THE PRESENCE OF B-m-HYDROXYPHENYLHYDRACRYLIC ACID IN THE URINE OF PATIENTS WITH ADHD AND OTHER NEURODEGENERATIVE METABOLIC DISORDERS

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INDEX

ABBREVIATIONS

5-HIAA	5-Hydroxy-indole-acetic acid
5-HT	Serotonin
A	
A	Adrenaline
ABC	ATP-binding cassette
ADHD	Attention-Deficit Hyperactivity Disorder
AGHS	Aandag-gebreks-hiperaktiwiteit-sindroom
AP	Apical
ATP	Adenosine triphosphate
B	
BBB	Blood-brain barrier
BCH	2-aminobicyclo-(2,2,2)heptane-2-carboxylic acid
BCSFB	Blood-cerebrospinal fluid barrier
BH_4	Tetrahydrobiopterin
BHHA	β-*m*-hydroxyphenylhydracrylic acid
BHHS	β-*m*-hidroksifenielhidrakrielsuur
BL	Basolateral
BM	Basal membrane
BSTFA	N, O-bis-(trimethylsilyl) trifluoracetamide
C	
Ca	Calcium
CB	Conditioned blocking
Cl	Chloride
CNS	Central nervous system
COMT	Catechol-O-methyl-transferase
CYP3A4	Cytochrome P450 3A4
D	
D1	Dopamine 1 receptor

D2	Dopamine 2 receptor
DA	Dopamine
DMI	Desipramine
DOPAC	3,4-dihydroxyphenylacetaldehyde
DOPEG	3,4-dihydroxyphenylglycol
DSM-IV	Diagnostic and Statistical Manual for Mental Disorders, volume IV

G

GC-MS	Gas chromatography-Mass spectroscopy
GI	Gastro intestinal
GPR	G-protein-coupled receptor
GSH	Glutathione

H

HCl	Hydrochloric acid
HD	Hartnup's disease
HDL	High-density lipoprotein
Hg	Mercury
$HgCl_2$	Mercury chloride
HVA	Homovanillic acid

L

LI	Latent inhibition
L-Phe	L-Phenylalanine

M

MDR	Multi drug resistance
MeHg	Methylmercury
MHPG	3-methoxy-4-hydroxyphenylglycol
MAO A	Monoamine oxidase A
MAO B	Monoamine oxidase B
MPH	Methylphenidate
MRI	Magnetic resonance imaging
MRP	Multidrug resistance-associated protein
MS-MS	Mass spectroscopy- Mass spectroscopy

MVM	Microvillous membrane

N

Na	Sodium
NA	Noradrenaline

P

PCMBS	*p*-chloro-mercuriphenyl sulfonic acid
PEP	Phosphoenol pyruvate
PEPT1	Peptide transporter 1
PEPT2	Peptide transporter 2
PET	Positron emission tomography
P-gp	P-glycoprotein
Phe	Phenylalanine
Pi	Phosphate
PKU	Phenylketonuria

S

SPECT	Single photon emission computerized tomography

T

TMD	Transmembrane domain
TMS	Trimethylsilane
Tyr	Tyrosine

GLOSSARY

A

Absorption: Movement of materials across an epithelial layer from body cavity or component toward the blood.

Aggregate: A sum total of many heterogenous things taken together.

Agonist: A drug or substance having a specific cellular affinity that produces a predictable response similar to an endogenous substance.

Albumin: The most abundant protein component of blood.

Anaerobic: Anaerobic refers to an environment or a condition, which is free of oxygen or describes a micro organism which can grow in the absence of oxygen.

Antagonist: Any agent, such as a drug that exerts an opposite action to that of another or competes for the same receptor sites.

Antioxidant: Antioxidants protect key cell components by neutralizing the damaging effects of "free radicals," natural metabolites of cell metabolism. Free radicals form when oxygen is metabolized, or burned by the body. They travel through cells, disrupting the structure of other molecules, causing cellular damage. Such cell damage is believed to contribute to aging and various health problems.

Apical: Portion of plasma-membrane facing the lumen.

Aqueous: Literally, watery. Term is used to describe solutions of substances dissolved in water.

Astrocyte: A star-shaped cell, comparatively large which supports the nerve cells (neurons) of the brain and spinal cord.

Asymptomatic: Without obvious signs or symptoms of disease.

Attention deficit hyperactivity disorder: A lifelong developmental disorder that involves problems with attention span, impulse control, and activity level. Typical behaviours include: fidgeting or squirming; difficulty remaining seated when required; distractibility; difficulty waiting for turns in groups; difficulty staying on task with chores or play activities; difficulty playing quietly; excessive talking; inattention; and engaging in physically dangerous activities without considering the consequences.

B

Basolateral: Sides of epithelial cell facing away from the lumen, faces the blood side of the cell.

Bioconcentration: Entails the uptake and accumulation of chemical substances in the tissues of an organism through the food chain.

Blood-brain barrier: Group of cells that form a special, impermeable lining in the blood vessels of the brain. The blood-brain barrier is made up of astrocytes and prevents toxic substances in the blood from entering the brain.

Blood-cerebrospinal fluid barrier: A barrier located at the tight junctions who surround and connect the cuboidal epithelial cells on the surface of the choroid plexus; capillaries and connective tissue stroma of the choroid do not represent a barrier to protein tracers or dyes.

C

Caco-2 cell: Human intestinal villus tip cell.

Capillary: The smallest of the body's blood vessels. Oxygen and glucose pass through capillary walls and enter the cells. Waste products such as carbon dioxide pass back from the cells into the blood through capillaries.

Catecholamine: Any of various substances (as epinephrine, norepinephrine, and dopamine) that contain a benzene ring with two adjacent hydroxyl groups and a side chain of ethylamine and that function as hormones or neurotransmitters or both.

Chromosome: A structure of compact intertwined molecules of DNA found in the nucleus of cells. Chromosome contains the cell's genetic information. Humans normally have 46 chromosomes.

Conditioned blocking: Rapidly passing suppression of learning about a stimulus.

D

Depolarise: To deprive of polarity, to reduce to an unpolarised condition.

Derivatization: An analytical technique in which the surface organic group such as carbonyl, hydroxyl, and carboxyl are treated with selective organic reagents prior to analysis.

Distribution: The act of distributing or spreading or apportioning.

E

Endogenous: Originating within or produced by the body.

Enzyme: A protein that induces or accelerates a chemical reaction.

Epithelial: Having to do with the layer of cells that cover or line an external surface or cavity.

Exocytosis: A process of cellular secretion or excretion in which substances contained in vesicles are discharged from the cell by fusion of the vesicular membrane with the outer cell membrane.

Exogenous: Originating or produced outside the body.

External: Connected with the outside or an outer part; exterior.

Extracellular: Outside of the cell.

F

Fenestrae: Small openings closed by membranes.

G

Gene: The unit of heredity. A gene contains hereditary information encoded in the form of DNA and is located at a specific position on a chromosome in a cell's nucleus. Genes determine many aspects of anatomy and physiology by controlling the production of proteins. Each individual has a unique sequence of genes, or genetic code.

Globus pallidus: The smaller and more medial part of the lentiform nucleus of the brain, separated from the putamen by the medullary lamina and divided into external and internal portions closely connected to the striatum, thalamus and mesencephalon.

H

Hartnup's disease: A congenital metabolic disorder characterized by aminoaciduria, pellagra like, light-sensitive skin rash, and a temporary cerebellar ataxia.

Heterogenous: Consisting of elements that are not of the same kind or nature.

Hydrophilic: Literally, "water-loving"; polar or charged compounds that is soluble in water.

I

Inhibition: One of the responses caused by specific neurotransmitters binding to receptors on a neuron. Inhibition decreases the probability that neurotransmitters will be released by the neuron. Prevention/ blocking.

Intracellular: Inside of the cell.

K

K*m*: The Michaelis constant, the substrate concentration that produces half-maximal velocity.

L

Latent inhibition: Delay in reacting on a stimulus following pre-exposure to it.

Ligand: A molecule that binds to a receptor protein.

Lipase: An enzyme used to digest fats and remove greasy stains

Lipophilic: Literally means "fat-loving." A synthetic substance is lipophilic (attracted to fat) if it dissolves much more easily in lipid than it does in water. It is often difficult for an organism to excrete lipophilic substances, so they tend to accumulate in fatty tissues. It is also known as hydrophobic.
Luminal: Surface on the side of the lumen.

M

Metabolite: A metabolite is a substance that takes part in the process of metabolism, which involves the breakdown of complex organic constituents of the body with the liberation of energy for use in bodily functioning. The various compounds that take part in or are formed by these reactions are called metabolites.
Mucosa: The three layers of the gastro intestinal tract wall nearest to the lumen. The three layers are the epithelium, lamina propria and muscularis mucosa.

N

Neurotransmitter: Any one of numerous chemicals that modify or result in the transmission of nerve impulses between synapses.

P

Paracellular pathway: The space between adjacent cells of an epithelial through which some molecules diffuse as they cross the epithelium.
Parenchyma: The internal functional tissues of an organ, as opposed to supporting or structural tissues.
Parkinson's disease: A progressive and degenerative movement disorder with primary motor symptoms: rigidity (stiffness of the limbs and joints), bradykinesia/akinesia (slowness of movement/absence of movement), tremor (involuntary rhythmic shaking of a limb, the head, mouth or tongue; or the entire body), and postural instability (impaired balance and coordination). Results when dopaminergic cells in the substantia nigra degenerate, causing a loss of the chemical dopamine.
pH: a logarithmic scale used to describe the acidity or alkalinity of a solution. Water has a neutral pH of 7. A pH below 7 is acidic; a pH above 7 is alkaline (or basic).
Phenylketonuria: An inherited metabolic disorder in which there is a deficiency or the absence of the enzyme phenylalanine hydroxylase. This enzyme deficiency leads to high levels of the amino acid phenylalanine and low levels of tyrosine, causing mental retardation and other health problems. Dietary restriction of phenylalanine can reduce or eliminate these problems.

Phospholipid: A fatty compound that contains phosphate. Phospholipids make up much of the outer membranes of cells and organelles.

Psychosis: Any major mental disorder of organic or emotional origin characterized by a gross impairment in reality testing.

R

Rate limiting: Slowest process involved in a process.

Reactivity: Tendency of a substance to undergo chemical reaction with the release of energy.

Receptor: A sensory nerve ending that responds to various kinds of stimulation.

S

Signal detection: Noticing of a signal.

Submucosa: Connective-tissue layer under mucosa in gastro intestinal tract.

Synapse: The region surrounding the point of contact between two neurons or between a neuron and an effector organ, across which nerve impulses are transmitted through the action of a neurotransmitter.

T

Transcellular pathway: Crossing an epithelium by movement into an epithelial cell, diffusion through the cytosol of that cell, and exit across the opposite membrane.

Threshold: Membrane potential to which excitable membranes must be depolarized to initiate an action potential.

Toxin: Any poisonous substance that can cause disease.

V

Vesicle: A membrane-bound structure used to shuttle molecules within the membrane.

Villi: Fingerlike projections into the gut transit space.

X

Xenobiotic: A xenobiotic is a chemical which is not a natural component of the organism exposed to it. Synonyms: drug, foreign substance or compound, exogenous substance or compound.

UITTREKSEL

β-*m*-Hidroksifenielhidrakrielsuur (BHHS) is 'n aromatiese organiese suur wat dikwels in urine van pasiënte waargeneem word. Die diagnostiese waarde van die suur is tans onbekend. Vroeëre verslae het daarop gedui dat die verbinding waarskynlik bakterieel van oorsprong kan wees.

Met die doel om die diagnostiese waarde van BHHS te ondersoek is organiese suur GC-MS analises op meer as 1000 pasiënte uitgevoer. Die kreatinienwaarde vir elke urienmonster is bepaal en die volumes interne standaard en derivateringsagent wat bygevoeg moes word is met behulp daarvan bepaal sodat 'n konstante hoeveelheid kreatinien telkens geanaliseer is. Die konsentrasie BHHS is bepaal deur gebruik te maak van 'n interne standard, 3-fenielbottersuur. Negentien pasiënte met verhoogde BHHS vlakke se urien is verder ondersoek vir die teenwoordigheid van ander abnormale metaboliete.

Die konsentrasies van die BHHS het gewissel van spoorhoeveelhede tot meer as 1000 mmol/mol kreatinien. Ander metaboliete wat teen verhoogde konsentrasies teenwoordig was het ingesluit *p*-hidroksifenielasynsuur, *m*-hidroksihippuursuur, *p*-hidroksihippuursuur en *p*-hidroksifeniellaktaatsuur. Unieke metaboliete is ook geïdentifiseer in die urien en sluit in: fenielakrilielglisien, bensoïelsuksiensuur, fenielasetielglisien en β-*m*-hidroksifenielhidrakrielglisien. Verhoogde vlakke van fenielasynsuur en feniellaktaat is ook waargeneem in die urien van twee pasiënte. Kliniese simptome by die meeste pasiënte was nie akuut nie. Een pasiënt het 'n pankreasektomie ondergaan om onbekende redes. Aandag-gebreks-hiperaktiwiteit-sindroom (AGHS) en hiperaktiwiteit was die algemeenste simptoom waarneembaar in die kliniese profiele van die pasiënte. Omrede fenielasynsuur en fenielmelksuur (wat benewens BHHS by twee pasiënte aangetoon is) aanduidend van 'n fenielalanienmetaboliese defek kan wees, is 'n fenielalanienbelading op diè twee pasiënte uitgvoer. Die fenielalanienbelading het 'n verlaging in die absorpsie van fenielalanien getoon. Net soos fenielalanien is tirosien ook teen verlaagde hoeveelhede geabsorbeer. Die Phe belading is opgevolg met 'n triptofaan belading. Triptofaanabsorpsie was egter normaal wat die moontlikheid van Hartnup se siekte uitskakel. Aspartaambelading het egter wel tot verhoogde fenielalanienvlakke gelei wat daarop dui dat die peptiedabsorpsie normaal plaasgevind het.

Die metaboliete wat in die urien van die pasiënte gevind is dui op moontlike bakteriële oorsprong. Verbindings soos bensoïelsuksiensuur word slegs deur anaerobe mikro-organismes geproduseer. Die meeste van die ander metaboliete wat teen abnormale konsentrasies teenwoordig was word ook gesien as bakteriële metaboliete wat in uriene uitgeskei word as gevolg van wanvertering of wanabsorpsie. Een

van die pasiënte in die studie het dan ook 'n pankreasektomie ondergaan en wanvertering kan in diè geval verwag word.

Hierdie studie moet gesien word as 'n loodsstudie en baie meer navorsing sal nog gedoen moet word om 'n finale antwoord te kan gee. Voorlopig wil dit egter voorkom of BHHS uitgeskei word as gevolg van wanvertering of wanabsorpsie soos wat vermoed is. Dit wil egter voorkom of die verbinding 'n belangrike indikator kan wees van defekte wat tot hierdie abnormaliteite aanleiding kan gee.

ABSTRACT

β-m-Hydroxyphenylhydracrylic acid (BHHA) is an aromatic organic acid frequently detected in the urine of patients. The diagnostic value of this acid is as yet unknown. Previous reports indicated that BHHA may be of bacterial origin.

With the aim to establish the diagnostic value of BHHA organic acid GC-MS analyses were conducted on more than a thousand patients. The creatinine value for each urine sample was determined and the volumes of acid and derivatization reagent adjusted accordingly to facilitate the analysis of constant amounts of creatinine. The concentration of BHHA was determined using an internal standard, 3-phenylbutyric acid. The urine of nineteen patients that showed elevated BHHA were further analysed to determine the presence of other abnormal metabolites.

Phenylalanine (Phe) loading was also done on one of these samples and Phe and tyrosine (Tyr) were measured. The effect of aspartame on the concentration of Phe in this patient was also tested. A mercury test was also done on one of the patients, to determine the role that mercury plays in the transport of amino acids.

The concentrations of BHHA ranged from trace amounts to more than a 1000 mmol/mol creatinine. Other metabolites with elevated levels in the urine of the nineteen patients included: *p*-hydroxyphenylacetic acid, *m*-hydroxyhippuric acid, *p*-hydroxyhippuric acid and *p*-hydroxyphenyllactic acid. These patients also excreted a few unique metabolites: benzoylsuccinic acid, phenylacrylylglycine, phenylacetylglycine and β-*m*-hydroxyphenylhydracrylglycine. Two of the patients also displayed elevated levels of phenylacetic acid and phenyllactate in their urine. One patient had undergone a pancreasectomy for reasons unknown to us. Clinical symptoms in most cases were not acute. ADHD and hyperactivity were the most common symptoms present in the clinical profile of these patients. Because phenylacetic acid and phenyllactic acid (that were, besides BHHA, present in two of the patients) may be indicative of a phenylalanine metabolic defect, a phenylalanine loading was carried out on two of the patients. The Phe loading caused a decreased absorption of Phe. Subsequently this was followed up with a tryptophan loading. Tyrosine absorption, analogous with the phenylalanine, was reduced but tryptophan absorption was normal, excluding Hartnup's Disease (HD). Aspartame loading did however; effect higher Phe levels, indicating that peptide absorption was normal.

The metabolites found in the urine of the patients suggest that they may be of a bacterial origin. Compounds such as benzoylsuccinic acid are produced only by anaerobic microorganisms. Most of the

other metabolites excreted at abnormal concentrations can also be characterised as bacterial metabolites excreted in the urine as a result of maldigestion or malabsorption. One patient in this study had undergone a pancreasectomy in which case malabsorption would be expected.

This investigation should be seen as a pilot study and quite extensive research is still required before a final conclusion will be reached. Currently it seems that BHHA is excreted as a result of malabsorption or maldigestion, as was expected, but it does appear however, that BHHA may be an important indicator of defects that may cause these abnormalities.

CHAPTER 1

INTRODUCTION

A large amount of absorptive intestinal membrane transporters play an important part in absorption and distribution of several nutrients, drugs and prodrugs. Several transport systems at the blood brain barrier (BBB) and intestine are involved: these include uptake transporters of nutrients, such as amino acids, hexoses, monocarboxylates, amines, carnitine, peptides, etc. and the efflux transporters, such as p-glycoprotein and multiple organic anion transporters.

Amino acid transporters, in parallel with peptide transporters, accomplish the uptake of amino acids, eg. phenylalanine from food in the small intestine, their release into blood, and subsequent uptake of amino acids from the blood into tissues such as liver or skeletal muscle, or the reabsorption of amino acids from the urine along the kidney nephron. In the central nervous system, amino acid transporters regulate the transport of amino acids across the BBB or are involved in the reuptake of neurotransmitter amino acids such as glycine, aspartate, or glutamate from the synaptic cleft.

The L system is a system that shows a broad transporting reactivity around neutral amino acids with branches or rings in their side chains. System L is a Na^+-independent system that works through trans-stimulation. Phenylalanine is one amino acid that is transported through this system (Gazzola *et al.*, 1980:935).

Major attention during the past decade has been given to the importance of multidrug transport proteins and the resistance of cells to multiple cytotoxic drugs. Resistance of human neoplastic cells against some anticancer drugs is being associated with over expression of p-glycoprotein (P-gp). P-gp's are transporters belonging to the ATP-binding cassette (ABC) protein family. They act by using energy from ATP hydrolysis to pump molecules out of cells. ABC multidrug transporters are not only found in human cells, but also in micro organisms. P-gp effluxes many structurally unrelated drug molecules from various tissues and organs, including the brain, intestine, liver, kidney and lymphocytes, hence altering the distribution of drugs throughout the body (Van Veen *et al.*, 2001:365) (Wang *et al.*, 2002:412).

Malabsorption refers to a number of disorders in which nutrients from food are not absorbed properly in the small intestine. Under normal circumstances foods are digested in the stomach and nutrients are absorbed into the bloodstream, mainly through the small intestine. Malabsorption may occur if a disorder interferes with the digestion of food and then interferes directly with the absorption of nutrients.

Malabsorption of different nutrients causes different symptoms, for example, a malabsorption of certain sugars can cause explosive diarrhoea, abdominal bloating and flatulence (Ebert, 2001:49).

In the gastrointestinal tract a number of anaerobic bacteria can be found that also metabolizes certain food and substances consumed. Metabolites form when these anaerobic bacteria break down phenylalanine. These metabolites include phenylpropionic acid, cinnamic acid, phenyllactic acid and isocaproic acid (Hamid *et al.*, 1997:130-131).

The German physician, Heinrich Hoffman, first described attention-deficit disorder with or without hyperactivity in 1845. ADHD is outlined as a syndrome which starts early in life, is more common in boys and is characterized by symptoms like hyperactivity, impulsivity, distractibility and excitability. These children usually also experience other symptoms like aggressive and antisocial behaviour, learning problems and emotional lability (Cantwell, 1975:3). It has been suggested that ADHD may be caused by neurotransmitter defects in the brain (Oades, 2002:97).

Dopamine (DA) is a neurotransmitter formed in the body through a complex process; the process involves phenylalanine (Phe) that undergoes enzymatic conversion to form DA. Phe is an essential amino acid, which means that Phe is essential to human health but cannot be manufactured by the body. Amino acids form the building blocks of protein. The body also converts Phe into other brain chemicals, tyrosine and thyroid hormones (Rodwell, 2000:311).

It is also known that natural products containing Phe is used to treat ADHD, compared to harmful effects of Ritalin (New Ideas, 2004:2). Methylphenidate (MPH), the active compound in Ritalin®, is one of the drugs that can be used to decrease symptoms of both attentional deficiency and impulsivity/hyperactivity (Overtoom *et al.*, 2003:2). We speculate that ADHD children using Ritalin® may also have elevated Phe levels. Natural products used for ADHD treatment also elevate Phe levels.

Phenylketonuria (PKU) is a metabolic disorder that occurs in people who lack an enzyme, phenylalanine hydroxylase that is required to properly metabolise Phe, which results in elevated levels of Phe and Phe metabolites. These metabolites, as well as organic acids, can be detected in the urine of such patients by using gas chromatography – mass spectroscopy.

