
3-HYDROXYISOBUTYRIC-DITMS	11.114	185	21561	22.96
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.33	229	6777	7.22
3-HYDROXY-ISO-VALERIC-DITMS	12.7	242	17260	18.38
BENZOIC-TMS	13.025	254	5346	5.69
PHOSPHORIC-TRITMS	15.05	328	8688	9.25
SUCCINIC-DITMS	15.802	356	36516	38.89
UREA-DITMS	16.451	380	78345	83.43
4-DEOXYTETRONIC-TRITMS	17.555	420	13835	14.73
ERYTHRO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.809	429	8499	9.05
3-METHYLGLUTACONIC-DITMS	19.566	493	3227	3.44
PYROGLUTAMIC-DITMS	21.368	559	19082	20.32
PIPECOLIC-DITMS	21.371	559	18525	19.73
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	21.557	566	5764	6.14
3-HYDROXYPHENYLACETIC-DITMS	23.521	638	24285	25.8
m-HYDROXYBENZOIC-DITMS	23.988	655	11625	12.38
p-HYDROXYPHENYLACETIC-DITMS	24.152	661	36723	39.10
FURAN-2,5-DICARBOXYLIC-DITMS	24.264	665	7103	7.56
m-HYDROXYPHENYLPROPIONIC-DITMS	25.957	727	4450	4.74
p-HYDROXYMANDELIC-TRITMS	27.383	779	5594	5.96
HIPPURIC-TMS	28.074	805	210440	224.10
CITRIC-TETRATMS	28.471	819	45810	48.78
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	28.742	829	36623	39.00
VANILGLYCOLIC-TRITMS	29.147	844	11901	12.67
ISOVANILHYDRACRYLIC-TRITMS	31.031	913	9069	9.66
p-HYDROXYHIPPURIC-DITMS	34.302	1032	6523	6.95
DIOCTYLPHTALATE-TMS	38.449	1184	15363	16.36
18.80 min INTERNAL STANDARD	20.911	543	246504	262.5

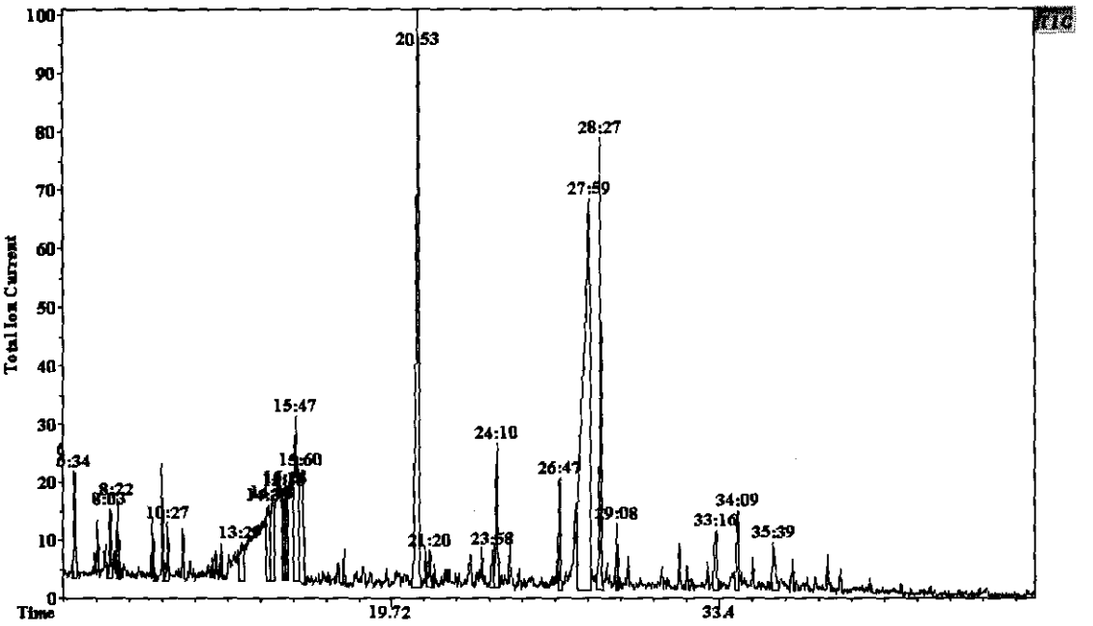

Figure A45: *GC-MS total ion chromatogram of the urine sample on day three after the intake of grapefruit juice of subject CM 11*

Table A45: Interpretation of GC-MS spectrum of urine sample of day three of subject CM 11.

Name	R.T.	Scan	Tot.Signal	mg/g creat
LACTIC-DITMS	8.073	74	25848	29.51
2-HYDROXYISOBUTYRIC-DITMS	8.273	81	11381	12.99
GLYCOLIC-DITMS	8.41	86	53558	61.15
OXALIC-DITMS	9.841	138	45460	51.90
p-CRESOL-TMS	10.252	153	74676	85.26
3-HYDROXYISOBUTYRIC-DITMS	11.114	184	24953	28.49
2-METHYL-3-HYDROXYBUTYRIC-DITMS	12.332	229	6287	7.18
3-HYDROXY-ISO-VALERIC-DITMS	12.688	242	17841	20.37
BENZOIC-TMS	13.025	254	9617	10.98
PHOSPHORIC-TRITMS	15.074	329	11657	13.31
SUCCINIC-DITMS	15.815	356	48268	55.11
UREA-DITMS	16.094	367	70167	80.11
4-DEOXYTETRONIC-TRITMS	17.555	420	9873	11.27
ERYTHRO-2,3-DIHYDROXYBUTYRIC-TRITMS	17.806	429	12847	14.67
HYDROCHINON-DITMS	18.592	458	4856	5.54
PYROGLUTAMIC-DITMS	21.377	560	25932	29.61
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	22.173	589	5102	5.83
3-HYDROXYPHENYLACETIC-DITMS	23.519	638	12328	14.08
m-HYDROXYBENZOIC-DITMS	23.989	655	13322	15.21
p-HYDROXYPHENYLACETIC-DITMS	24.176	662	65461	74.74
FURAN-2,5-DICARBOXYLIC-DITMS	24.274	666	3316	3.79
t-ACONITIC-TRITMS	26.797	758	37919	43.29