Hartnup's disease (HD) is a disease characterized by defective transport of neutral amino acids, including alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan and histidine.

These amino acids can be found in increased amounts in the urine of these patients (Symula *et al.*, 1997:102).

The aim of this study was to analyse the urine of children with ADHD for the presence of abnormal organic acids or abnormal concentrations of normal organic acids. Subsequently an organic acid namely *β-m*-hydroxyphenylhydracrylic acid (BHHA) was found in the urine of these patients.

We will attempt to determine if the presence of BHHA is the cause of a Phe malabsorption in the intestine or a defect in the Phe metabolic pathway. During this study it will also be investigated if the Phe malabsorption is the result of malfunctioning of P-gp, peptide transporter or the amino acid L-transport system and if together with these factors elevated mercury concentrations in patients, which influences transport systems could contribute to ADHD.

CHAPTER 2

LITERATURE OVERVIEW

2.1 INTESTINAL ABSORPTION

The gastrointestinal (GI) system consists of the mouth, pharynx, esophageus, stomach, small intestine, large intestine and rectum. The overall function of the GI is to process ingested foods into molecular forms that can be transferred, along with salts and water, from the external environment to the body's internal environment, where these nutrients can be distributed to the cells by the circulatory system (Vander *et al.*, 1998:551).

The stomach plays a vital role in the absorption of substances because it is in this organ that substances are digested. The stomach is a sacklike organ between the esophagus and the small intestine. The stomach also functions as a storage place for ingested foodstuffs: substances can be dissolved here and regulate the rate at which the ingested material empty into the small intestine (Figure 2.1).

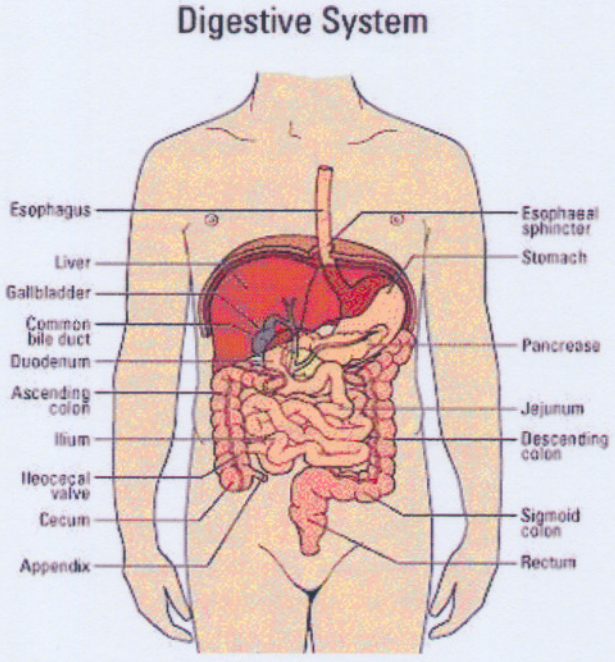


Figure 2.1 Figure of the gastrointestinal tract (Gibson, 2004:1).

The products of digestion cross the epithelial cells and enter the blood and/ or lymph. The small intestine is divided into three segments, the duodenum, followed by the jejunum and then the ileum. Most of the absorption occurs in the first two segments (Vander *et al.*, 1998:554).

2.1.1 Structure of the gastrointestinal wall

Most of the GI tract's wall is highly convoluted, a feature that greatly increases the surface area available for absorption (Figure 2.2). From the stomach to the big intestine the surface is covered by a single layer of epithelial cells linked together along the edges of their luminal surfaces by tight junctions. In this epithelial layer exocrine cells are located that secrete mucus into the lumen of the tract and endocrine cells that release hormones into the blood. Under the epithelial layer another layer of connective tissue is found called the lamina propria. The lamina propria is separated from underlying tissues by a thin layer of smooth muscle, the muscularis mucosa. These three layers combined are known as the mucosa.

Under the mucosa a second connective tissue layer is found the submucosa that contains a network of nerve cells, the submucous plexus, and blood and lymphatic vessels whose branches penetrate into the underlying layer of smooth muscle called the muscularis externa. Finally, surrounding the outer surface of the tube is a thin layer of cells and connective tissue called the serosa.

The small intestine also has another layer known as villi, which are fingerlike projections into the gut transit space. The surface of each villus is covered with a single layer of epithelial cells whose surface membranes form small projections called microvilli. It is the microvilli that make absorption possible by increasing the absorptive area many fold (Vander *et al.*, 1998:557-558).

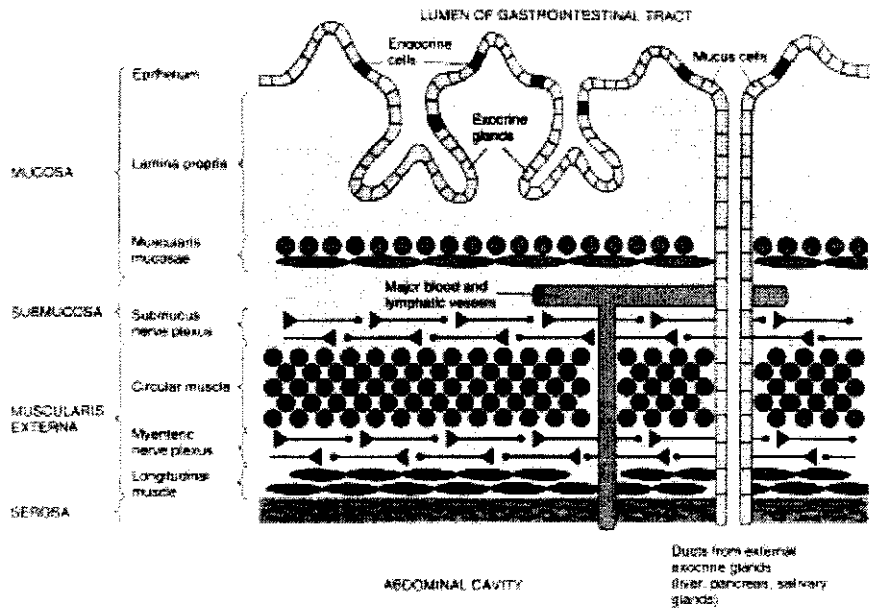


Figure 2.2 Structure of the gastro intestinal wall (Vander *et al.*, 1998:557).

2.1.2 Absorption processes

Absorption is the movement of particles from the GI tract to the blood and lymph. Substances can be absorbed through 3 processes, which include:

a) Passive diffusion: this is the movement of the particle across the cell membrane from a high concentration to a low concentration without the use of cellular energy.

b) Facilitated diffusion: this process occurs through the membrane with the help of a carrier. The substance can't move through the cell membrane, it then binds with a carrier and is transported through the membrane. On the other side the carrier and substance separate and the substance can be transported to where it is needed.

c) Active transport: this process drives against a concentration gradient. The substance moves from a low to a higher concentration. The substance also uses a carrier to cross the cell membrane (Venter, 99:10, 23, 24, 28).

Compounds such as nutrients can thus utilize carrier-mediated routes, either energy-dependent or passive transporters, in the small intestine (Figure 2.3). Studies of the multidrug resistance phenomenon have focused on a membrane glycoprotein (termed P-glycoprotein). P-glycoprotein (P-gp) is not only highly expressed in cancer cells but also expressed in normal intestinal and colonic epithelial cells. This system has been found to mediate drug transport in a secretory direction. Another secretory transporter in the intestine is the multidrug resistance-associated protein (MRP). It has been reported that P-gp transports cationic and neutral compounds as substrates while MRP family may play a role in the efflux of anionic compounds. In addition peptides and proteins are degraded into small peptide subunits and amino acids by peptidases and proteases in the gastrointestinal tract to be absorbed across the intestinal mucosa. Recently H^+-coupled peptide transporters, PEPT1 and PEPT2 have been cloned and well characterized. Di-and tri-peptides are actively transported into the cells by peptide transporters after ingestion of proteins (Ano *et al.*, 2004:250).

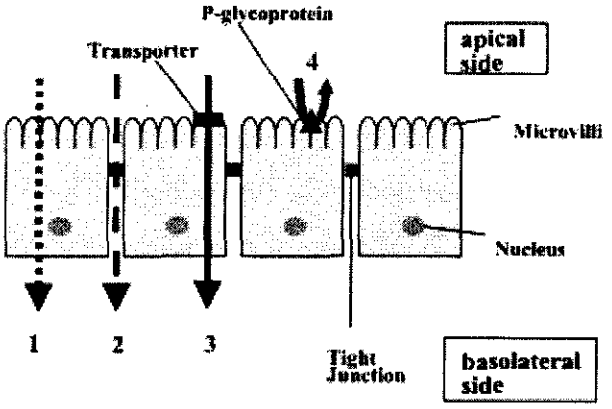


Figure 2.3 Pathways for drug transport across the intestinal epithelium: (1) passive transcellular, (2) passive paracellular, (3) active carrier-mediated routes, and (4) P-glycoprotein efflux system (Ano *et al.*, 2004:250).

2.2 BLOOD-BRAIN BARRIER ABSORPTION

The brain is shielded against potentially toxic substances by the presence of two barrier systems: The blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). The BBB's surface area is approximately 5000-fold greater than that of the BCSFB and is therefore considered to be the major route for the uptake of endogenous and exogenous ligands into the brain parenchyma (Demeule *et al.*, 2002:339).

2.2.1 Structure of the BBB

The BBB is formed by brain capillary endothelial cells that are closely sealed by tight junctions. Brain capillaries possess few fenestrae and few endocytic vesicles as compared to capillaries of other organs. The extracellular matrix, astrocytes, pericytes and microglial cells surround the endothelial cells. The close association of endothelial cells with the astrocyte foot processes and the basement membrane of capillaries are important for the development and maintenance of the BBB properties that permit rigorous control of blood-brain exchange (Demeule *et al.*, 2002:339).

2.2.2 Function of the BBB

The tight junctions in the BBB prevent significant passive movement of small hydrophilic molecules from the blood to the brain, but specialized transport systems mediate the entry of essential substances such as amino acids, choline, glucose, monocarboxylic acids, amines, thyroid hormones, purine bases and nucleotides. Larger hydrophylic molecules do not cross the BBB to any significant extent aside from

specific proteins such as lactoferrin, transferrin and low-density lipoprotein, which are taken up by receptor-mediated endocytosis (Figure 2.4).

The BBB is frequently a rate-limiting factor for the penetration of drugs from the brain. Due to the amphophilic nature of cell membranes, lipid solubility is an important determinant of passive BBB permeability. The overall hydrophilic/ lipophilic balance of a molecule appears to be a good predictor of BBB permeability (Demeule *et al.*, 2002:339-340).

Substances that dissolve readily in the lipid components of the plasma membranes enter the brain quickly. The properties of the BBB also explain why lipids cannot serve as a significant energy source for the brain when glucose supplies are low. Lipids are transported in the plasma, bound to albumin; a type of plasma protein and the resulting lipoprotein aggregate cannot cross the BBB.

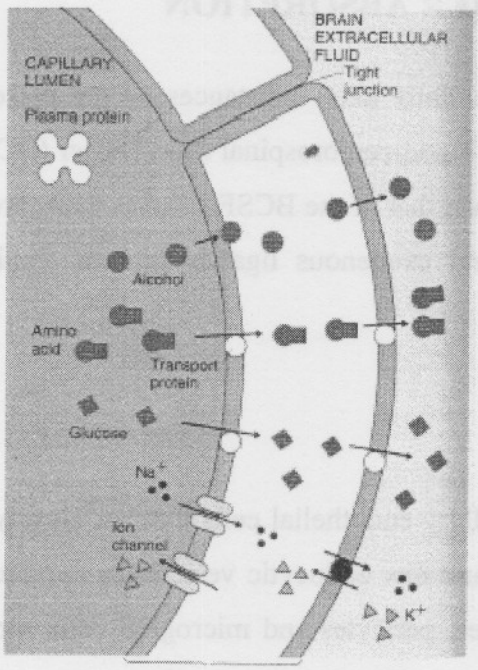


Figure 2.4 Movements across the BBB (Vander *et al.*, 1998:220).

Another important role of the BBB plays is its ability to retain cerebral neurotransmitters in the brain. This is also a result of the brain capillary endothelial cells that are connected by tight junctions. Cerebral transmitters and their metabolites cannot be transported across the BBB via paracellular passive diffusion. This structural barrier plays a key role in the efficient turnover of cerebral transmitters into their metabolites to be pumped out of the brain. If there were no pathway for eliminating hydrophilic substrates from the brain to the circulating blood, there would be significantly higher concentration of hydrophilic substrates such as homovanilic acid in the brain interstitial fluid. P-gp also plays a role in eliminating hydrophilic substrates from the brain to the circulating blood (Terasaki & Hosoya, 1999:196).

2.3 PEPTIDE TRANSPORT

Absorptive intestinal membrane carriers play an important part in absorption and distribution of several nutrients. The targeting of drugs or prodrugs to absorptive membrane carriers tends to influence bioavailability and distribution of these drug substrates (Steffansen *et al.*, 2004:3).

Suggested intestinal peptide transporters include peptide transporters PEPT1, and PEPT2. Both are plasma membrane proteins with similar topology. They contain 12 predicted membrane-spanning domains (TMD) and N- and C-termini that faces the cytosol (Figure 2.5). These proton-coupled transporter proteins are present predominantly in epithelial cells of the small intestine, mammary gland, lung, choroid plexus and kidney, but are also found in other cell types. The proton gradient and the membrane potential provide the driving force for peptide uptake into intestinal epithelial cells via the proton-dependent peptide transporter located in the apical membrane. Substrates for PEPT1 and PEPT2 include di- and tripeptides as well as several peptidomimetic compounds, such as β-lactam antibiotics and selected peptidase and protease inhibitors (Steffansen *et al.*, 2004:3).

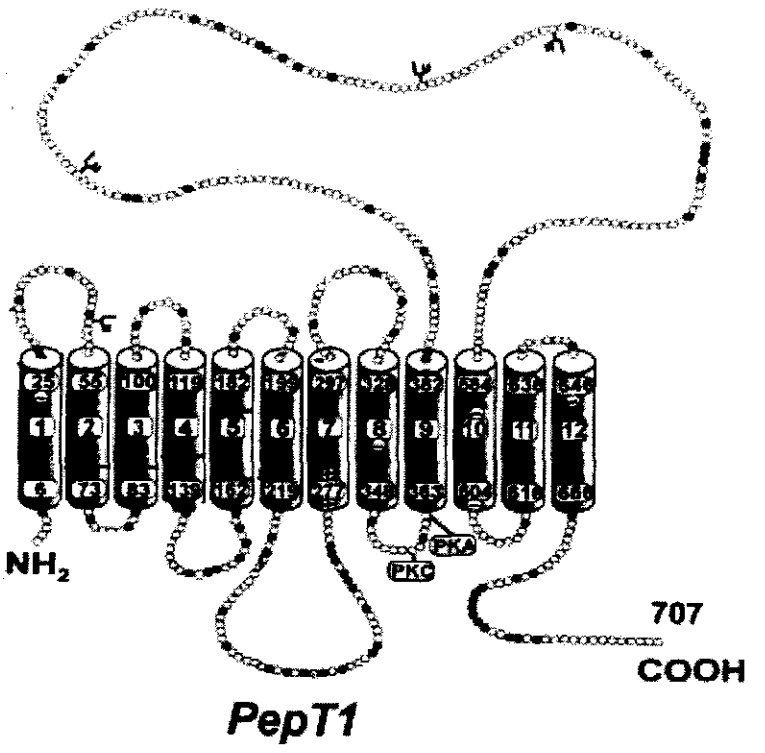


Figure 2.5 A schematic model of PEPT1 (Lee, 2000:S43).

2.4 AMINO ACID TRANSPORT

All twenty standard amino acids found in proteins are α-amino acids. These amino acids possess a carboxyl group and an amino group bonded to the same carbon atom (the α carbon). They differ from each other in their side chains, or R groups, which vary in structure, size, and electronic charge. All of these properties contribute to the solubility of the amino acids in water (Wagner *et al.*, 2001:1079).

Amino acid transporters fall into different families and are distinguished by their functional properties (specificity of amino acids transported, transport mechanism and coupling ions) and their molecular similarity or dissimilarity. Many of these amino acid transporters have first been described as transport systems in tissues or cell cultures and were only identified over the past few years at the molecular level.

Currently, nine amino acid transport systems have been reported to be present at the brain capillary endothelium of the blood-brain barrier (BBB). These systems differ in substrate specificity, inhibition by model ligands (eg. methylaminoisobutyric acid) and transport dependence on sodium transport. The systems are:

- System L: mediates high affinity, Na^+ - independent uptake of neutral amino acids with large, neutral side chains, including L-leucine, L-phenylalanine, L-tryptophan, L-tyrosine, L-isoleucine, L-methionine and L-valine.
- System y^+: mediates moderate affinity, Na+ - independent uptake of amino acids with cationic side chains, including L-arginine, L-lysine and L-orthinine.
- System T: mediates high affinity, low capacity transport of thyroid hormone T3 and T4.
- System x-: mediates Na^+- independent, high affinity uptake of amino acids with anionic side chains, including L-glutamate and L-aspartate.
- System A: Na^+- dependent active transport system that transports small neutral amino acids, including L-alanine, L-serine, L-cysteine.
- System $B^{0,+}$: Na^+ -dependent, expresses affinity for both neutral and basic amino acids.
- System ASC: Na^+- dependent, shows affinity for small neutral amino acids, including L-alanine, L-serine and L-cysteine.
- System β: Na^+- and Cl^-- dependent system that transports β-amino acids, including β-alanine and taurine.
- System X-: mediates Na^+- dependent transport of anionic amino acids, L-glutamate and L-aspartate (Smith, 2000:1017S-1018S).

The intestinal transport of amino acids has been investigated in various animal models that involve membrane vesicles. These have shown that the essential large neutral amino acid L-phenylalanine (L-Phe) crosses the brush border membrane via simple passive diffusion as well as via Na^+ -dependent and

Na^+-independent carriers in the human intestine. Phenylalanine is mainly transported through the above-mentioned System L, because it is a large neutral amino acid. In a study done by Berger and co-workers (2000), L-Phe transport across Caco-2 cell monolayers grown on microporous filters showed that apical (AP) to basolateral (BL) transport of L-Phe is temperature dependent, saturable and inhibited by metabolic inhibitors. The study also showed that the AP uptake exhibits characteristics very different from those of the BL uptake. The major carrier in the AP uptake resembles the Na^+-dependent $B^{0,+}$ system, whereas the carriers involved in the BL uptake include the Na^+-dependent $B^{0,+}$ and ASC systems as well as the Na^+- independent L system (Berger *et al*., 2000:2780-2781).

2.4.1 System L

System L shows a broad reactivity towards neutral amino acids with branches or rings on the side chain, is Na^+ - independent and exhibits strong exchange properties. The system works through *trans*-stimulation. The Site L-specific *trans*-stimulation appears to sustain a large part of the activity of System L under physiological conditions (i.e. when cells are not depleted of relevant amino acids) and would support entry and accumulation of Site L-reactive amino acids in exchange with other amino acid substrates previously concentrated by a more steeply uphill system (Gazzola *et al.*, 1980:935). An example of this is leucine that exhibits strong trans-stimulation by other neutral amino acids such as glycine in transplacental transport. It appears to be transported almost exclusively by system L. Leucine moves from the microvillous membrane (MVM) to the basal membrane (BM) (Figure 2.6) (Jansson, 2001:145).

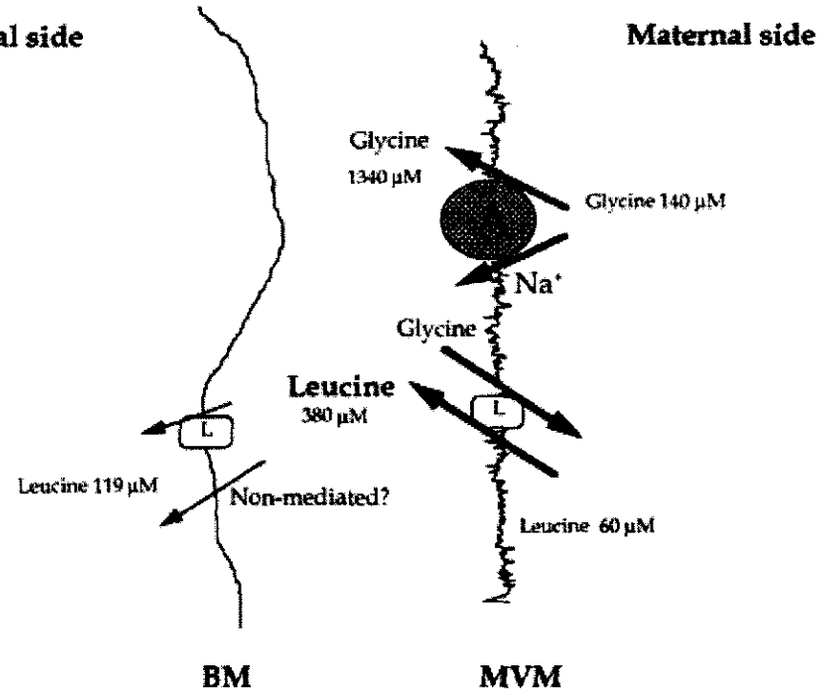


Figure 2.6 Mechanisms for transplacental transport: system L. Leucine appears to be transported almost exclusively by system L, a transporter that exhibits strong trans-stimulation by other neutral amino acids such as glycine. The steep, outwardly directed glycine gradient represents a possible driving force for the uphill accumulation of leucine into the syncytiotrophoblast (Jansson, 2001:145).

System L conveys the Na^+-independent transport of large branched and aromatic neutral amino acids in almost all types of cells. System L is differentiated from related transporters by its ability to transport the two model substances, 2-aminobicyclo-(2,2,2)heptane-2-carboxylic acid (BCH) and 3-aminobicyclo(3,2,1)octane-3-carboxylic acid. On the basis of the affinity for its substrate two subtypes have been described; LAT1 with a high affinity (i.e. in the micromolar range), and LAT2, with a lower affinity (i.e. in the millimolar range). LAT1 transports large neutral amino acids, such as phenylalanine and leucine, whereas LAT2 transports small neutral amino acids, such as L-alanine, L-glycine, L-cysteine, L-serine and glutamine. System L transport activity has been described in a variety of cells and organs such as the brain, spleen, thymus, testis, skin, liver, placenta, skeletal muscle, and stomach (Wagner *et al.*, 2001:1077).

The endothelial cells of cerebral capillaries are joined together by tight junctions forming the blood-brain barrier (BBB). Thus, hydrophilic nutrients, such as amino acids, require the presence of carriers in the respective luminal and abluminal membranes to reach the brain. System L, characterized by affinity for a

broad spectrum of neutral amino acids (especially large neutral amino acids) is equally distributed between the luminal and abluminal membranes; therefore System L is in a position to facilitate neutral amino acid movement between blood and brain (Lee *et al.*, 1996:19129).

2.5 P-GLYCOPROTEIN

2.5.1 Function

P-glycoprotein is a large plasma membrane protein of the ATP binding cassette family of transporter proteins. The human body contains 48 different ATP-binding cassette (ABC) transporter genes. These ABC genes can further be divided into 7 subfamilies (Table 2.1). Each of these subfamilies consists of a different number of genes and these genes are present on different chromosomes and play different roles in cells. Mutations of some of the genes cause diseases and irregularities in the human body (Table 2.2) (Dean *et al*, 2001:1156-1160).

P-gp appears to be a major transporter at the blood-brain barrier that acts as a guardian of the central nervous system by preventing the accumulation of many drugs in the brain. P-gp is also involved in the excretion of toxic compounds by renal proximal tubules and hepatic canacular membranes and in the secretion of endogenous molecules from adrenal glands. P-gp has been found to be involved in cytokine secretion from lymphocytes, dendritic cell migration, steroid secretion from adrenal glands and lipid (cholesterol) transport (Demeule *et al.*, 2002:340).

P-glycoprotein is present in the endothelial cells that form the blood-brain barrier and is functionally active in transporting drugs from the brain (or basolateral) side to the blood (apical or luminal) side of these cells. P-gp can prevent the accumulation of many compounds, including a variety of drugs, in the brain (Schinkel, 1999:180). P-gp can also interact with drug metabolizing enzymes, like the 3A4 isozyme of cytochrome P450 (CYP3A4). These two shares may substrates and inhibitors and have a common tissue distribution. P-gp is present in many human tissues such as the liver, kidney, intestines and adrenal glands as well as in blood-tissue barriers including the placenta, testis capillaries and brain capillaries (Demeule *et al.*, 2002:340-341).

Table 2.1 Gene subfamilies and their functions

SUBFAMILY	NAME	NUMBER OF MEMBERS	DIFFERENT GENES AND THEIR FUNCTIONS
ABCA (ABCA1-10,12-13)	ABC1	12	ABCA1-Disorders of cholesterol metabolism and high-density lipoprotein biosynthesis. ABCA4-Transports Vitamin A, thus plays a part in the visual cycle.
ABCB (ABCB1-11)	MDR	11	ABCB1-Present in the blood-brain barrier and liver. Phenotype for cancer cells. ABCB4, 12-Present in the liver, involved in bile salt secretion ABCB6-8, 10-Function in iron metabolism.
ABCC (ABCC1-12)	MRP	12	ABCC7 (CFTR)-Role in exocrine secretions ABCC1-3-Transports drug conjugates to glutathione and other organic compounds.
ABCD (ABCD1-4)	ALD	4	ABCD1-4-Regulation and transport of very long chain fatty acid.
ABCE (ABCE1)	OABP	1	ABCE1-Is produced in response to infection by certain viruses.
ABCF (ABCF1-3)	GCN20	3	ABCF1-3-Associated with the ribosome and activation of the elf-2 α-kinase.
ABCG (ABCG1-2,3?,4-6)	WHITE	5 (+1?)	ABCG2-A drug resistance gene. ABCG1-Cholesterol transport regulation ABCG5, 8-Transports sterols in the intestine and liver.

Table 2.2 Mutations of some of the genes cause diseases and irregularities in the human body (Dean *et al*, 2001:1156-1160)

DISEASE	EXPLANATION
Cystic fibrosis	Develops as a result of a mutation on the ABCC7 (CFRT) gene. It's the most common fatal childhood disease. Other disorders caused by this gene include inadequate secretion of pancreatic enzymes leading to nutritional deficiencies, bacterial infections of the lung, obstruction of the vas deferens, leading to male infertility.
Adrenoleukodystrophy	X-linked recessive disorder, there is no apparent correlation to ABCD1. Symptoms include neurodegenerative phenotypes with onset in late childhood. Characterized by adrenal deficiencies.
Sulfonylurea Receptor	ABCC8 is a high-affinity receptor of the drug sulphonylurea. Sulfonylureas increase insulin in patients with non-insulin-dependent diabetes. The bond that forms inhibits an associated potassium channel, the patient then develops familial persistent

	hyperinsulinemia.
Bile salt transport disorders	Mutations on the ABCB4 gene cause intrahepatic cholestasis during pregnancy. Mutations on ABCC2 cause problems in the transport of organic anion transport, because it is expressed on the canalicular side of the hepatocyte.
Retinal Degeneration	The ABCA4 gene transport Vitamin A derivates, which play a role in the visual cycle. Mutations on this gene result in retinitis pigmentosa or macular dystrophy with loss of central vision.
Mitochondrial iron homeostasis	Mutations on ABCB7 are associated with X-linked anemia and ataxia.
Sterol transport deficiencies	Tangier disease is characterized by deficient efflux of lipids from peripheral cells and a very low high-density lipoprotein (HDL). It is the result of alteration on the ABCA1 gene. This mutation also cause hypolipidemia, because it has a role in regulating HDL in the blood

2.5.2 Mechanism

Borst & Schinkel (1997) described p-gp as a membrane vacuum cleaner recognizing molecules that do not belong in the membrane and removing the foreign molecules out of cells.

It appears that the intestinal metabolism of drugs could be changed as a function of p-gp activity without either inhibiting or inducing CYP3A4 enzymes. If the p-gp efflux is inhibited, the drug will pass through the intestine without difficulty and the inhibiting will result in a decreased metabolism of the drug since there are less enzymes (Figure 2.7)(Benet & Cummins, 2001:S6-S7).

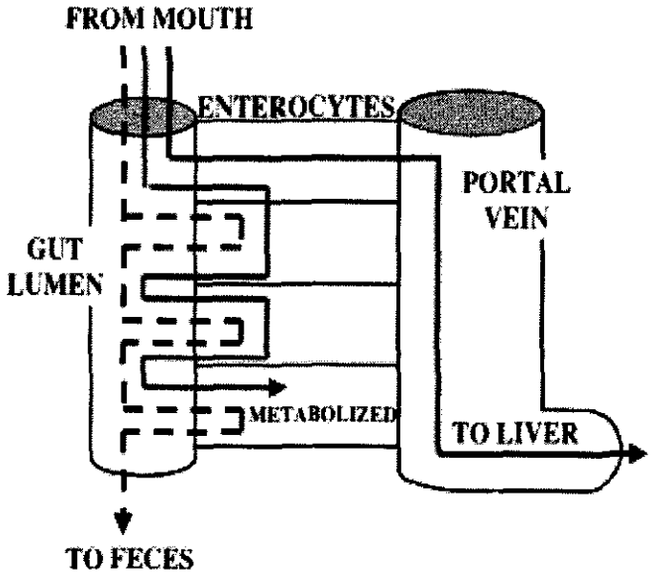


Figure 2.7 Schematic depicting the potential effects of P-glycoprotein efflux on access of drugs to intestinal CYP3A enzymes (Benet & Cummins, 2001:S6).

The elucidation of the primary structure of p-gp and its deduced transmembrane topology initially led to the idea that p-gp literally function as pumps. According to this hypothesis, the 12 transmembrane segments come together to form a drug pore and drugs are transported through this pore with the help of energy generated by the hydrolysis of ATP (Borst & Schinkel, 1997:220-221).

Substrates of the MDR1 gene product, the major p-gp form responsible for multidrug resistance, tend to be hydrophobic or amphiphilic. It has been suggested that only those molecules able to insert themselves into the inner leaflet of the membrane, may serve as p-gp substrates. These molecules eventually partition into the membrane according to a process determined by their physicochemical characteristics. From within the membrane, the substrates may bind to p-gp and either be translocated to the outer leaflet of the membrane (flippase activity), or transported across the p-gp molecule, and directly expelled into the external aqueous phase (Figure 2.8). This is called drug flippase (Zimniak *et al.*, 1999:110-111).

Drug flippase works on the principle that the mobility of the phospholipids within the membrane is high, while the flipping between the membrane leaflets is very low (Figure 2.8). This is the result of the polar head groups of the phospholipids that cannot pass the hydrophobic interior of the membrane. There are certain enzymes that can speed up this flipping reaction. They are called flippases or phospholipid translocators (Borst & Schinkel, 1997:220-221).

Flippase activity affects the distribution in the body. P-gp is localised on the apical membrane of secretory cells, where it plays the role of secreting xenobiotics and metabolites into the intestinal lumen, urine and bile. It also protects the brain from toxins and metabolites that can accumulate. However, p-gp can cause drug resistance in certain cell types by inhibiting therapeutic drug accumulation in target cells, which hinders certain drugs (especially for the treatment of HIV and cancer) reach their targets (Hansten *et al.*, 2001:590).

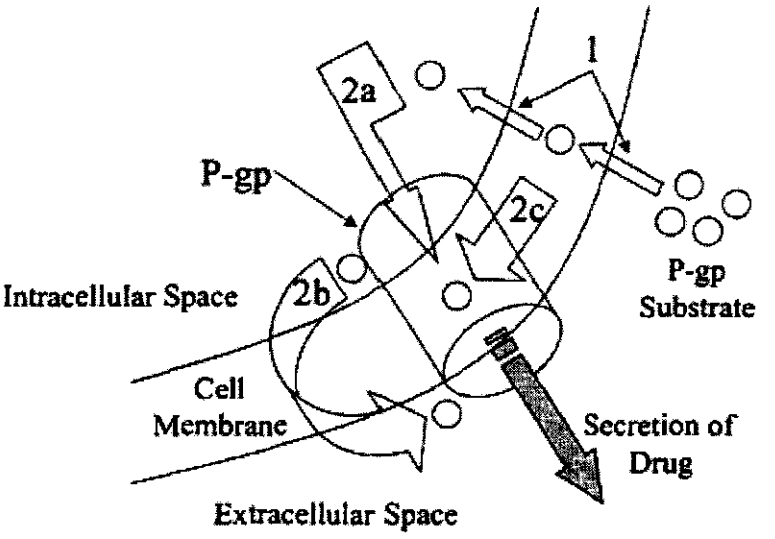


Figure 2.8 Proposed mechanisms by which P-gp secretes substrates. (1) Passive drug uptake across cell membrane. (2a) Formation of hydrophobic channel (pore) between the intracellular and extracellular space. (2b) Flippase activity whereby the drug is flipped from the inner leaflet to the outer leaflet of the cell membrane. (2c) "Vacuum cleaner model" in which drug interacts with P-gp in the lipid bilayer and is subsequently secreted back into the extracellular space (Matheny *et al.*, 2001:779).

2.6 MALABSORPTION

Malabsorption refers to a number of disorders in which nutrients from food are not absorbed properly in the small intestine. This process occurs if a disorder interferes with the digestion of food and nutrients. Absorption of nutrients into the bloodstream can be affected by disorders that injure the lining of the small intestine. Infections (bacterial, viral or parasitic), drugs such as neomycin and alcohol, celiac disease and Crohn's disease all injure the intestinal lining. Disorder that affect the remaining layers of the intestinal wall such as blockage of the lymph vessels by lymphoma (cancer of lymphatic system) and poor blood supply to the small intestine, also reduce absorption.

Symptoms of malabsorption are caused by the increased passage of unabsorbed nutrients through the digestive tract or by the nutritional deficiencies that result from inadequate absorption (Ebert, 2001:49). Diseases of malabsorption are characterized by diarrhoea (stool volume of more than 250 g/day) that stops with fasting (associated with an osmotic gap). Osmolarity of the stool is calculated by using the concentrations of the main cations, sodium and potassium, added together and doubled to account for the associated anions. The calculated stool osmolality should be within 60 mEq/L of the measured stool osmolality. If this value is greater, there is osmotic diarrhoea (Ebert, 2001:59-60).

Most diseases of malabsorption and maldigestion are characterized by diarrhoea or loss of more than 7% of dietary fat. Isolated carbohydrate or protein malabsorption may occur without diarrhoea. The most common causes of maldigestion and malabsorption are given in Table 2.3.

Table 2.3 Most common causes of maldigestion and malabsorption (Ebert, 2001:59-60).

MALDIGESTION	MALABSORPTION
Impaired micelle formation	Diffuse small bowel disease (celiac sprue, Crohn's disease)
Reduced delivery of bile salts to duodenum (biliary obstruction)	Short bowel syndrome
Impaired bile salt synthesis (liver disease)	Lack of brush border enzymes (disaccharidase deficiencies or general loss due to rapid epithelial cell turnover)
Deconjugation of bile salts (baterial overgrowth)	Bacterial overgrowth
Increased loss of bile salts (terminal ileal disease or resection)	Lymphatic obstruction
Impaired digestion	
Pancreatic insufficiency	
Inactivation of lipase by low pH	
Improper mixing (gastric surgery)	
Rapid transit (hyperthyrodism)	

2.7 ATTENTION-DEFICIT-HYPERACTIVITY-DISORDER (ADHD)

2.7.1 Introduction

Attention-Deficit Hyperactivity disorder (ADHD) is a clinically heterogeneous condition. ADHD has been called hyperkinetic or hyperactive syndrome in the past. This condition is the most common heritable and behavioural disorder of childhood. The condition has been classified into three sub-types depending on the predominance of symptoms. For instance ADHD could be (a) predominantly inattentive (b) hyperactive-compulsive and (c) combined inattentive and hyperactive type. The third type is the most common type in children and adults (Shastry, 2004:469).

Epidemiological studies suggest that the syndrome is three to four times higher in males than in females. ADHD occurs approximately in 3-5% of school-age children in Western countries or 5-10% worldwide. The observed male to female difference is not due to an X-linked gene but considered due to familial factors. The affected patients exhibit a continuous pattern of inattention, which includes carelessness in

schoolwork, poor academic performance, disobedience, often avoiding tasks which require mental efforts and/or hyperactivity. These symptoms can occur before the age of seven when the patient is either at home or school (Shastry, 2004:469).

Although the etiology of ADHD is unknown, family, twin and adoption studies provided strong evidence for genetic factors as causative components of the disorder. In some cases environmental factors such as a chaotic family, child abuse, substance abuse and school situations have also been implicated (Shastry, 2004:470). It is likely that multiple factors such as biological and psychosocial are involved in manifesting the disorder. Several studies such as single photon emission computerized tomography (SPECT), magnetic resonance imaging (MRI) and positron emission tomography (PET) have identified an abnormality in different brain regions (Shastry, 2004:469).

ADHD indicates an impairment that is referred to as a poor ability to maintain or sustain attention. ADHD has been associated more with the poor perceptual detection thresholds (in signal detection terms, d-prime) than the identification thresholds (beta-criterion), thus the children experience more difficulties in detecting a foreign stimulus than identifying a foreign stimulus, although a few studies show an impairment of beta in ADHD patients (Oades *& Müller*, 1997:95-96).

2.7.2 Organic acids

It has been suggested that ADHD is associated with elevated levels of organic acids or abnormal organic acids in these patients' urine. In a case study involving 15 ADHD boys and 16 controls, the urinary catecholamine excretion was measured. Dihydroxyphenylalanine, dopamine, noradrenaline (NA), adrenalin (A), 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylglycol (DOPEG) were assayed by high-pressure liquid chromatography with electrochemical detection. It was found that the ADHD patients had lower levels of DOPEG in their urine than the controls. Lower urinary adrenaline levels could also be detected in the hyperactive boys. These results were consistent with previous reports of abnormal NA and A metabolism (Hanna *et al.*, 1996:63).

In another study the following metabolites were found in the urine of ADHD patients: homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG). These two compunds are organic acids that are metabolites of catecholamines (Castellanos *et al.*, 1994:306). In addition, DOPEG levels in the urine were also decreased (Hanna *et al.*, 1996:63).

2.7.3 Symptoms

The symptoms of ADHD are usually present from an early age. Children that have been diagnosed with ADHD have always seemed to have an unusual amount of energy, didn't need as much sleep and wore out things like shoes faster than other children their age. Other symptoms include:

- fidgetiness
- inability to sit still for any length of time
- talking a great deal
- inability to keep hands to themselves
- distractibility
- impulsivity
- excitability
- antisocial behaviour
- cognitive and learning disabilities
- depression
- low self-esteem
- lack of ambition (Cantwell, 1975:4-10).

It seems that there is a difference in the extent of the impulsivity, hyperactivity and inattention in boys and girls - boys having more severe symptoms. The symptoms threshold in the Diagnostic and Statistical Manual for Mental Disorders Volume IV (DSM-IV) is most appropriate to boys, because more boys were used in the study. Girls have to have a higher threshold in DSM-IV criteria to be diagnosed with ADHD relative to other girls, than boys relative to other boys need to. Therefore the cut off score must be adjusted for sex (Barkley, 2003:81).

A selective attention deficit is reflected by a changed signal-detection (response to a stimuli) strategy. An alternative explanation of the attention-deficit is an ability to suppress actively the processing of and response to irrelevant stimuli. Tests have been done to diagnose patients with impairment in signal-detection:

- Latent inhibition (LI): Delay in reacting upon a stimulus following pre-exposure to it, without any consequence. LI was reported in younger children (5-6 years), and not in older children.
- Conditioned blocking (CB): Refers to the rapidly passing suppression of learning about a stimulus added during conditioning to another one. It was found that patients with ADHD could not process irrelevant information, thus no normal development of CB. Children with ADHD older than 11 years showed a decreased CB compared to normal controls, while children younger than 11 years actually showed an increase in CB (Oades & Müller, 1997:95-96).

2.7.4 Role of mercury in ADHD

Mercury (Hg) is the second most toxic element on earth. The amount of mercury found in one mercury thermometer is enough to pollute a small lake. Mercury toxicity has been linked to a large number of diseases, including arthritis, Alzheimer's disease, multiple sclerosis, fibromyalgia, lupus, chronic fatigue syndrome, depression, autism, bipolar disorder, schizophrenia, learning disabilities and ADHD (Schettler, 2001:813).

Mercury poisoning in cases where the presence of the metal goes undetected is often initially diagnosed as a psychiatric disorder. Symptoms include extreme shyness, indifference to others, active avoidance of others, lack of interest, mental confusion, irritability, aggression, tantrums, anxiety, fearfulness and emotional lability (Bernard *et al.*, 2000:4).

Mercury has been contaminating our environment for years. The two largest sources of mercury in the environment are coal-fired power plants and municipal waste incinerators, which burn consumer products containing mercury (Gallagher, 2004:1). Mercury is a metallic element that cannot be broken down by any method, and it possesses unusual qualities not found in other metals because it is liquid at room temperature and evaporates very quickly when heated. Mercury escapes readily through smokestacks and is spread widely by winds before it descends to earth. There, the metal is bioconcentrated in the fatty tissue of animals. This is especially true in fish that are higher up in the food chain, like tuna or swordfish. Mercury is a well-known neurological poison, which causes all the symptoms of ADHD, such as hyperactivity and poor concentration (Bernard *et al.*, 2000:1).

Many other common sources for mercury exposure have been identified. These include:

- dental amalgams, it is believed that children who have mercury containing amalgam fillings in their mouth and grind their teeth are at risk of accumulating high mercury levels in their fatty reservoirs;
- release into the air by coal burning plants;
- fish and shellfish, especially tuna, salmon and swordfish;
- some older paint products;
- thermometers and blood pressure gauges (especially if mercury from broken instruments is spilled on carpets);
- fluorescent light bulbs.

Another source of mercury comes from vaccines. Thimerosal, which contains 49.6% ethylmercury is a preservative added to many vaccines. Children receive many vaccinations before the age of 5: as many as

9 vaccinations on the same day and are exposed to quantities of mercury that exceed safety guidelines (Yazbak & Yazbak, 2002:284). The U.S. Environmental Protection Agency has recently developed a reference dose for mercury of 0,1 μg Hg/kg/day and estimates that Hg exposure at this level is likely to result in hair Hg levels of about 1 ppm (Schettler, 2001:815).

It is also important to note that mercury present in a mother's body is passed to her baby through the placenta, and later, through breast milk. The present generation of mothers also received more vaccines and has more immune disorders than any previous generation. Given this, the era during which the mother was vaccinated play a role in the severity of the mercury intoxication of their children (Yazbak & Yazbak, 2002:284).

Recently a study was done to assess the complications associated with the administration of live virus vaccines around the critical period of a pregnancy. The study involved 76 mothers, where 18 of these mothers were vaccinated just before or after conception and 58 of the others were vaccinated in the postpartum period. Of the mothers studied 76% had one or more children diagnosed with autistic spectrum disorder and 13 other mothers (17%) had children with severe ADHD and significant development delays. The outcome of the study was only done on a single pregnancy of each mother. Concluded in the study was that over 80% of the boys experienced major difficulties: 21 out of 47 boys having been diagnosed with autism and 12 others having ADHD and/or developmental delay. Two boys were identified as having other medical or educational issues. Only 6 of the boys born were normal. Six of the girls were diagnosed as being autistic, 2 had ADHD and/or developmental delay, and another three had other significant health issues. Fifteen of the girls were born normal. The study concluded that if possible mothers should only be vaccinated after breastfeeding was terminated to prevent harmful Hg to be distributed to their children (Yazbak & Yazbak, 2002:285).

A pertinent characteristic of mercury is the great variability in its effects measured in individuals. At the same exposure level, some individuals will be affected severely while others may be asymptomatic. An example is acrodynia, a condition that arose in the early twentieth century from mercury in teething powders and where only 1 in 500-1000 children given the same dose contracted the disease. Studies in mice and humans indicate that susceptibility to mercury effects arises from genetic status, in some cases including a propensity to autoimmune disorders. Mercury studies in mice and humans consistently report greater effects on males than on females, similar to ADHD. At high doses both sexes are equally affected, but at low doses only males are affected (Bernard *et al.*, 2000:10).

Mercury is eliminated from the body by chelating with selenium after which this chelate is expelled from the body (Warnitz, 2004:1).

2.7.4.1 The effect of mercury on amino acid transport

The uptake of methylmercury (MeHg) in astrocytes in the central nervous system (CNS) occurs via the neutral amino acid L-system. MeHg preferentially accumulates in astrocytes where MeHg inhibits astrocytic glutamate uptake and increases glutamate's efflux, thus increasing extracellular glutamate concentrations and sensitizing neurons to excitotoxic injury. Glutathione (GSH) plays a critical role in modulating MeHg neurotoxicity. An important relationship between MeHg toxicity and GSH levels has been demonstrated in a study done by Miura *et al.* (1993) in a MeHg-resistant rat PC12 cell line. MeHg toxicity can be enhanced by decreasing intracellular GSH levels, or decreased by increasing intracellular GSH levels. This protective effect of GSH might be due to direct conjugation and efflux of MeHg with GSH, to GSH acting as an intracellular buffer, thus limiting the effective MeHg concentration available for interaction with other macromolecules (Shanker *et al.*, 2001:160).

The neutral amino acid, cysteine, is the rate-limiting substrate for the biosyntheses of GSH, and the maintenance of optimal intracellular GSH levels is dependent upon the extracellular availability and transport of cysteine into cells. The L-system plays a part in cysteine uptake. A study was done to determine the sensitivity of cysteine uptake transport to MeHg in rat astrocytes (Shanker *et al.*, 2001:160). This study concluded that adequate GSH levels in astrocytes in both *in vivo* and *in vitro* conditions are essential for the astrocyte's protection against antioxidants and free radicals, as well as providing a vehicle for extracellular transport of MeHg. MeHg inhibits the astrocytic uptake of cysteine, the key precursor to GSH biosynthesis. Cysteine and MeHg competes for the transporters in the amino acid transport system and therefore neurotoxicity occurs (Shanker *et al.*, 2001:163).

In another study Schaeffer and co-workers (1973) attempted to identify some of the functional chemical groups in the mucosal membrane of the rabbit ileum that facilitate amino acid transport across the membrane. The role of the sulfhydryl groups was examined using *p*-chloro-mercuriphenyl sulfonic acid (PCMBS) on Phe influx across the brush border membrane. This organic mercury reacts with protein sulfhydryl groups with relatively high specificity and appears to penetrate biological membranes rather slowly. They concluded that PCMBS rapidly inhibits 80-90% of Phe influx. The inhibitory effect could not be reversed by prolonged washing of the tissue with Na-free solution, and there was no significant recovery of ability to transport Phe. It can be seen that PCMBS inhibits amino acid influx as a result of reaction with sulfhydryl groups in the brush border membrane (Schaeffer *et al.*, 1973:142).

Lugea and co-workers (1994) studied the effect of $HgCl_2$ on galactose and phenylalanine (Phe) uptake in rat everted intestinal rings. The study concluded that the Na^+- dependant Phe transport was totally blocked in the presence of $HgCl_2$. It seemed that the Phe transport was more sensitive to $HgCl_2$ than the galactose transport. The inhibition of these intestinal transport systems by $HgCl_2$ might be due to its

interaction with ligands of the transport proteins located in the luminal membrane of enterocytes (Lugea *et al.*, 1994:167).

2.7.5 Role of dopamine in primary symptomatology in ADHD

In view of the dependence of CNS function on the function of biogenic amines as neurotransmitters, astonishingly few studies are published on the parameters that influence amine activity in normal human development or on the changes associated with child and adolescent psychopathology (Oades, 2002:97).

The motor and cognitive symptoms of ADHD are mediated differently. This can be seen in the fact that the attentional and the hyperactive-impulse factors in the presentation of symptoms can be separated. The difference in the two symptom groups can be seen according to their heritability, as well as the difference between time-action and dose-response curves for stimulant effects on each group (Solanto, 2002:66).

2.7.5.1 Motor overactivity

Dopamine (See 2.8 Dopamine) dysfunction in the striatum or in the cortical regulation of dopamine in the striatum is involved in the pathophysiology of ADHD. The selection, initiation and execution of voluntary motor responses are under control of the dorsal striatum. The indirect prefrontal-striatal-thalamic-cortical circuit projects through the external segment of the globus pallidus and produce a net inhibition of the cortical output. The result is excessive motor output, because of insufficient dopaminergic activity. This is in contrast with Parkinson's disease where there is a direct dopamine circuiting. Parkinson's disease is characterized by excessive dopaminergic activity in the internal segment or insufficient inhibition in the external segment (Solanto, 2002:66).

2.7.5.2 Cognitive dysfunction

Cognitive dysfunction is the abnormal or impaired functioning of a person's conscious intellectual activity. The prefrontal cortex in the brain is very sensitive to the neurochemical environment such that both excessive D1 stimulation, as in stress, as well as insufficient stimulation causes working memory deficits. It has been shown that, with respect to dopamine, D1 agonists enhance and D1 antagonists impair the working memory function (Solanto, 2002:66).

A direct prefrontal-striatal-thalamic-cortical circuit extends from the prefrontal cortex through the internal segment of the globus pallidus, feeding back to give amplification or the cortical output. Cognitive dysfunctioning happens via disinhibition. Exhaustion of dopamine in the circuit results in difficulty to

initiate movement like in Parkinson' disease. Cognitive dysfunction in Parkinson's disease is in contrast with ADHD because of the indirect dopamine-circuiting characteristic of ADHD (Solanto, 2002:66-67).

2.7.6 Monoamine metabolic status and conditioned blocking

Although it has been known since the 1930s that psychostimulants are effective in the treatment of many children with ADHD, the idea of an altered catecholamine metabolism in these children only caught on 25 years ago (Oades, 2002:97).

During a case study it was found that ADHD children excreted more 5-hydroxy indole acetic acid (5-HIAA), a serotonin metabolite, than the controls. It was also found that dopamine metabolism plays a role in information processing related to conditioned blocking. Increased dopamine utilization leads to an increase in conditioned blocking. An impairment of conditioned blocking can be correlated with an increase of the unmetabolized parent amine, especially in relation to the noradrenaline levels. However the serotonine activity is negatively related to conditioned blocking. This means that with an increase in serotonine (5-HT) metabolism there is a decrease in conditioned blocking and thus leads to an increase of 5-HT utilization in children with ADHD (Oades, 2002:98-100).

2.7.6.1 Noradrenaline

In a study done by Oades (2002:98) it was psychopathologically significant to understand the relationship between the levels of activity of one monoamine to the other. Noradrenaline (NA) has often been reported to be low in groups in groups of ADHD children. More recent results are consistent for low plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) and 3,4-dihydroxyphenylglycol (DOPEG) levels in ADHD samples. The balance of DA versus NA activity is critical for ADHD, as of course this balance is for normal frontal lobe function in attention, working memory and behavioural inhibition. It is important to see that in normal subjects levels of monoamines and metabolites alone fall from childhood across adolescence, showing different patterns across development. DA activity rises steadily across adolescence, while NA activity drops at puberty before steadily rising to adult levels (Figure2.9).

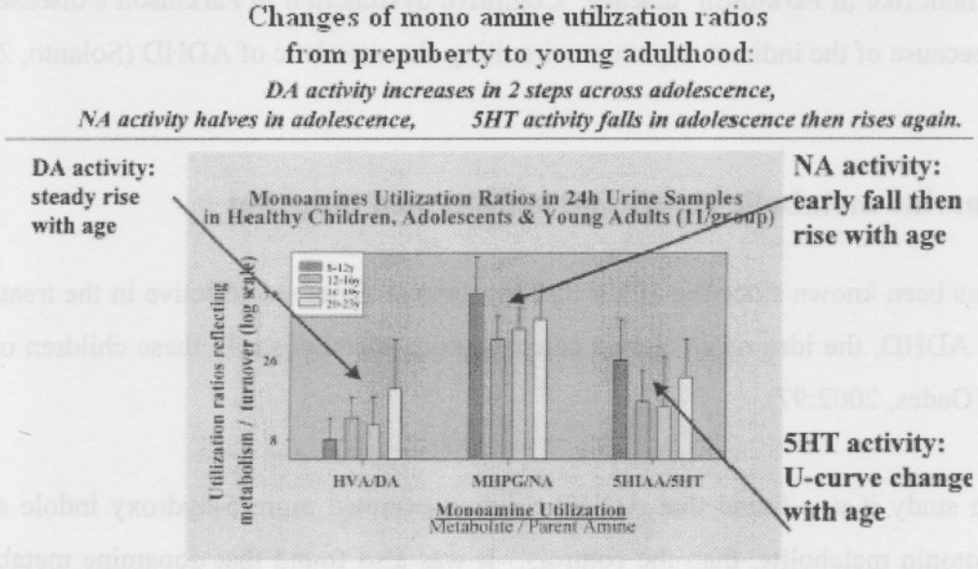


Figure 2.9 The development of the turnover or utilization ratios for dopamine (DA)and noradrenaline. The development of the turnover or utilization ratios for dopamine (DA), noradrenaline (NA) and serotonin (5-HT) as indicators of general monoamine activity from 24-h urine samples across four age-groups of healthy children, adolescents and young adults (8–22 year olds). (Oades, 2002:98).

Oades concluded that NA activity for ADHD children is lower than in healthy controls (Figure 2.10). The right diagram in figure 2.10 shows that the DA activity (HVA/MHPG) is higher in the ADHD group than in the controls. This increased ratio HVA/MHPG may reflect low levels of MHPG and/or high levels of unused noradrenaline (Figure 2.10). In ADHD they experience relatively high DA activity to the relatively low level of NA activity, thus explaining the widely-reported impaired performance of ADHD children. The findings of Oades advance the hypothesis that DA is hyperactive with respect to NA activity, but often hypoactive with respect to 5-HT activity in certain manifestations of ADHD (Oades, 2002:98).

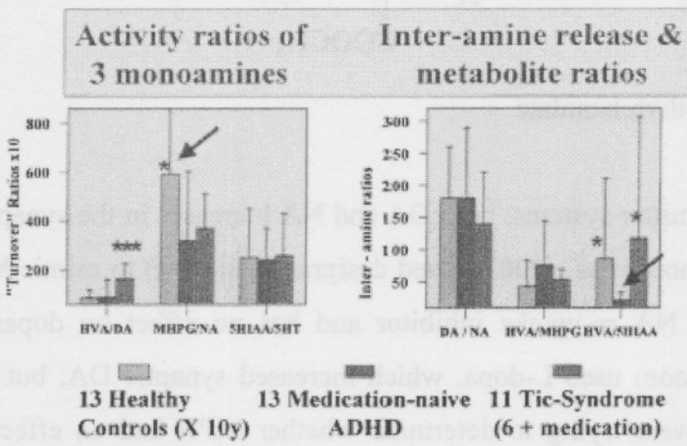


Figure 2.10 On the left, the turnover ratios for dopamine (DA), noradrenaline (NA) and serotonin (5-HT) as indicators of general monoamine activity from 24-h urine samples for groups of healthy children, ADHD children and complex-tics/Tourette syndrome. On the right, the ratio of the catecholamines, the ratio of their metabolites and the ratio of the dopamine to serotonin metabolites (Oades, 2002:99).

2.7.6.2 Serotonin (5-HT)

Recent evidence shows that there is an apparent increase in serotonin metabolism in ADHD patients. This is an unexpected finding because increased 5-HT metabolism is at odds with an association with impulsivity recorded in non-ADHD cases. This only shows the different opinions people have about the meaning of 'impulsivity' in ADHD and non-ADHD context. While there can remain little doubt that 5-HT activity may modulate features of the ADHD syndrome, the mechanism requires attention. The effect of raising or suppressing 5-HT activity is dependant on the dopamine activity.

Oades (2002:100) indicated that the study of HVA/5-HT ratios in ADHD has certain pitfalls. Figure 2.10 shows that while DA utilization may gradually increase across adolescence, that for 5-HT may show more of an inverted U-pattern with maturation. Nonetheless, if this caveat is put to one side, it may be seen in figure 2.9 that in the control group HVA/5-HT ratio is much depressed compared to the other two groups studied.

2.7.7 Effect of medication on ADHD

Methylphenidate (MPH), the active compound in Ritalin®, is one of the drugs that can be used to decrease symptoms of both attentional deficiency and impulsivity/hyperactivity (Figure 2.11).

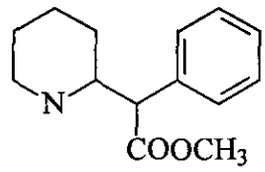


Figure 2.11 Structure of Methylphenidate

MPH affects two neurotransmitter systems: both DA and NA increases in the synaptic cleft. MPH blocks the re-uptake of NA. Overtoom *et al.* (2003:) used desipramine (DMI) to mimic MPH's effect in a case study. DMI is a selective NA re-uptake inhibitor and has no effect on dopaminergic activity. In comparison with this, Overtoom used L-dopa, which increased synaptic DA, but had no effect on NA. With this experiment they were trying to determine whether MPH had an effect on inhibition and/or attention and if the action of MPH was more dopaminergic than noradrenergic. They found that the effect could not be only one or the other. It could not only be noradrenergic seeing that DMI, which works on the noradrenergic pathway, would have improved the attention, which was not the case. The effect could not be totally dopaminergic either because L-dopa that works dopaminergically did not have an effect on attentional measures either (Overtoom *et al.*, 2003:6).

Although the study above showed that MPH had a positive effect on attention, there was no effect on the percentage of inhibition of response related processes. In contrast with this there were other studies that found that MPH had an effect on the impulsivity (Overtoom *et al.*, 2003:2). There are two possible answers for this: One being the way in which impulsivity is defined; different neural systems might be underlying the different operationalizations of impulsivity and the other explanation for the absence of an ameliorating effect of MPH on inhibition may be that the dose of MPH used was too low (Overtoom *et al.*, 2003:6).

Hyperactivity and perhaps poor motor impulse control in ADHD can be the result of increased dopaminergic activity in the striatum. This can be deduced from the observation that there is increased striatal activity in adolescents with ADHD compared to their controls as well as high cerebrospinal fluid homovanillic acid (HVA) levels in children with ADHD. High levels of HVA can be correlated to increased severity and a more positive response to stimulants. Hyperactivity is lowered by stimulants through reduced dopaminergic activation in the striatum (Solanto, 2002:68).

Aspartame (aspartyl-phenylalanine methyl ester), which is a Phe derivative, fulfils the same role as Ritalin® (Figure 2.12). Ritalin® elevates Phe levels in the body and therefore plays a role in the forming

of neurotransmitters, seeing that Phe are converted to dopamine, adrenaline and noradrenaline. Aspartame helps to keep the symptoms of ADHD under control.

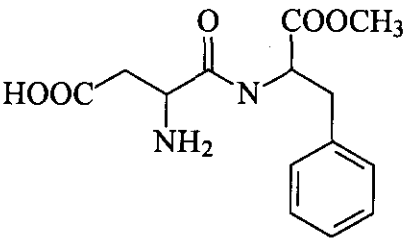


Figure 2.12 Structure of Aspartame

Too much Phe is also dangerous, because in excessive quantities it acts as a neurotoxin and excites the neurons in the brain to the point of cellular death. People with PKU should especially be careful not to consume too much Phe seeing that they lack the enzyme to break Phe down. Too much Phe can cause irreversible brain damage and death, especially when used in high quantities or during pregnancy. ADHD, emotional and behavioural disorders can all be triggered by too much Phe in the daily diet (Starr Hull, 2002).

A new product namely Attend®, containing amino acid combinations, essential fatty acids, lipid complexes, homeopathic medicines, hormone precursors and the precursors to specific neurotransmitters are being used to improve the lives of children and adults with ADHD. The amino acids Attend® contains promote the production of various neurotransmitters and enzymes critically needed in brain metabolism. Amino acids allow smooth, balanced cognition and fluid transition from thought to disciplined action. This product also aids in the reduction of stress, frustration and cognitive overload. Attend® addresses the symptoms of ADHD without the harmful Ritalin® (MPH) side effects. Attend® contains Phe which is used to synthesize dopamine and norepinephrine in the human body (New ideas, 2004) (Figure 2.13).

2.8 DOPAMINE

Dopamine is a central neurotransmitter and possesses important intrinsic pharmacological properties. Dopamine is a substrate for both mono-amine oxidases and carboxy-oxy-methyl transferase and thus is ineffective when administered orally. Large amounts of DA are found in brain areas like the basal ganglia, the nucleus accumbens, the olfactory tubercle, the central nucleus of the amygdala, the median eminence and restricted fields of the frontal cortex (Hoffman *et al.*, 1996:120).

There are basically six steps involved in DA acting as a neurotransmitter: synthesis, storage, release, binding, reuptake and degradation.

2.8.1. Synthesis

The synthesis of dopamine (figure 2.13) involves a process driven by enzymes. The reaction catalysed by tyrosine hydroxylase is rate limiting because it is most easily saturated with the substrate (Murray, 2000:840-841).

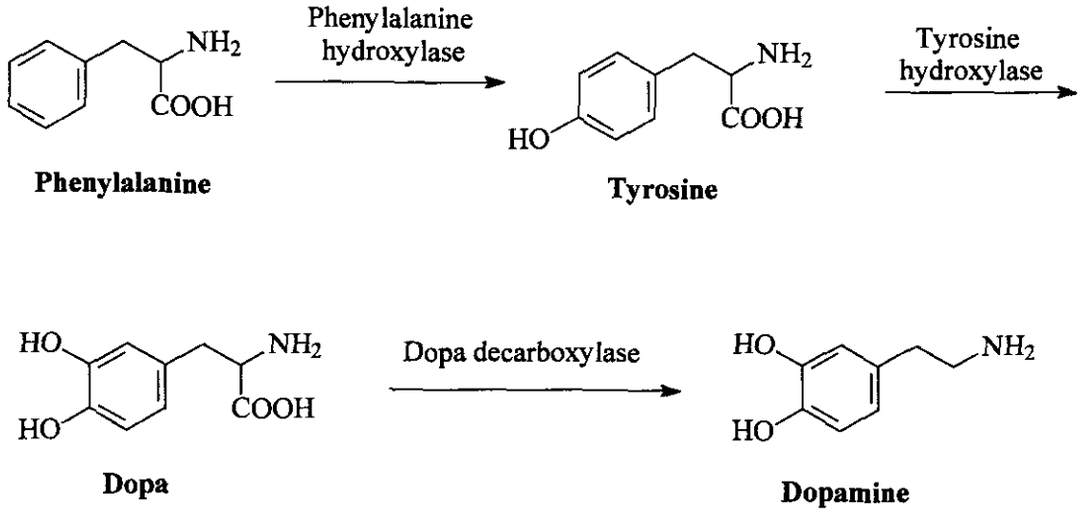


Figure 2.13 Steps in the enzymatic synthesis of Dopamine (Hoffman *et al.*, 1996:120).

2.8.2. Storage

DA is stored in synaptic vesicles. Entry of DA into the vesicles is driven by a pH gradient established by a protein present in the vesicular membrane that pumps protons into the vesicle at the expense of ATP (Figure 2.14) (Balfour *et al.*, 1998:1022).

2.8.3. Release

DA release involves exocytosis; it includes the fusion of the vesicles with the presynaptic membranes. In the resting state (the voltage difference between the inside and outside of the cell in absence of excitatory or inhibitory stimulation), single quanta are released spontaneously, resulting in small miniature endplate potentials (depolarization of motor end plate and then release of dopamine). When a nerve ending is depolarized by transmission of a nerve impulse, this process opens voltage-sensitive Ca^{2+} channels, permitting an influx of Ca^{2+} from the synaptic space into the nerve terminal. This Ca^{2+} plays an essential role in the exocytosis that releases DA into the synaptic space (Figure 2.14).

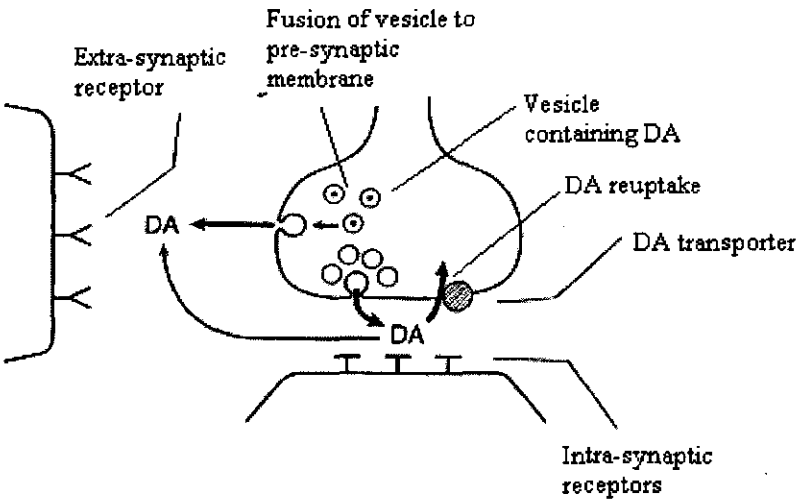


Figure 2.14 Diagrammatic representation of a DA terminal. This figure summarizes the two putative mechanisms by which DA may be released into the extrasynaptic space and gain access to extrasynaptic DA receptors. It may either escape from the synaptic cleft or be released directly into the extrasynaptic space from vesicles that release neurotransmitter preferentially in response to burst firing. Dopamine is recycled and can again be stored in vesicles to be reused (Balfour *et al.*, 1998:1022).

2.8.4 Binding

After its release DA binds to its postsynaptic receptors. The amine reaches its receptors by diffusion across the synaptic cleft. There are at least five different DA receptors, known as D1, D2, D3, D4 and D5. The different receptor isoforms may be grouped into the D1-receptor family (D1 and D5) and the D2-receptor family ($D2_{short}$, $D2_{long}$, D3 and D4). In the brain D1-receptors and D2-receptors are much more abundant in the striatum and substantia nigra neurons than the other three subtypes, D3, D4 and D5 (Ohno *et al.*, 1987:1938). Dopamine receptors belong to the G-protein-coupled receptor (GPR) family which includes receptors such as serotoninergic, adrenergic and neuropeptide receptors. The common structural features of the GPR's are: the seven hydrophobic transmembrane domains, the extracellular amino terminus, the cytoplasmic C-terminus domain and the G-protein-coupling sites in the third plasmic loop (Ogawa, 1995:3). They produce their effector actions by affecting adenylyl cyclase positively or negatively or in at least one case, by affecting another signalling system like phospholipase C and the polyphosphoinositide cycle (Murray, 2000:840-841).

2.8.5 Reuptake

Reuptake of dopamine occurs. The reuptake is achieved by a high-affinity transporter, which uses ATP, present in the presynaptic membrane. The recycled DA can again be incorporated into synaptic vesicles and reused as a transmitter (Figure 2.14) (Balfour *et al.*, 1998:1022).

2.8.6. Degradation

Degradation of dopamine happens in the synaptic cleft or follow reuptake within the presynaptic terminal. Monoamine oxidase B (MAO B) is present in the outer membrane of mitochondria within the presynaptic terminal and is also present in the synaptic cleft. MAO B and MAO A are distinguished from each other by the different substrates they metabolize and their reactions to inhibitors. Both of them act on DA and 3,4-dihydroxyphenylacetaldehyde (DOPAC) is formed. DOPAC is then later converted to homovanilic acid by the action of catechol-O-methyl-transferase (COMT). Another pathway exists as well where COMT converts the dopamine first to form 3-methoxytyramine, which is then converted to homovanillic acid by MAO (Figure 2.15). The level of homovanilic acid in the cerebrospinal fluid can be used to follow dopamine metabolism in the brain (Murray, 2000:840-841).

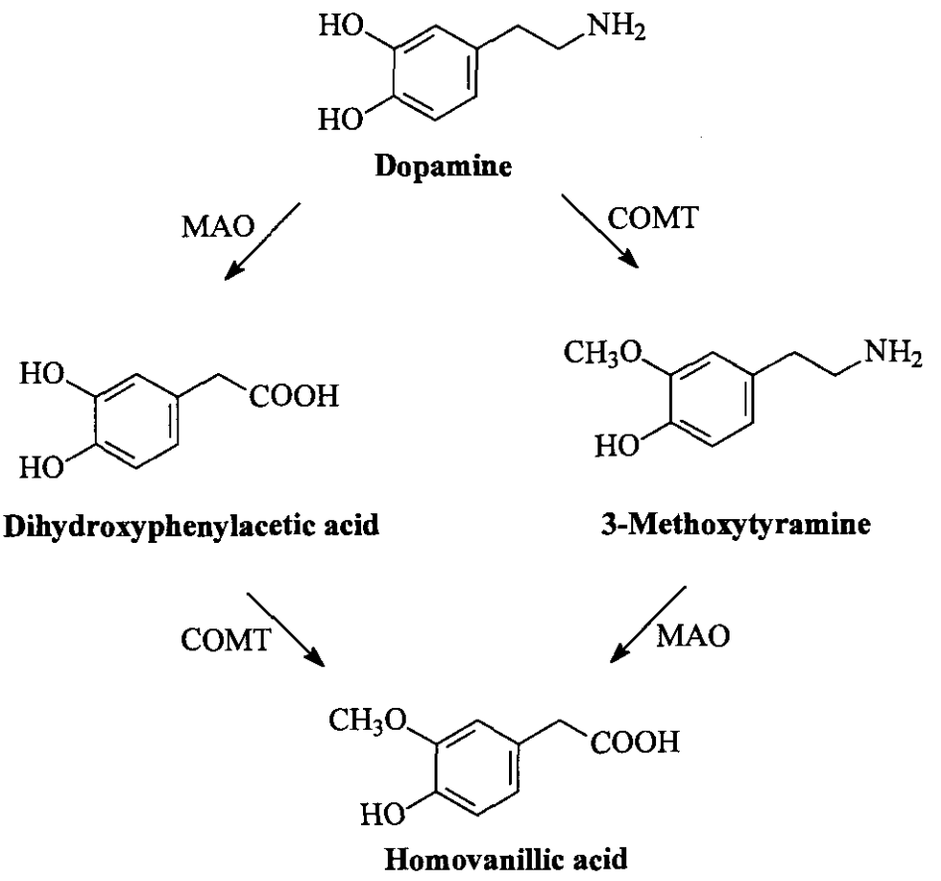


Figure 2.15 Degradation of dopamine (Granner, 2000:589).

2.9 PHENYLALANINE

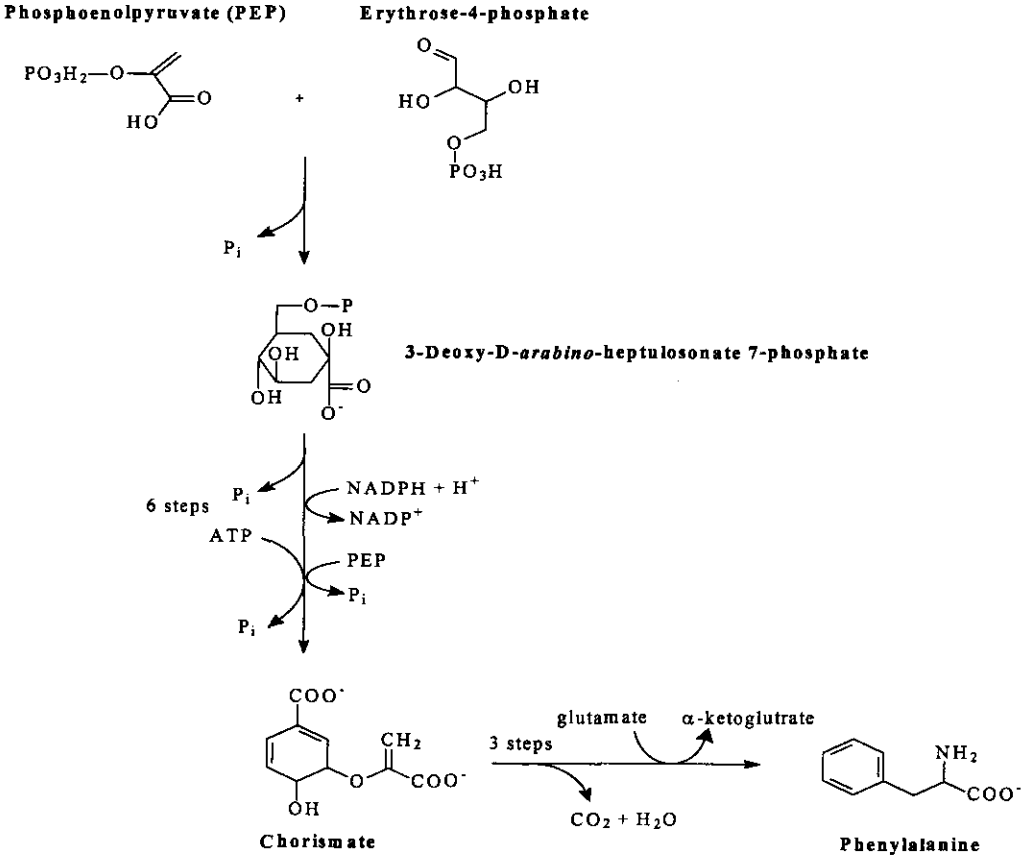
As mentioned earlier Phenylalanine is an essential amino acid. Amino acids form the building blocks of protein. Phenylalanine is available in three chemical forms:

- L-phenylalanine, the natural form found in proteins throughout the body
- D-phenylalanine, a mirror image of L-phenylalanine that is synthesized in a laboratory
- DL-phenylalanine, a combination of the previous two forms ((Rodwell, 2000:29).

The body converts phenylalanine into tyrosine, another amino acid essential for making proteins, certain brain chemicals and tyroid hormones ((Rodwell, 2000:311).

2.9.1 Synthesis

The shikimate (common aromatic biosynthesis) pathway plays a pivotal role in the production of precursors for aromatic compounds in microorganisms and plants. In microorganisms, the pathway serves primarily in the production of the aromatic amino acids required for protein synthesis. In plants, the pathway provides the precursors of phenylalanine, tyrosine and tryptophan, but also precursors for a very diverse range of other aromatic compounds derived from chorismate, the end product of the shikimate pathway (Weaver & Herrmann,



997:346).

Figure 2.14 Synthesis of phenylalanine (Paustian, 2004:6).

Synthesis of the aromatic amino acids begins with the synthesis of chorismate, which is an important intermediate for many biosynthetic pathways. Phosphoenol pyruvate (PEP) and erythrose 4-phosphate serve as beginning substrates for the synthesis of chorismate. NADPH + H^+ and one ATP are used to form every chorismate (Figure 2.16). In the sixth cycle of the synthesis another phosphoenol pyruvate molecule is added to the growing molecule (Paustian, 2004:5). Chorismate is converted to phenylpyruvate in two steps and phenylalanine is synthesized by a transamination reaction with glutamate (Figure 2.16). No energy is required for this reaction.

2.9.2 Transport

Phenylalanine is mainly transported through System L, because Phe is a large neutral amino acid. In a study done by Berger (2000) the transport of L-Phe across Caco-2 cell monolayers grown on microporous filters showed that apical (AP) - to basolateral (BL) transport of L-Phe is temperature dependent, saturable and inhibited by metabolic inhibitors. The study also showed that the AP uptake exhibits characteristics very different from those of the BL uptake. The major carrier in the AP uptake resembles the Na^+ -dependent $B^{0,+}$ system, whereas the carriers involved in the BL uptake include the Na^+ - dependent $B^{0,+}$ and ASC systems as well as the Na^+ - independent L system (Berger *et al.*, 2000:2780-2781).

2.9.3 Anaerobic metabolism

In a study done by Hamid *et al.* (1997) to determine the inhibitory effect of metronidazole on the anaerobic metabolism of phenylalanine the following products were formed:

- phenylpropionic acid
- cinnamic acid
- phenyllactic acid
- phenylacetic acid
- isocaproic acid

The cells from the organism *Peptostreptococcus anaerobius* used in the study produced phenylpropionate from Phe as the main product (Figure 2.17). The cells produced hydroxylated products, i.e. phenyllactate, along with other non-hydroxylated products. The hydroxylated products were not the main end products, but under aerobic conditions the main end products would be the hydroxylated products (Hamid *et al.*, 1997:130-131).

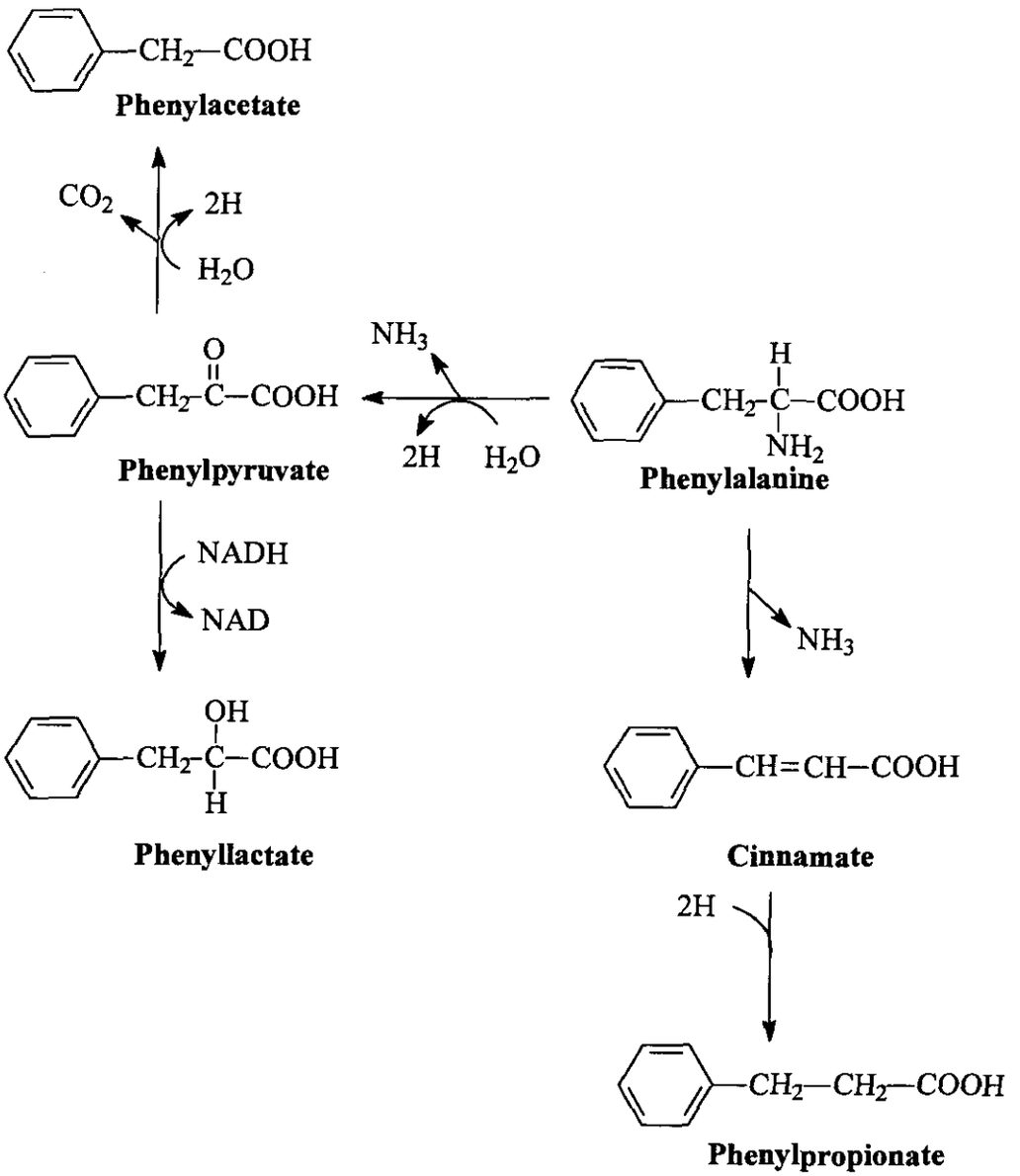


Figure 2.17 Proposed phenylalanine degradation pathways (Hamid *et al.*, 1997:133).

2.9.4 Diseases linked to phenylalanine

2.9.4.1 Phenylketonuria

Phenylkentonuria (PKU) is a rare metabolic disorder that occurs in people who are missing an enzyme, phenylalanine hydroxylase, required to properly metabolise phenylalanine, which then results in elevated levels of phenylalanine and phenylalanine metabolites in the blood and urine. PKU is inherited as an autosomal recessive trait, which means that it a genetically defaulted autosome with a characteristically defect. The mutation that causes PKU is located on chromosome 12. Newborn screening is very important for the diagnosis of this disease and has largely eliminated mental retardation as a consequence of this disease. Early diagnosis is made when high phenylalanine and low tyrosine levels are detected

during the screening of a newborn. The symptoms tend to appear between three and six months of age and include the following:

Symptoms in newborns:

- Symptoms are usually absent
- May be abnormally drowsy or listless
- Feeding difficulty
- Light hair, skin and eyes
- Eczema
- Severe mental retardation if not treated

Symptoms in children

- Seizures
- Nausea
- Vomiting
- Hyperactivity
- Aggressive behaviour
- Clumsy
- Poor coordination
- Abnormal gait
- Abnormal posturing
- Self-injurious behaviour
- Mousy body odour

The only treatment for PKU is diet restricted in phenylalanine. Phenylalanine must be restricted starting in the first few weeks of life. Infants with PKU should be breast fed along with phenylalanine free special formula. A diet should be planned with adequate calories and amino acids to ensure sufficient growth and development.

Certain vegetables, fruits, grains and other low phenylalanine foods should be added later. Regular milk, cheese, eggs, meat and fish should not be allowed. These patients must remain on a restricted diet throughout life. A restricted diet, started early and well maintained, makes normal development possible and prevents mental retardation (Kolpuru, 2000:3).

2.9.4.2. Hartnup's disease

Hartnup's disease (HD) is biochemically characterized by defective transport of neutral amino acids, including tryptophan, across the intestine and kidney. This suggests a defect in the Na^+ dependant System B^0 (See 2.4 Amino acid transport) that transports these amino acids. This disease is probably autosomal recessive. HD occurs at a frequency of approximately 1 in 40 000 in urine amino acid screens and has an infantile or childhood onset. This disease was named after the family in which it was first discovered (Torres-Zamorano *et al.*, 1997:259).

In HD patients' kidney amino acid transport has not been directly assessed, but plasma amino acid levels are usually normal or low, indicating a transport defect in the kidney tubule. The intestinal amino acid

transport defect is similar to that of the kidney, at least in some patients. Using a direct measure of transport, several investigators have reported deficient amino acid uptake by the brush border of intestinal mucosa (Symula *et al.*, 1997:102).

HD differs from other amino acid transport deficiencies like cystinuria or lysinuric protein intolerance, in which only a few structurally similar amino acids are affected. HD affects the transport of a large number of neutral amino acids, including alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan and histidine (Symula *et al.*, 1997:102)

Signs and symptoms include the following:

- Pellagra-like skin rash, intermittent red, scaly rash across the face, neck, hands and legs, which is caused by a niacin deficiency (Symula *et al.*, 1997:102)
- Neurologic exacerbations
- Cerebellar atrophy
- Gliosis
- Growth failure and developmental delay
- Episodic personality disorder and uncontrolled temper
- Psychosis
- Episodic cerebellar ataxia (Neurohelp, 2004).

Attacks of this disease are triggered by exposure to sunlight, emotional stress and sulphonamide drugs and last for about two weeks. 50% of children progressively develop mental retardation.

HD can be diagnosed by an increase in urine amino acid levels (5-10 times normal amounts) as well as elevated amino acid levels in the faeces. Plasma levels of the affected amino acids' levels are normal to low. There are large amounts of indicans, mainly indoxyl sulphate, excreted especially after L-tryptophan loading. These patients also excrete abnormally high levels of nonhydroxylated indole metabolites (Symula *et al.*, 1997:102).

Administration of nicotinic acid appears beneficial in improving neurologic and dermatologic symptoms. Normally, oral amino acid challenge results in a transient increase in plasma levels of the amino acid, but in HD this effect is much reduced and and may be delayed (Symula *et al.*, 1997:102).

It is generally agreed that the clinical features of HD are due to the defective intestinal absorption of tryptophan, rather than to excessive amounts of tryptophan that is excreted through a defect in the kidney

tubules. This agreement is based on the concept that with a normal absorption, the renal losses, even while important, would not in themselves contribute sufficiently to establish such a florid clinical picture. This can be seen in the fact that when tryptophan ethyl ester is fed to children with HD, serum tryptophan promptly increases and the clinical signs of the disease are reversed. A tryptophan deficiency is thus the main cause of HD (Liberato, 2004:1).

A study (Symula *et al.* 1997) involving mice were done to understand the physiology and genetics of amino acid transport, as well as the genetic and environmental factors that resulted in the transition from a healthy to a pathological state of HD in mice. Among several recessive mutations in the mouse, isolated on the basis of delayed clearance of a Phe challenge, one type was defective in amino acid transport and provided a possible model for HD. This mouse model was named HPH2. The outcome of the study revealed that in HPH2 mice the levels of amino acids were elevated in their urine. The study also suggested that the delay in Phe clearance was not due to a defect in Phe catabolism. For assessment of whether the mutant mouse was defective in amino acid transport, plasma and urine amino acids from mutant and non-mutant mice were quantitated. The results of amino acid screening suggested a specific deficiency in amino acid transport in the mutant mouse's kidney, as could be deduced from the observation of elevated levels of amino acid accumulated in the vesicles of the kidney cells (Symula *et al.*, 1997:102).

2.10 β-*m*-HYDROXYPHENYLHYDRACRYLIC ACID (BHHA)

BHHA is a polyphenolic organic acid (Figure 2.18). A phenolic molecule is often characteristic of a plant species or even of a particular organ or tissue of that plant. It is therefore impossible to know precisely the nature of all the polyphenols we ingest. It is however, desirable to know the main classes of the polyphenols consumed the main foods that contain them and their contents in these foods (Scalbert & Williamson, 2000:2074S).

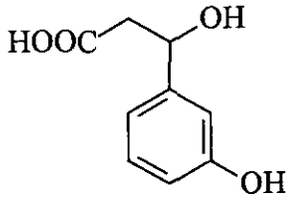


Figure 2.18 Structure of BHHA

This metabolite (BHHA) has been unequivocally identified as one of the major phenolic acids commonly present in human urine. It is suggested that BHHA is derived from an unknown compound in the diet and that it will be fruitless to attempt to ascribe any significance to a variation in their excretion. BHHA was observed in larger amounts in the urine of mentally ill patients (Armstrong & Shaw, 1956:276).

Polyphenols, such as BHHA, are receiving increasing interest from consumers and food manufacturers for several reasons. Polyphenol-rich foods or beverages help to prevent disease (Scalbert & Williamson, 2000:2073S). It is also an antioxidant and together with other dietary antioxidants, such as vitamin C, vitamin E and cartenoids, are thought to protect body tissue against oxidative stress. The chemical structure of polyphenols will affect their biological properties: bioavailability, antioxidant activity, specific interactions with cell receptors and enzymes and other properties (Scalbert & Williamson, 2000:2073S).

2.10.1 Metabolism by gut flora

Polyphenols that are not absorbed in the stomach or small intestine, will be carried to the colon (Figure 2.19). In addition, polyphenols that are absorbed, metabolised in the liver and excreted in the bile will also reach the colon but in a different chemical form such as a glucuronide. This different chemical form applies to polyphenols that are directly transported back from the enterocyte to the small intestine (Schalbert & Williamson, 2000:2087S).

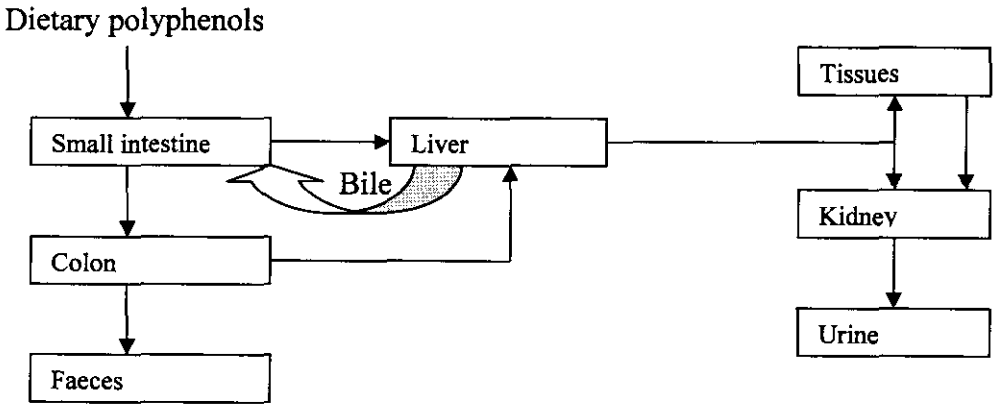


Figure 2.19 Possible routes for consumed polyphenols in humans (Scalbert & Williamson, 2002:2087S).

Unlike enzymes in human tissues, colonic microflora catalyze the breakdown of the polyphenol itself to more simple compounds, such as phenolic acids. For example: In a study by Baba *et al.* (1983) quercetin-3-O-rhamnoside was incubated anaerobically with human intestinal bacteria. Quercetin, 3,4-dihydroxyphenylacetic acid and 4-hydroybenzoic acid were found as metabolites. No unchanged quercetin-3-O-rhamnoglucoside and quercetin were found in human urine after the administration of the

original compounds, but metabolites from breakdown by colonic microflora, 3-hydroxy-phenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxytoluene and β-*m*-hydroxyphenylhydracrylic acid were present in the urine. This speculates that BHHA is a metabolite that is formed in the human body by microflora in the intestine (Schalbert & Williamson, 2000:2087S).

We will attempt to determine with this study if the presence of BHHA is the cause of a Phe malabsorption in the intestine or a defect in the Phe metabolic pathway. We will also investigate if the Phe malabsorption is the result of malfunctioning of P-gp, peptide transporter or the amino acid L-transport system and if together with elevated mercury concentrations in patients, all which influence transport systems, could contribute to ADHD.

Chapter 3 describes all the experimental procedures employed to test the hypotheses of the study. Chapter 4 contains the results obtained and in Chapter 5 conclusions are drawn from all the results obtained.

CHAPTER 3

METHODS

This study protocol was approved and done in accordance with the guidelines stipulated by the Ethics Committee for use of humans at the North West University.

In order to determine the diagnostic value of β-*m*-hydroxyphenylhydracrylic acid a case study of more than a thousand patients was used. The urine of the patients were analysed for BHHA using GC-MS analysis. The urine was also analysed for the presence of other abnormal organic acids.

3.1 DETERMINATION OF URINARY ORGANIC ACIDS

For the determination of urinary organic acids, a traditional method, employing gas chromatography-mass spectrometry was used.

3.1.1 Chemicals and reagents

Chemicals and reagents used in this section are listed in table 3.1.

Table 3.1 Chemicals and reagents used in the determination of urinary organic acids.

Chemical or reagent
Hydrochloric acid
3-Phenylbutyric acid
Ethyl acetate
Diethyl ether
Sodium sulphate (anhydrous)
Nitrogen
TMCS
BSTFA

3.1.2 Instrumentation

The instrumentation and conditions employed in the analysis of urinary organic acids are summarised in table 3.2.

Table 3.2 Analytical conditions in the GC-MS determination of urinary organic acids

Software	Hewlett Packard, Palo Alto, CA
GC system	Hewlett Packard 6890 series GC system (Hewlett Packard, Palo Alto, CA)
MS system	Hewlett Packard 5973 Mass Selective Detector (Hewlett Packard, Palo Alto, CA)
Column	Permabond® SE30 fused silica capillary column, 25m x 0.32 mm and film thickness, 0.25 μm.
Mobile phase	Helium
Flow rate	2ml/min
Temperature programming	Initial temperature of 70°C for two minutes, then increased to 280°C with increments of 5°C/min. Final temperature maintained for 3 min.
Software used for processing of mass spectral data	Wsearch32® WsearchPro® AMDIS®

3.1.3. Creatinine determination

The creatinine content of urine samples was determined prior to extraction. The creatinine content provides an estimate of the urine concentrate. The urinary organic acid content can then be calculated and expressed in terms of the creatinine content, thus allowing for intra individual physiological differences in the rats. Creatinine was determined using a Technicon RA-100 analyser. Urine was diluted 10 or 20 times and analysed according to the prescriptions of the manufacturer (Miles Inc., Tarrytown, NY).

3.1.4 Organic acid extraction

The volume of urine used for extraction of organic acids was determined according to the creatinine values (mg%). [mg% = μmol/litre x 10/1000 x 11.312]. For creatinine values < 100mg%, 1 ml of urine

was used, for creatinine values between 100 mg% and 135 mg%, 0.5 ml of urine was used, and for values > 135 mg%, 0.25 ml of urine was used. This volume was transferred to a 15 ml glass tube (Pyrex®) and ± 6 drops of HCl (5 M) were added to adjust the pH to 1. Internal standard (3-phenylbutyric acid) was added [volume IS in µl = 5X creatinine mg%]. Organic acids were extracted using two solvents: first 6 ml distilled ethyl acetate was added and the samples vigorously shaken for 30 min. The samples were centrifuged for 3 min at 300 rpm and the organic phases aspirated into clean tubes. Thereafter 3 ml of distilled diethyl ether was added to the aqueous phase and again shaken for 10 min. After centrifugation, the organic layer was added to the ethyl acetate phase. The organic phase was dried over two spatulas of Na_2SO_4 and centrifuged, where after it was transferred to clean smaller glass tubes (Kimax®). The organic phase was evaporated to dryness under nitrogen at 40 °C and the dry samples were frozen at –18 °C.

Prior to injection into the GC-MS system, BSTFA [volume in µl = 3X mg% creatinine] and TMCS [volume in µl = 0.6 X mg% creatinine] were added to the dried samples, which were then incubated at 60 °C for 1 hour to form TMS derivatives.

The results were analysed with the computer aided programme AMDIS®.

3.2. ABSORPTION STUDIES

The nineteen patients with the highest BHHA levels were chosen for more in depth investigation. Diagnostic and clinical profiles were drawn up for each of the nineteen patients with the aid of their respective medical doctors. Phenylalanine loading (100 mg/kg body weight) was performed on two of these patients. The required amount L-Phe was dissolved in orange juice and administered at 0 hour. Urine and blood samples were taken at 3, 7 and 11 hours after loading. This was followed with a tryptophan loading while one of the patients also received aspartame.. Another patient, suffering from PKU, also received a Phe loading dosage. Three hours after the L-Phe loading, tetrahydrobiopterin (BH_4, 20 mg/kg body weight) was administered to this patient.

3.2.1 Phenylalanine determination using MS-MS

Phenylalanine and tyrosine, after the loading, were determined by mass spectrum- mass spectrum (MS-MS).

Stable isotopes for quantification were purchased from Cambridge Isotope laboratories, Andover, MA 018810-5413 USA. The equivalent of 10 µl of urine samples were enriched with 400 µl of the

methanol/stable isotope mixture, which contained 5.77 nmol/ml of d_5-phenylalanine as quantification isotope, vortexed and the methanol evaporated on a Techne DB-3 Dri-Block at 70 °C under a stream of nitrogen. The samples were then butylated with methanolic-HCl (3M HCl in methanol) and the excess derivatization reagent was evaporated under nitrogen. The derivatized mixtures were dissolved in 100 µl of a 99:1 water/acetonitrile mixture that contained 0.1% formic acid. The HPLC used was a Hewlett Packard 1090 (Waldbronn, Germany). Sample introduction was via a Hewlett-Packard 1090 autosampler and samples were subjected to chromatographic separation on a Phenomenex Luna 5µ C18(2), 250mm x 2.00mm column. The mobile phase was a mixture of acetonitrile and water and a gradient was effected, starting at 1% asetonitrile and increased linearly to 60% asetonitrile after 20 minutes. The flow rate was 200 µl/ min and the HPLC-oven kept constant at 45 °C. The Micromass Quatro II instrument was operated in the MRM acquisition mode to monitor the specified transitions for butylated derivatives of tyrosine (m/z 238 - 136), phenylalanine (m/z 222- 120) and d_5-phenylalanine (m/z 227-125). The electrospray capillary tip was set at 3.5 kVolts, the HV lens at 0.5 kVolts while the cone lens was held at 35 volts. The source temperature was 90°C and resolution in both mass analyzers on 14.5. Collisional induced decomposition was achieved with argon as collision gas and a collision energy setting of 12 volt. The deteruated phenylalanine stable isotope was used to quantify both phenylalanine and tyrosine levels in patient samples.

CHAPTER 4

RESULTS

4.1 ORGANIC ACIDS

The GC-MS analysis showed that the concentration of BHHA ranged from minute concentrations to more than a 1000 mmol/mol creatinine. Individual BHHA concentrations are given in Appendix I. A scatter plot to indicate the distribution of the BHHA levels are displayed in Figure 4.1.

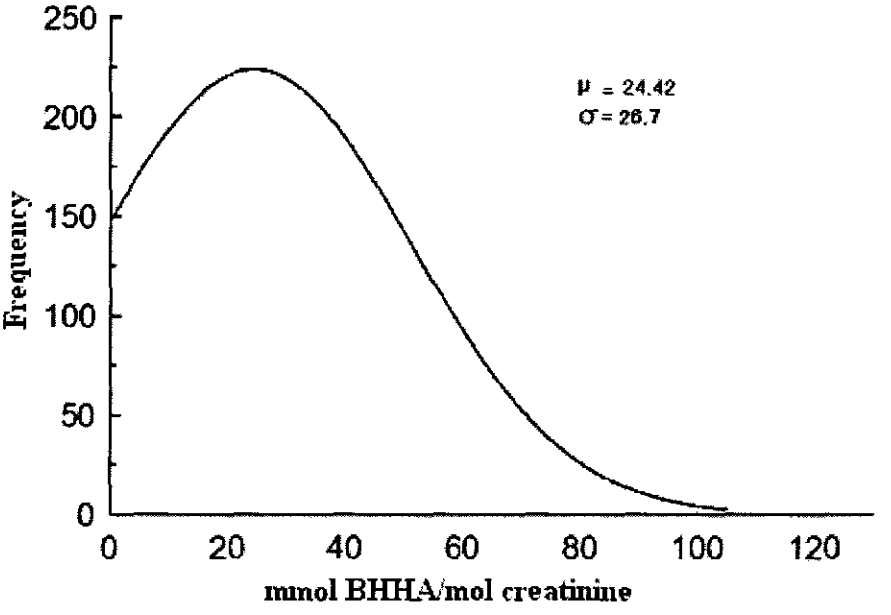


Figure 4.1 Scatter plot of BHHA concentration (μ – average, σ - standard deviation).

Figure 4.1 shows that most of the patients had fairly low concentrations BHHA, where some patients had significantly elevated BHHA concentrations. Nineteen patients had drastically elevated levels of BHHA, the levels ranging from 700-16 000 mmol/mol creatinine (Table 4.1).

Table 4.1 Patients with elevated BHHA levels

	PATIENT NUMBER	BHHA CONCENTRATION
1	12/09/05/03	753.034
2	01/24/01/03	725.336
3	06/21/01/03	728.213
4	07/17/01/03	715.129
5	07/09/04/03	762.291
6	03/12/05/03	862.130
7	06/14/04/03	866.163
8	20/24/04/03	1967.719
9	03/23/01/03	1121.135
10	06/27/02/03	1981.953
11	02/25/03/03	1495.186
12	07/31/03/03	1901.912
13	09/04/02/03	2479.481
14	11/20/02/03	2634.311
15	04/04/04/03	2515.687
16	11/14/05/03	6291.271
17	06/20/02/03	8352.801
18	04/29/02/03	9909.199
19	12/20/03/03	15622.730

Figure 4.2 gives an example of a chromatogram of a patient with abnormally elevated levels of BHHA. This chromatogram can be compared with a chromatogram of a normal subject in Figure 4.3. The identification of the peaks in Figure 4.2 and 4.3 are given in Table 4.2. The chromatograms of the patients in the elevated group (Table 4.1) are displayed in Appendix II.

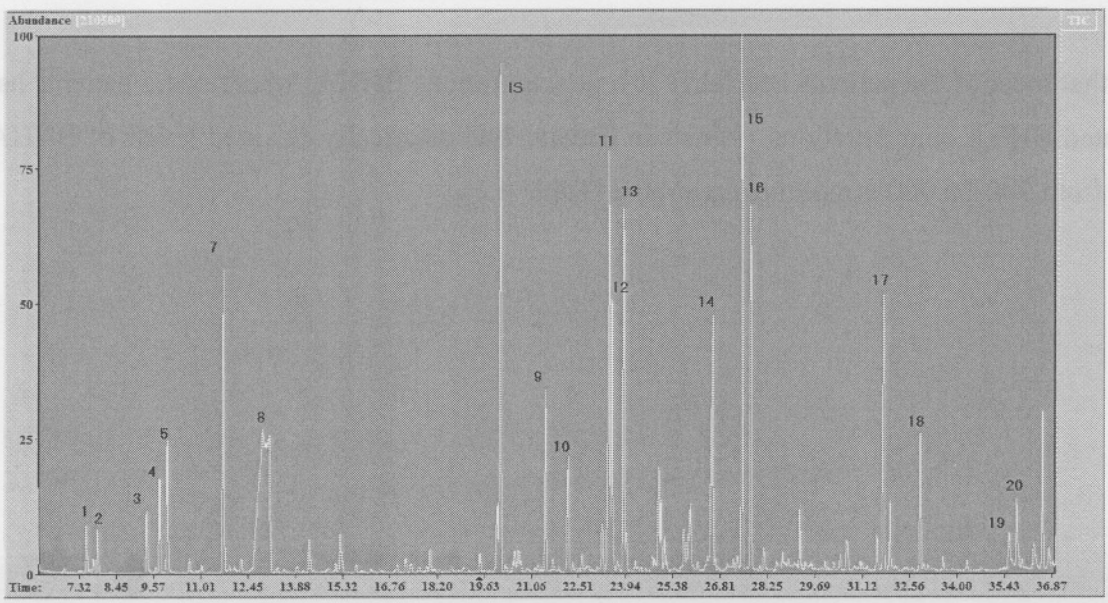


Figure 4.2 A typical chromatogram of a patient with abnormally elevated BHHA levels.

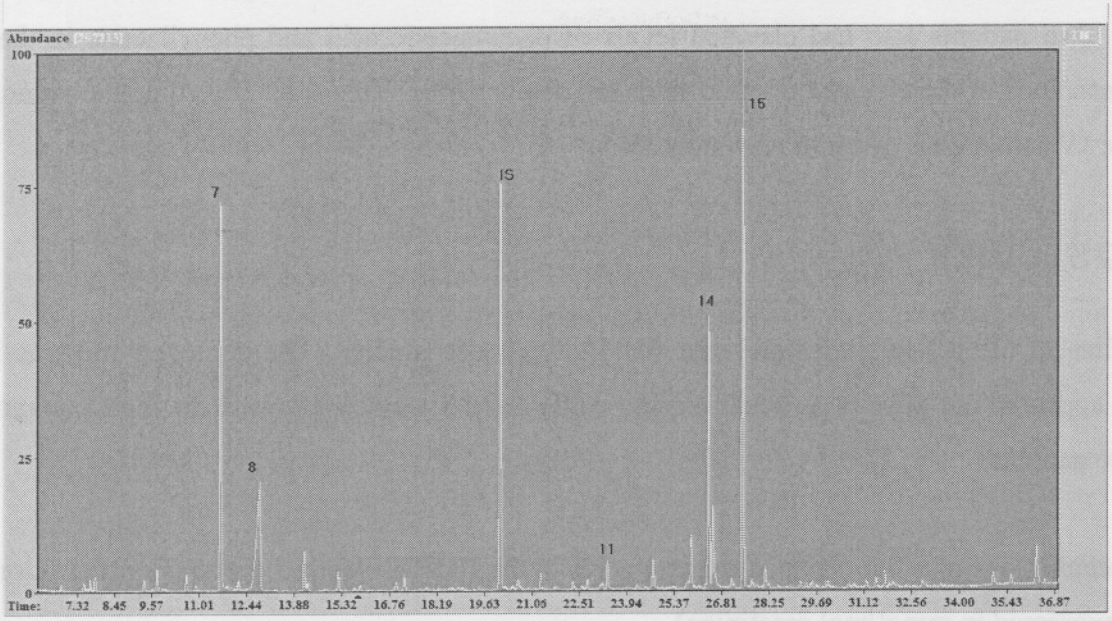


Figure 4.3 A typical chromatogram of a patient with normal organic acid levels.

Table 4.2 Identification of peaks in Figure 4.2 and 4.3.

COMPONENT	PEAK (Figure 4.2)	PEAK (Figure 4.3)
Lactic- DITMS2	1	
Glycolic acid-TMS	2	
Oxalic acid-DITMS	3	
p-Cresol-TMS	4	
m-Cresol-TMS	5	
p-Cresol-TMS	6	
Urea	7	7
Urea	8	8
5-Hydroxymethylfuranecarboxylic acid-DITMS	9	
Pipecolic acid-DITMS	10	
p-Hydroxyphenylacetic acid-DITMS	11	11
5-Hydroxymethyl-2-furanecarboxylic-DITMS	12	
Furan-2,5-dicarboxylic-DITMS	13	
Hippuric-TMS	14	14
Citric-TETRATMS	15	15
B-m-Hydroxyphenylhydracrylic-TRITMS	16	
p-Hydroxyhippuric acid-DITMS	17	
Phenylacetylglutamine	18	
Isovanilylglycine	19	
Benzoylsuccinateglycine	20	
Internal Standard	IS	IS

Patients with elevated BHHA levels also showed elevated levels of other organic acids (mass spectrum of BHHA in Appendix III), including: *p*-hydroxyphenylacetic acid, *m*-hydroxyhippuric acid, *p*-hydroxyhippuric acid and *p*-hydroxyphenyllactic acid. These patients also excreted a few unique

metabolites: β-*m*-hydroxyphenylhydracrylglycine, phenylacetylglycine, phenylacrylylglycine (isovalylylglycine) and benzoylsuccinic acid. The mass spectrums of the unique organic acids are given in Appendix III. Two patients also had elevated levels of phenylacetic acid and phenyllactate in their urine. The concentrations of the elevated organic acids are displayed in Table 4.3. The complete organic acid profiles of the 19 patients are given in Appendix IV.

4.2 LOADING STUDIES

The clinical profiles of the patients with elevated BHHA were also studied. The clinical symptoms in most of the cases appeared not to be of an acute origin. ADHD and hyperactivity were the most common symptoms in these patients.

Table 4.3 Concentrations of other elevated organic acids in the BHHA elevated group (Concentrations are expressed in mmol/mol creatinine)

	Patient 12/09/ 05/03	Patient 01/24/ 01/03	Patient 06/21/ 01/03	Patient 07/17/ 01/03	Patient 07/09/ 04/03	Patient 03/12/ 05/03	Patient 06/14/ 04/03	Patient 20/24/ 04/03	Patient 03/23/ 01/03	Patient 06/27/ 02/03	Patient 02/25/ 03/03	Patient 07/31/ 03/03	Patient 09/04/ 02/03	Patient 11/20/ 02/03
p-hydroxy-phenyl acetic acid	1606.584619	440.5435614	200.2014789	399.4928632	300.5316992	717.0852691	1404.631672	534.7259882	807.6856292	2958.127381	509.6618909	544.5540406	2027.637198	221.9326297
p-hydroxy-phenyll actic acid	213.5802872	72.4786812	24.44811906	49.6368223	6.683381213	40.26554482	39.37105277		72.76971401	294.5272184	106.2414361		209.9093676	363.2798399
p-hydroxy-hippuric acid	535.6396212	401.027496	396.9113299	591.611982	226.5199474	273.3389909		393.9862478	303.0254314	859.5550332	781.8502248	446.0696961	898.035068	
m-hydroxy-hippuric acid					236.2447438									
Phenyl acetic acid										42.24330461			32.19768384	
Phenyll actic acid														129.4269403

4.2.1 Phenylalanine loading

The Phe loading on patient 1 showed decreased systemic Phe absorption as indicated by the lower concentrations of Phe in the blood and urine (Table 4.4 and Table 4.5). The Tyr absorption was also decreased with low concentrations in the blood and urine (Table 4.4 and 4.5). The results of the Phe loading on patient 2 are displayed in Table 4.6 and Table 4.7 and shows decreased concentrations in the blood and urine. The results of Tyr levels in blood are displayed in Table 4.6 and indicate also decreased Tyr absorption. The above results are indicative of the patients' inability to absorb phenylalanine from the intestine.

Other amino acids in abnormal concentrations in patient 1 were ornithine, arginine, valine, leucine, lycine, homocystine, α-aminoadipic acid and carnosine (See Appendix V). Patient 2 showed abnormal amino acid concentrations for alanine, ornithine, arginine, homocystine, histidine, α-aminoadipic acid and carnosine (See Appendix V).

Tryptophan (protein diet) loading on patient 1 showed normal increased levels of tryptophan in blood (68 μmol/L - 269 μmol/L), thus eliminating the possibility of Hartnup's disease. Normal tryptophan levels in the blood of children should be 12- 69 μmol/L (Blau & Blaskovics, 1996:66). In Hartnup's disease, tryptophan loading would have had no effect on the tryptophan levels in the blood because the defect lies in the transport of tryptophan in the intestine.

Table 4.4 Phenylalanine and tyrosine concentrations in blood after loading with phenylalanine. (Patient 1)

TIME OF SAMPLE	PHENYLALANINE CONCENTRATION IN BLOOD (μMOL/L)	TYROSINE CONCENTRATION IN BLOOD (μMOL/L)	PHE/TYR RATIO IN BLOOD (REF. 0.8 ± 0.3, MAX 2.4)
Time 0h	47.7	34.1	1.4
Time 3h	102.1	78.5	1.3
Time 7h	65.0	50.0	1.3
Time 11h	39.1	26.1	1.5

Table 4.5 Phenylalanine and tyrosine concentrations in urine after loading with phenylalanine . (Patient 1)

TIME OF SAMPLE	PHENYLALANINE CONCENTRATION IN URINE (MMOL/MOL CREATININE))	TYROSINE CONCENTRATION IN URINE (MMOL/MOL CREATININE)	PHE/TYR RATIO (REF FOR PKU >2)
Time 2h	26.6	35.9	0.74
Time 3h	25.2	41.3	0.61
Time 4h	20.0	76.9	0.26
Time 5h	12.6	52.5	0.24
Time 6h	39.0	26.0	1.5

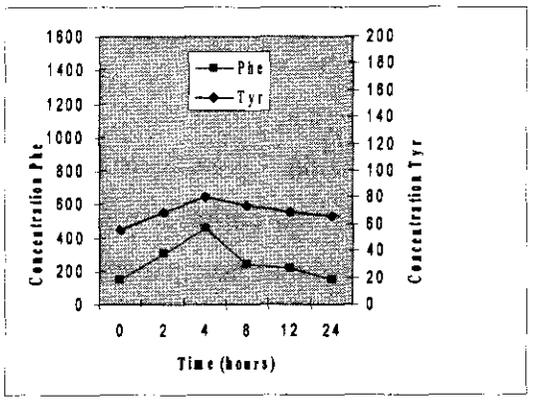
Table 4.6 Phenylalanine and tyrosine concentrations in blood after loading with phenylalanine (Patient 2)

TIME OF SAMPLE	PHENYLALANINE CONCENTRATION IN BLOOD (µMOL/L)	TYROSINE CONCENTRATION IN BLOOD (µMOL/L)	PHE/TYR RATIO (REF 0.8 ± 0.3, MAX 2.4)
Time 0h	5.776	6.275	0.9
Time 3h	14.982	11.797	1.3
Time 7h	4.455	6.336	0.7
Time 11h	6.676	5.183	1.3

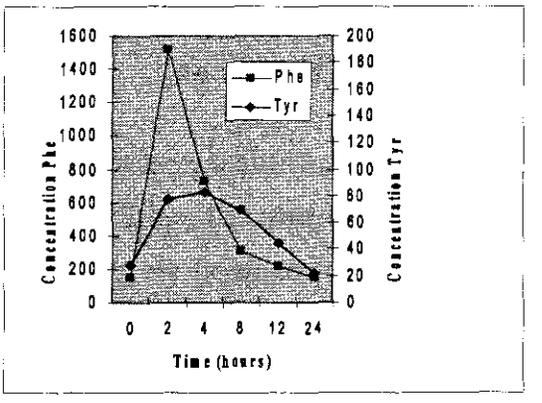
Table 4.7 Phenylalanine concentration in urine after loading with Phe (Patient 2).

TIME OF SAMPLE	PHENYLALANINE CONCENTRATION IN URINE (MMOL/MOL CREATININE))
Time 0h	No sample taken
Time 3h	2.85
Time 7h	14.83
Time 11h	10.87

Figure 4.4(a) is an example of a patient with a Phe absorption defect that was analysed in a study done by Mienie (1981). This graph gives an indication of the absorption levels of Phe and Tyr in the blood. The graph of a control subject in Mienie's study is also displayed in Figure 4.4(b). The graphs of patient 1 (a) and patient 2 (b) are shown in Figure 4.5. Our patients' absorption graphs seemed to be similar to those of Mienie in Figure 4.4(a).

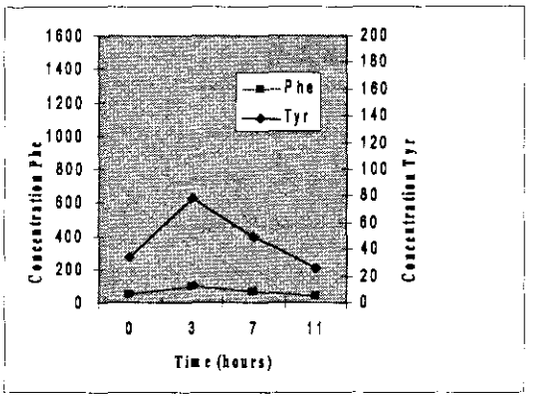


(a)

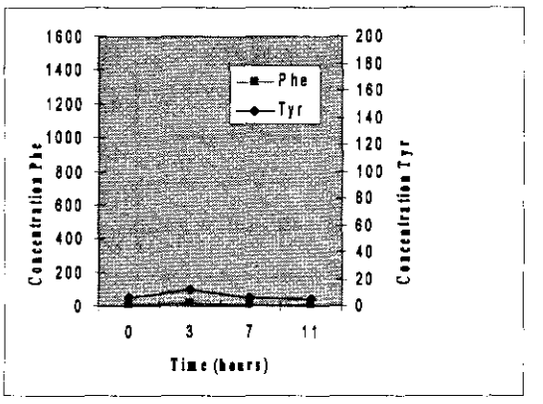


(b)

Figure 4.4 Typical absorption graph of Phe and Tyr absorption in Phe absorption defect patient (a) and normal subject (b). Concentrations are given in μmol/L (Mienie, 1981: 119, 143).



(a)



(b)

Figure 4.5 Absorption graphs of Phe and Tyr absorption in patient 1 (a) and patient 2 (b). Concentrations are given in μmol/L.

4.2.2 Aspartame loading

The aspartame loading, done on patient 1, showed increased Phe levels, indicating that peptide absorption was normal (aspartame is metabolised to Phe) (Table 4.8). The Phe/Tyr ratio (0.9) also seemed to normalise three hours after aspartame loading, the accepted ratio being 0.8 (Table 4.8).

Table 4.8 Phenylalanine and tyrosine concentration in blood after aspartame loading (Patient 1)

TIME OF SAMPLE	PHENYLALANINE CONCENTRATION IN BLOOD AFTER ASPARTAME (μMOL/L)	TYROSINE CONCENTRATION IN BLOOD AFTER ASPARTAME (μMOL/L)	PHE/TYR RATIO (REF. 0.8 ± 0.3)
Time 0h	119.3	94.9	1.3
Time 2h	187.7	87.3	2.2
Time 3h	137	161.0	0.9

4.2.3 Phe loading on a PKU patient

A patient with phenylketonuria (patient 3) also received phenylalanine loading. A comparison of the profile of the PKU patient with patient 1 and 2 should allow us to establish if our patients suffered from PKU. The Phe and Tyr levels of patient 3 are given in Table 4.9. Figure 4.6 shows how the Phe and Tyr levels vary over a period of time in a patient 3 with PKU who received Phe loading.

Table 4.9 Phenylalanine and tyrosine levels in blood after phenylalanine loading in a PKU patient (Patient 3)

TIME OF SAMPLE	PHENYLALANINE CONCENTRATION IN BLOOD (μMOL/L)	TYROSINE CONCENTRATION IN BLOOD (μMOL/L)	PHE/TYR RATIO (REF. 0.8 ± 0.3, MAX 2.4)
Time 0h	132.61	9.078	14.6
Time 3h (BH_4) added	256.163	48.416	5.3
Time 7h	164.246	17.054	9.6
Time 11h	508.363	84.157	6.0

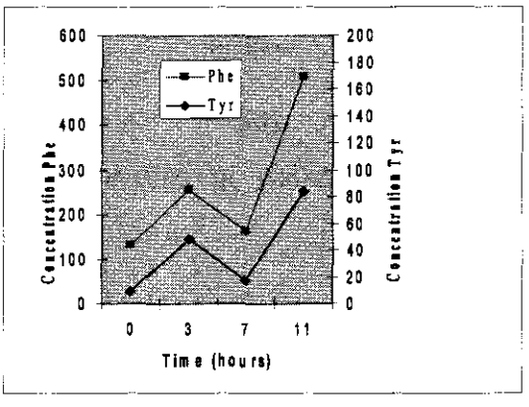


Figure 4.6 Typical absorption graph of Phe and Tyr absorption in blood in patient 3. Concentrations are given in μmol/L.

Comparing the results after Phe loading of the PKU patient (patient 3) with the results of patient 1 and patient 2, it show clearly that patient 1 and 2 did not suffer from PKU. Phe and Tyr concentrations in patients 1 and 2 decreased with time, because Phe and Tyr could not be absorbed, but both Phe and Tyr levels increased in patient 3 because PKU patients lack phenylalanine hydroxylase to metabolise Phe.

CHAPTER 5

CONCLUSION

This study was undertaken to identify the diagnostic value in ADHD of β-*m*-hydroxyphenylhydracrylic acid (BHHA). BHHA is an aromatic organic acid and likely a metabolite of phenylalanine. This metabolite is frequently found to be elevated in the urine of some patients, most of whom experience symptoms of attention-deficit hyperactivity disorder (ADHD).

This syndrome, ADHD, that usually starts early in life, is most common in boys and includes symptoms like hyperactivity, impulsivity, distractibility and excitability. Such children may also experience symptoms like aggressive behaviour, learning problems and emotional lability. There have been suggestions that these manifestations may be the result of neurotransmitter defects in the brain.

Dopamine is formed in the body through a long process involving phenylalanine (Phe) as a precursor. Phe is not only responsible for the formation of dopamine, but it is also converted into other brain chemicals, tyrosine and thyroid hormones. Phe is an essential amino acid and cannot be formed by the body and therefore needs to be taken in through food supplements. Some people have insufficient enzymes to metabolise Phe, causing the defect phenylketonuria (PKU), which results in elevated levels of Phe and Phe metabolites.

All nutrients are to be metabolised and absorbed to be useful in the human body. Substances need to be transported across membranes, a process often involving transporter proteins. Major transporters in the body include p-glycoproteins (P-gp), belonging to the ATP-binding cassette proteins. These transporters function by pumping substances out of cells using energy from ATP hydrolysis. P-gp effluxes substances from the brain, intestine, liver, kidney and lymphocytes, hence altering the distribution of substances.

Substances are mostly absorbed in the small intestine, from where they are distributed. Malabsorption arises when food are not properly absorbed in the intestine and could be caused by a number of factors such as drugs, diseases, etc.

We identified BHHA in the urine of a number of ADHD patients. In an effort to identify the origin of BHHA, a substantial database of patients' urine was subsequently analysed and the concentration of BHHA in these samples was measured. The concentration of the BHHA varied from trace amounts to concentrations of more than a 1000 mmol/mol creatinine. This finding of elevated BHHA levels led to a

further study aimed at determining the levels of other abnormal levels of organic acids. Other metabolites that were also found to be elevated in the urine of these patients were *p*-hydroxyphenylacetic acid, *m*-hydroxyhippuric acid, *p*-hydroxyhippuric acid and *p*-hydroxyphenyllactic acid. A few unique compounds were also excreted by these patients, including: benzoylsuccinic acid, phenylacrylylglycine, β-*m*-hydroxyphenylhydracrylglycine and phenylacetylglycine. These metabolites suggest a possible bacterial origin, considering that a compound such as benzoylsuccinic acid can only be produced by anaerobic micro organisms. Most of the other metabolites that were present can also be classified as bacterial metabolites, which are excreted in urine due to maldigestion or malabsorption.

Noticeably elevated concentrations of phenylacetic acid and phenyllactate were observed in the urine of two of the patients in the study. These two acids may be indicative of a Phe metabolic defect. Therefore Phe loading was done to determine its effect on Phe levels. Phe levels in the urine were found to be decreased after Phe loading, suggesting a decreased absorption of Phe and thus eliminating a Phe metabolic defect.

Phe loading was followed by tryptophan loading in these two patients. Tyrosine was also observed as being poorly absorbed, although tryptophan absorption was normal, thus eliminating Hartnup's disease (HD), for the reason that in HD, tryptophan levels are decreased in plasma, but elevated in urine. Patients with HD not only have a defect in their Phe levels, but also in tryptophan levels. In a study done by Symula *et al.* (1997) using a mouse model, it was concluded that the elevated Phe concentration in plasma after injecting Phe was not due to a deficiency in Phe catabolism, but a defect in amino acid transport. These findings lead us to postulate that Phe malabsorption or maldigestion could be the cause of the abnormal levels of Phe and Tyr found in the patients in our study. Aspartame, a dipeptide containing Phe, increased the Phe absorption when given to one of the patients, thus indicating normal peptide absorption.

It is known that mercury can lead to ADHD. In a study done by Shanker *et al* (2001) in rat PC12 cell line, the authors concluded that the Phe transport protein is inhibited by mercury. Lugea and co-workers (1994) also reported the inhibition of the Phe transport system by $HgCl_2$ in rat intestine, but it is still unknown if this same protein is also present in the human body. In our study a patient with elevated levels of BHHA also had concomitantly elevated mercury levels. Such a finding suggests that mercury has an effect on the amino acid transporter, but this remains speculative because this particular transport protein has not yet been identified in humans. Elevated mercury levels together with abnormal microorganisms in the body may therefore play a role in Phe absorption.

We argue that in cases of malabsorption or maldigestion of Phe, the intestinal bacteria would metabolise Phe to BHHA, causing significant levels of BHHA in the urine as found in our investigation. Excretion of BHHA in the urine may be indicative of ADHD as well as pancreatic disorders such as cystic fibrosis, Schmidt syndrome and Shwachman-Diamon syndrome – disorders that are currently very difficult to diagnose.

Another possible explanation for the elevated BHHA levels originating from a Phe deficiency may be a Phe absorption defect at the cell membrane, causing Phe levels in the cell to be decreased even more than the levels detected in our study. The specific defect that leads to this malabsorption is not yet known, but it is known that the defect can cause intestinal and cellular transport abnormalities, causing decreased cellular Phe and Tyr levels. Because dopamine is formed through a metabolic process from Phe, it can be argued that decreased Phe levels lead to decreased dopamine levels. Neurotransmitters play a role in ADHD, and most of the patients in this study suffering from ADHD have displayed significantly elevated levels of BHHA in their urine.

We conclude that elevated levels of BHHA in the urine may be used as a diagnostic tool for ADHD and hyperactivity, but that more research needs to be done to fully understand the intricate connection between the factors involved.

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APPENDIX I

Table of all the patients containing the patient's number and the concentration of β-m-hydroxyphenylhydracrylic acid in their urine

Patient number	Concentration of BHHA (mmol/mol creatinine)
01 . 05 / 05 / 03	16.919
01 . 22 / 04 / 03	38.913
01 . 22 / 04 / 3H	3.086
02 . 06 / 05 / 03	30.537
02 . 25 / 04 / 03	11.466
02 . 29 / 04 / 03	12.569
03 . 02 / 05 / 03	23.726
03 . 06 / 05 / 03	2.449
03 . 25 / 04 / 03	1.155
04 . 06 / 05 / 03	1.320
04 . 25 / 04 / 03	19.045
04 . 29 / 04 / 03	42.284
05 . 15 / 04 / 3H	17.982
05 . 25 / 04 / 03	4.686
07 . 14 / 05 / 03	7.191
07 . 24 / 04 / 3A	22.709
07 . 24 / 04 / 3B	25.046
07 . 24 / 04 / 3C	35.868
08 . 25 / 04 / 03	19.626
08 . 29 / 04 / 03	45.220
10 . 25 / 04 / 03	3.151
11 . 25 / 04 / 03	4.414
12 . 40 / 43 / AH	10.008
12 . 40 / 43 / CH	18.682
19 . 25 / 04 / 03	49.683
22 . 24 / 04 / 03	16.044
22 . 24 / 04 / 3H	9.095
22 . 40 / 43 / BH	3.830
22 . 40 / 43 / CH	12.830
01 . 19 / 06 / 03	15.344
01 . 25 / 06 / 03	27.890
02 . 24 / 06 / 03	17.443
02 . 25 / 06 / 03	21.930
03 . 19 / 06 / 03	4.453
03 . 23 / 06 / 03	40.149

04	20	06	03	13.816
04	24	06	03	4.848
05	26	05	03	15.091
06	25	06	03	14.080
07	26	05	3S	47.517
09	24	06	03	27.223
09	27	06	03	26.777
12	24	06	03	22.845
01	20	01	03	45.452
01	21	01	03	8.210
03	19	12	2H	1.882
04	09	01	3H	3.925
05	15	01	03	14.606
05	22	01	3H	2.056
06	15	01	03	43.970
07	23	01	03	42.141
08	22	01	3H	28.932
11	23	01	03	16.272
13	15	01	03	11.050
16	03	12	02	11.702
18	22	01	03	20.960
01	15	01	03	24.982
02	08	01	03	18.157
02	20	01	03	43.834
02	28	01	03	42.241
03	28	01	03	12.824
04	06	01	03	5.489
05	08	01	03	6.878
06	08	01	03	16.648
08	07	01	03	25.790
01	10	02	03	21.703
01	14	02	03	23.905
01	17	02	03	1.600
01	21	02	03	4.028
01	25	02	03	18.384
01	27	02	03	1.399
02	03	02	03	22.685
02	04	02	03	14.739
02	05	02	03	11.015
02	10	02	03	30.303
02	17	02	03	31.876
02	27	02	03	1.852

02	31	01	03	6.536
03	17	02	03	14.788
04	03	02	03	7.920
04	07	02	03	21.791
04	09	01	3H	3.925
04	31	01	03	36.742
05	04	02	03	29.618
05	22	01	3H	2.056
08	21	01	3H	2.422
08	22	01	3H	28.932
12	06	01	3H	1.171
14	24	02	03	27.182
06	06	02	03	18.622
06	07	02	03	30.513
07	07	02	03	14.687
07	27	02	03	4.197
08	06	02	03	49.128
08	24	02	03	43.486
09	03	02	03	24.447
09	10	02	03	25.960
10	03	02	03	46.859
10	03	02	3H	44.800
10	06	02	03	21.590
10	17	02	03	8.271
10	17	02	3H	1.236
11	03	02	03	13.733
12	10	02	3H	24.550
14	20	02	03	28.327
15	10	02	03	3.820
16	24	02	03	8.600
19	03	02	03	36.344
20	12	02	03	18.862
01	17	03	3H	8.060
01	24	03	03	0.512
01	27	03	3H	10.644
01	28	03	03	19.328
02	17	03	03	23.388
02	24	03	3H	40.791
02	25	03	3H	25.248
03	04	03	03	17.116
03	12	03	03	1.166
03	12	03	3H	12.529

03	, 14	, 03	/ 03	46.101
04	, 06	, 03	/ 03	8.374
04	, 25	, 03	/ 3H	14.631
05	, 05	, 03	/ 03	5.454
05	, 19	, 03	/ 03	32.763
05	, 25	, 03	/ 3H	27.853
06	, 05	, 03	/ 03	7.454
06	, 06	, 03	/ 03	11.239
06	, 28	, 03	/ 3H	18.797
07	, 25	, 03	/ 3H	23.315
08	, 05	, 03	/ 03	33.849
08	, 31	, 03	/ 3H	5.772
09	, 05	, 03	/ 03	15.845
20	, 12	, 02	/ 03	18.862
06	, 31	, 03	/ 03	8.031
10	, 20	, 03	/ 3H	6.647
11	, 20	, 03	/ 3H	13.148
01	, 01	, 04	/ 03	5.885
01	, 07	, 04	/ 03	23.065
01	, 15	, 04	/ 03	46.259
01	, 22	04	/ 03	38.913
02	, 02	, 04	/ 03	39.440
02	, 07	, 04	/ 03	7.513
02	, 16	, 04	/ 03	39.967
02	, 25	, 04	03	11.466
02	, 29	, 04	/ 03	12.569
03	, 01	, 04	/ 03	35.536
03	, 04	, 04	/ 03	1.039
03	, 25	, 04	/ 03	1.155
03	, 29	, 04	/ 03	2.462
04	, 07	, 04	/ 03	9.742
04	, 08	, 04	/ 03	11.202
04	, 09	, 04	/ 03	2.829
04	, 10	, 04	/ 03	21.696
04	, 23	, 04	/ 03	13.205
04	, 25	, 04	/ 03	19.045
04	, 29	, 04	/ 03	42.284
05	, 04	, 04	/ 03	41.687
05	, 07	, 04	/ 03	1.904
05	, 10	, 04	/ 03	43.006
05	, 10	, 04	/ 03	4.135
05	, 14	, 04	/ 03	91.331

05 , 25 , 04 / 03 4.686
06 , 07 , 04 / 03 19.753
06 , 11 04 03 33.048
07 , 01 , 04 / 03 10.141
07 , 07 , 04 / 03 40.131
07 , 10 , 04 / 03 6.938
08 , 02 , 04 / 03 38.714
08 , 04 , 04 / 03 6.546
08 , 07 , 04 / 03 4.423
08 , 25 , 04 / 03 19.626
08 , 29 , 04 / 03 45.220
09 , 04 , 04 / 03 1.307
09 , 07 , 04 / 03 7.592
09 , 08 , 04 / 03 4.517
09 , 15 , 04 / 03 29.432
09 , 16 , 04 / 03 2.014
09 , 17 , 04 / 03 31.672
10 , 07 , 04 / 03 22.514
10 , 25 , 04 / 03 3.151
11 16 04 03 6.911
11 , 25 , 04 / 03 4.414
19 , 25 , 04 / 03 49.683
22 , 24 , 04 / 03 16.044
01 , 22 , 04 / 3H 3.086
02 , 07 , 04 / 3H 14.690
04 , 04 , 04 / 3H 16.547
04 , 07 , 04 / 3H 4.839
04 , 11 , 04 / 03 13.934
04 , 23 , 04 / 3H 19.269
05 , 15 , 04 / 3H 17.982
06 , 07 , 04 / 3H 44.575
08 , 07 , 04 / 3H 6.169
09 , 07 , 04 / 3H 14.890
12 , 07 , 04 / 3I 8.911
16 , 24 , 04 / 3I 1.290
22 , 24 , 04 / 3H 9.095
05 , 02 , 05 / 03 56.080
05 , 14 , 04 / 03 91.331
05 , 29 , 04 / 03 81.295
08 , 29 , 04 / 3H 97.802
11 09 , 05 / 03 69.491
15 , 08 , 05 / 03 99.601

19	25	04	3H	50.650
20	07	05	03	90.284
01	20	06	03	55.822
01	24	06	03	99.072
03	21	01	03	81.309
04	17	01	03	57.143
05	17	01	`'03	97.430
05	21	01	03	66.341
06	23	01	03	55.633
09	15	01	03	60.540
17	22	01	03	58.558
22	22	01	03	63.756
01	05	02	03	60.695
01	17	01	3H	88.596
02	07	02	03	88.244
02	27	01	3H	74.679
03	07	02	03	64.236
03	10	02	03	75.417
03	26	02	03	76.646
03	31	01	03	69.208
04	11	02	03	64.272
05	07	02	03	65.063
05	13	02	03	80.447
06	04	02	03	64.974
06	17	02	03	98.340
08	07	02	03	69.719
09	07	02	03	98.368
11	27	02	03	71.982
13	03	02	03	66.981
14	03	02	03	96.591
15	03	02	03	73.220
17	03	02	03	73.354
19	12	02	03	70.293
02	06	03	03	70.101
03	24	03	03	50.536
05	03	03	03	74.960
19	12	02	03	70.293
B4	12	02	03	97.232
11	06	03	03	75.123
12	06	03	03	57.946
01	10	04	03	84.404
01	17	04	03	81.449

04	15	04	03	84.717
05	14	04	03	91.331
05	17	04	03	58.735
05	29	04	03	81.295
06	04	04	03	62.325
08	16	04	03	66.859
10	04	04	03	71.283
11	04	04	03	55.575
03	01	04	3H	51.735
05	23	04	3H	54.225
05	29	04	3H	63.479
07	07	04	3H	76.514
08	29	04	3H	97.802
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19	25	04	3H	50.650
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H5	23	04	3H	56.800
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06	24	04	3C	148.466
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10	28	05	03	116.198
02	09	01	3H	186.212
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02 , 13 / 01 / 03 257.110

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02	14	01	3H	280.927
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09	27	02	03	268.996
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06	21	01	03	728.213
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03	12	05	03	862.130
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20	24	04	03	1967.719
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04	10	07	00	10.297
03	17	02	00	10.529
01	13	04	00	10.594
03	14	06	00	10.642
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02	07	06	00	24.262
01	06	04	00	24.288
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04	02	08	00	24.416
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19	,	19	,	07	/	00	69.155
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02	,	03	,	04	/	00	78.115
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03	,	09	,	03	/	00	209.501
01	,	13	,	07	/	00	233.410
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03	,	06	,	03	/	00	244.597

01 , 02 , 06 / 00	244.842
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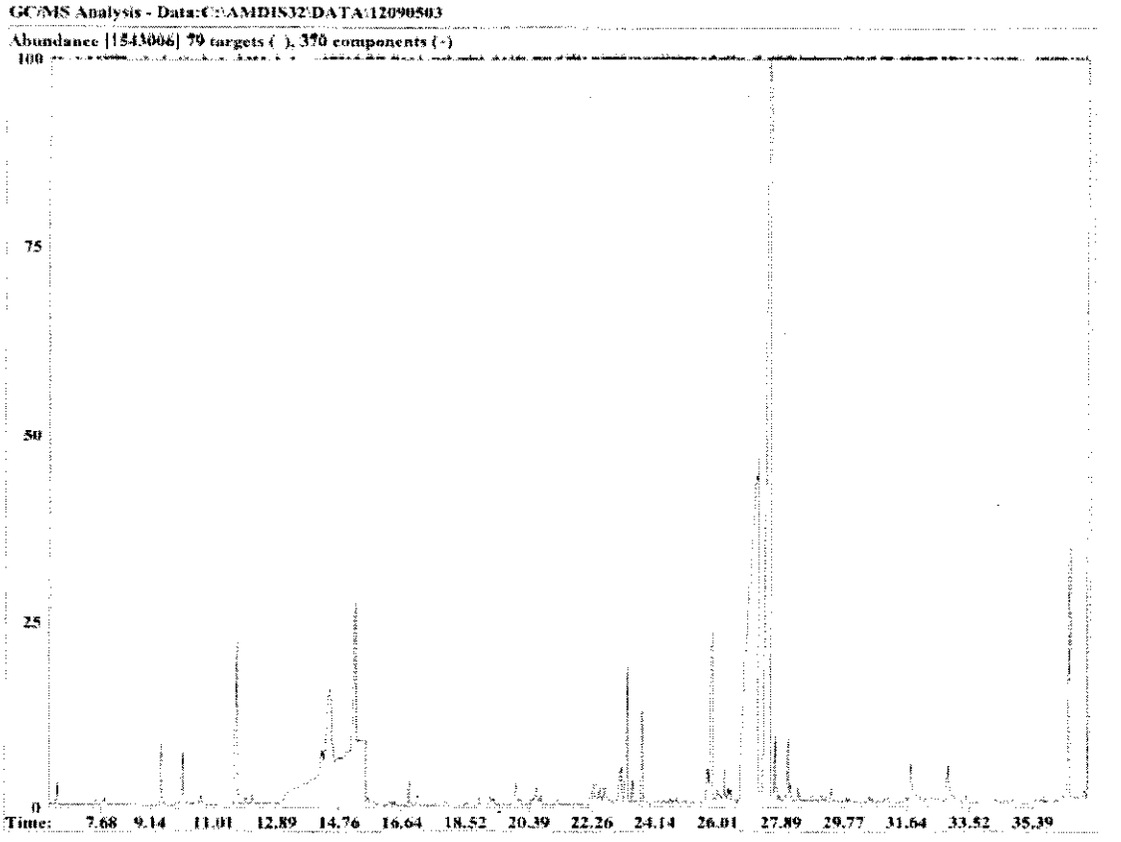
APPENDIX II


GC/MS Analysis - Data:C:\AMDIS32\DATA\12090503
Abundance [1543006] 79 targets ( ), 350 components ( )
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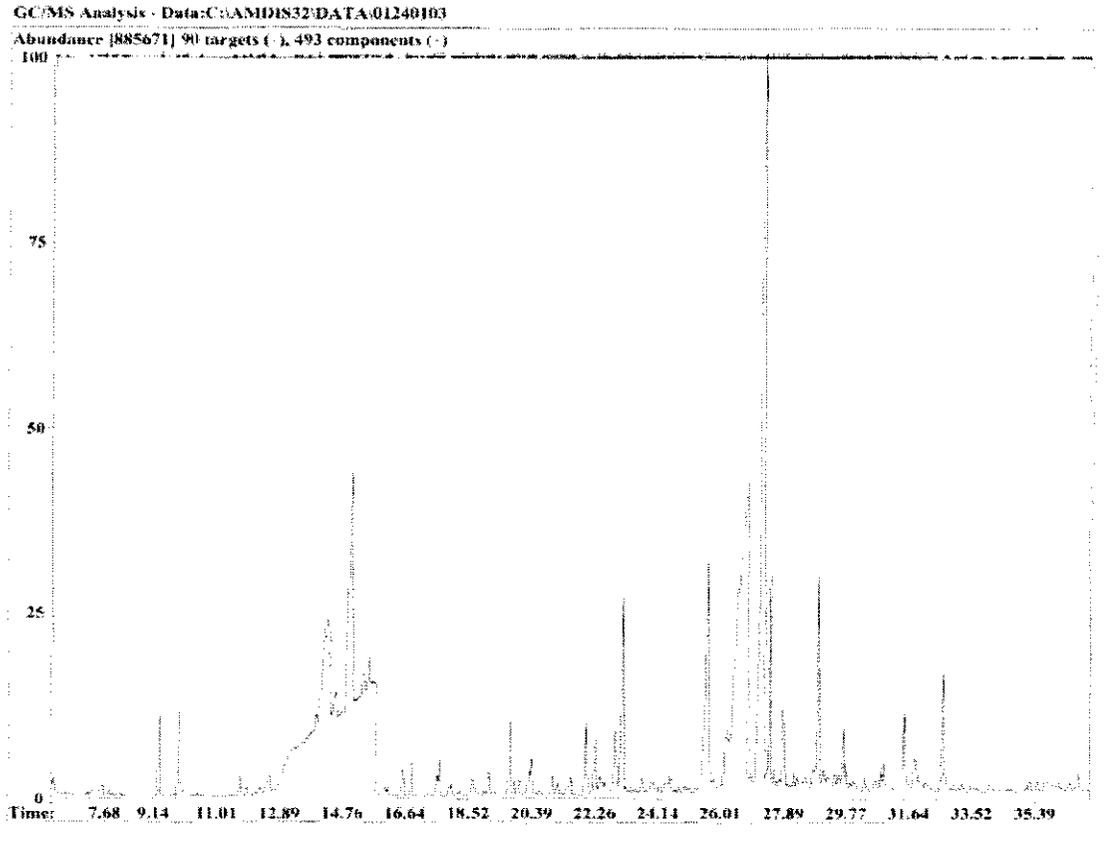
75
50
25
0
Time: 7.68 9.14 11.01 12.89 14.76 16.64 18.52 20.39 22.26 24.14 26.01 27.89 29.77 31.64 33.52 35.39


GC/MS Analysis - Data:C:\AMDIS32\DATA\01240103
Abundance [885671] 90 targets ( ), 493 components ( )
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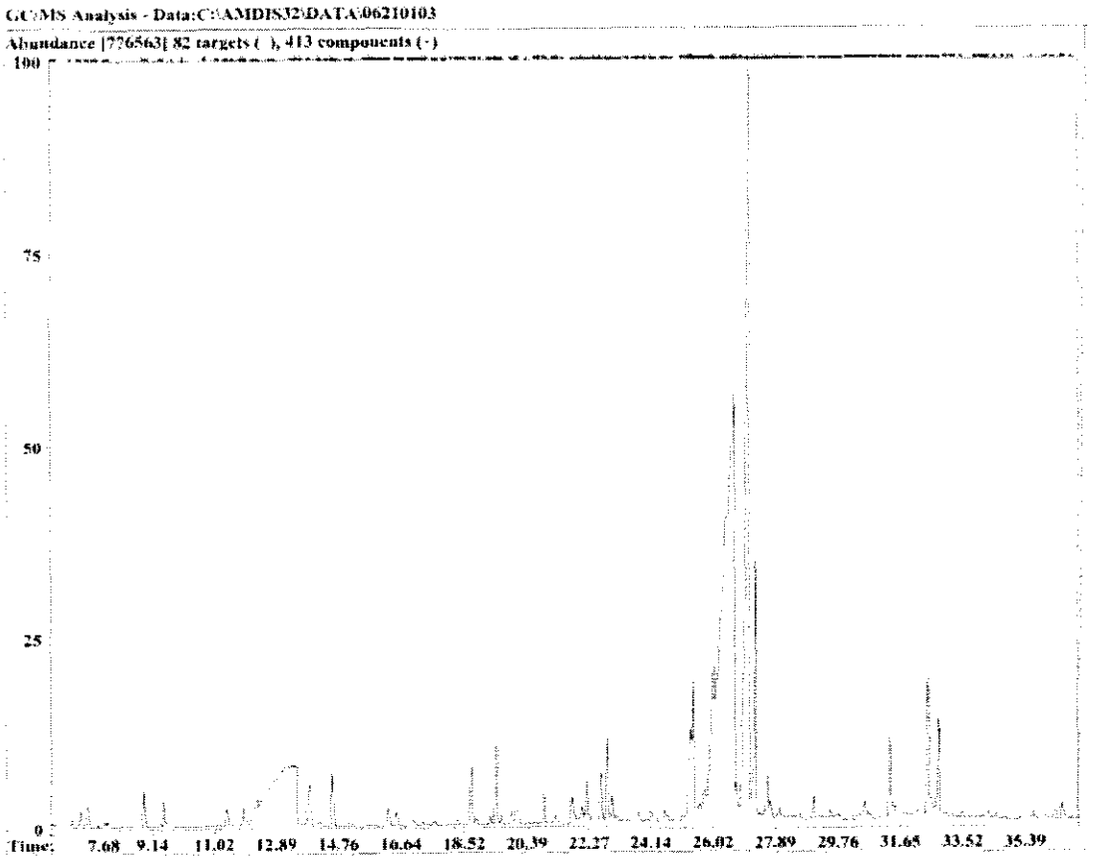
75
50
25
0
Time: 7.68 9.14 11.01 12.89 14.76 16.64 18.52 20.39 22.26 24.14 26.01 27.89 29.77 31.64 33.52 35.39


GC/MS Analysis - Data:C:\AMDIS32\DATA\06210103
Abundance |7765631| 82 targets ( ), 413 components (-)
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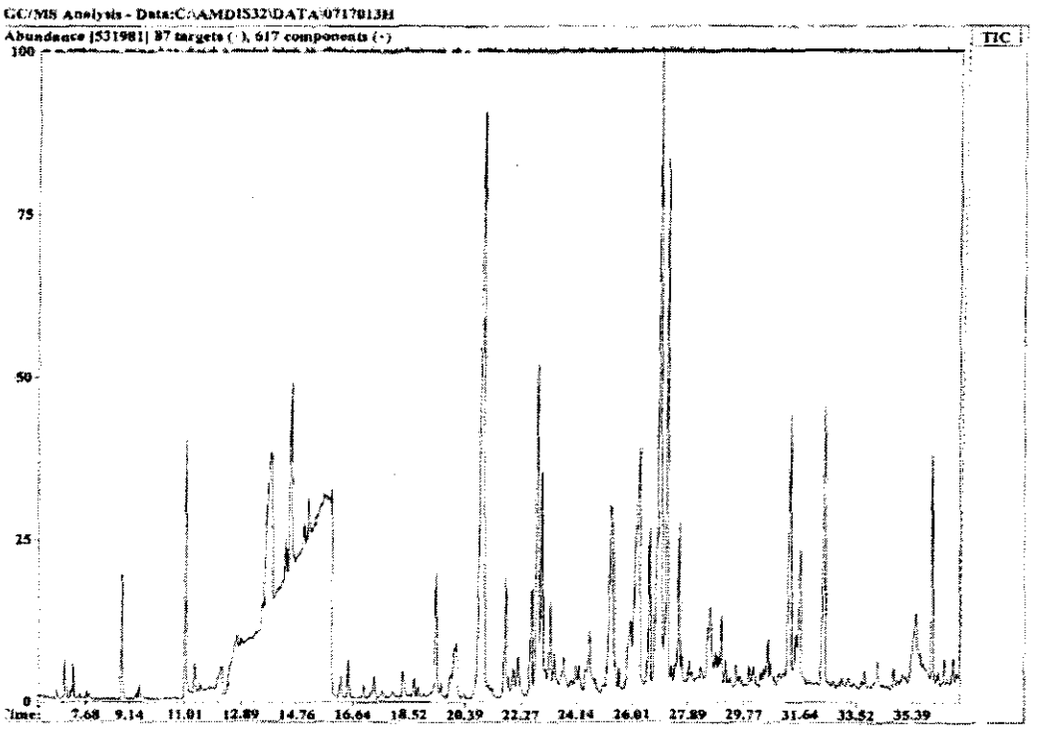
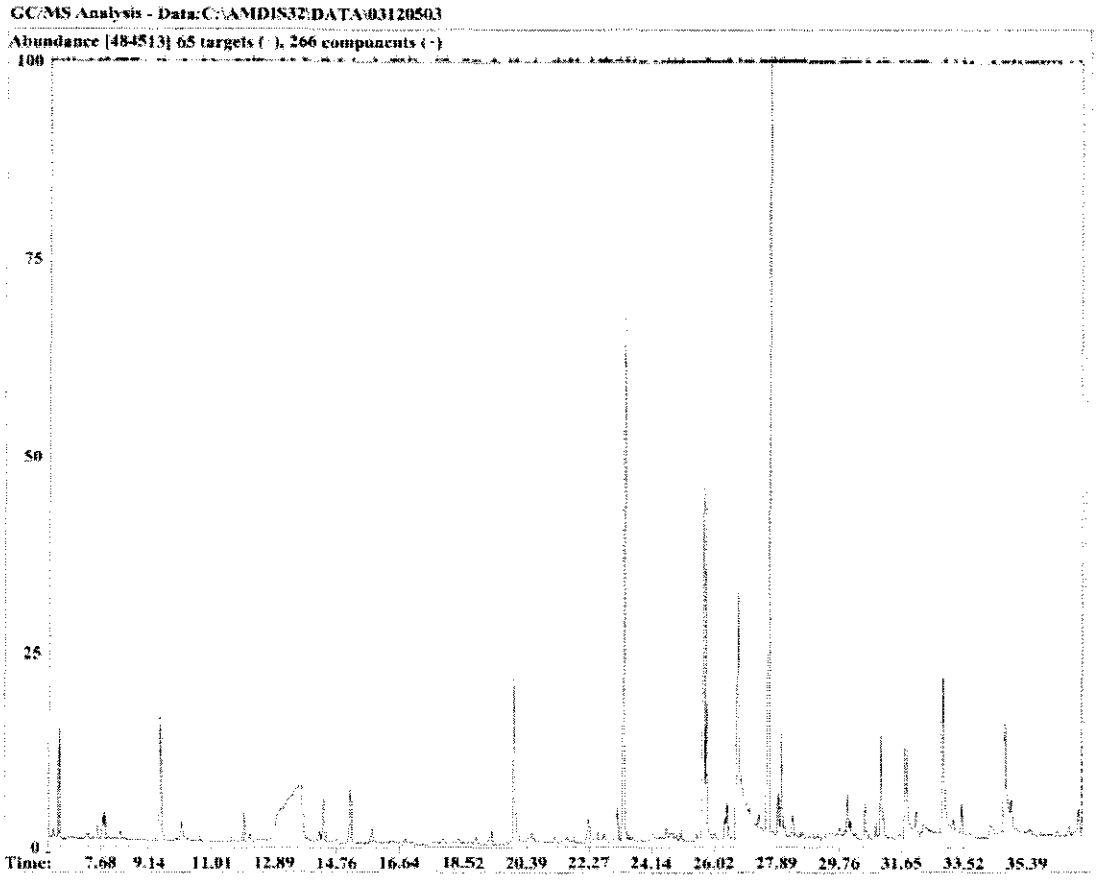
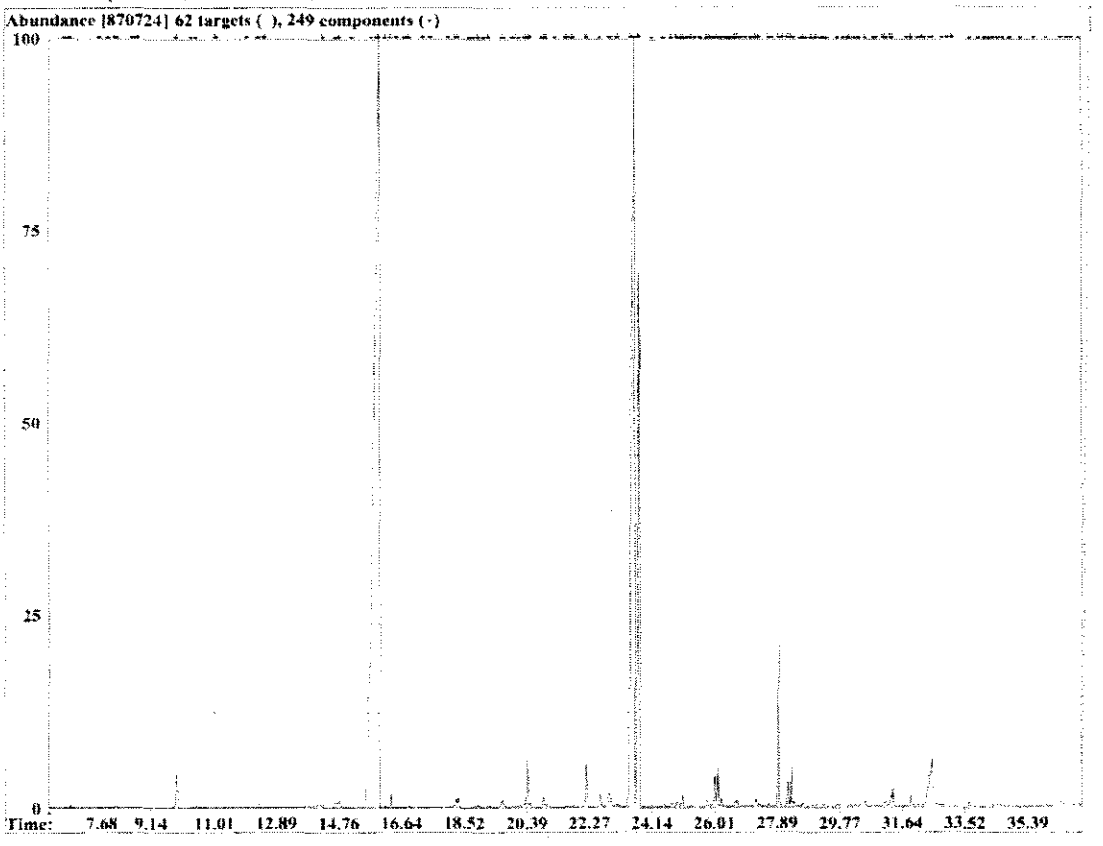
75
50
25
0
Time: 7.68 9.14 11.02 12.89 14.76 16.64 18.52 20.39 22.27 24.14 26.02 27.89 29.76 31.65 33.52 35.39

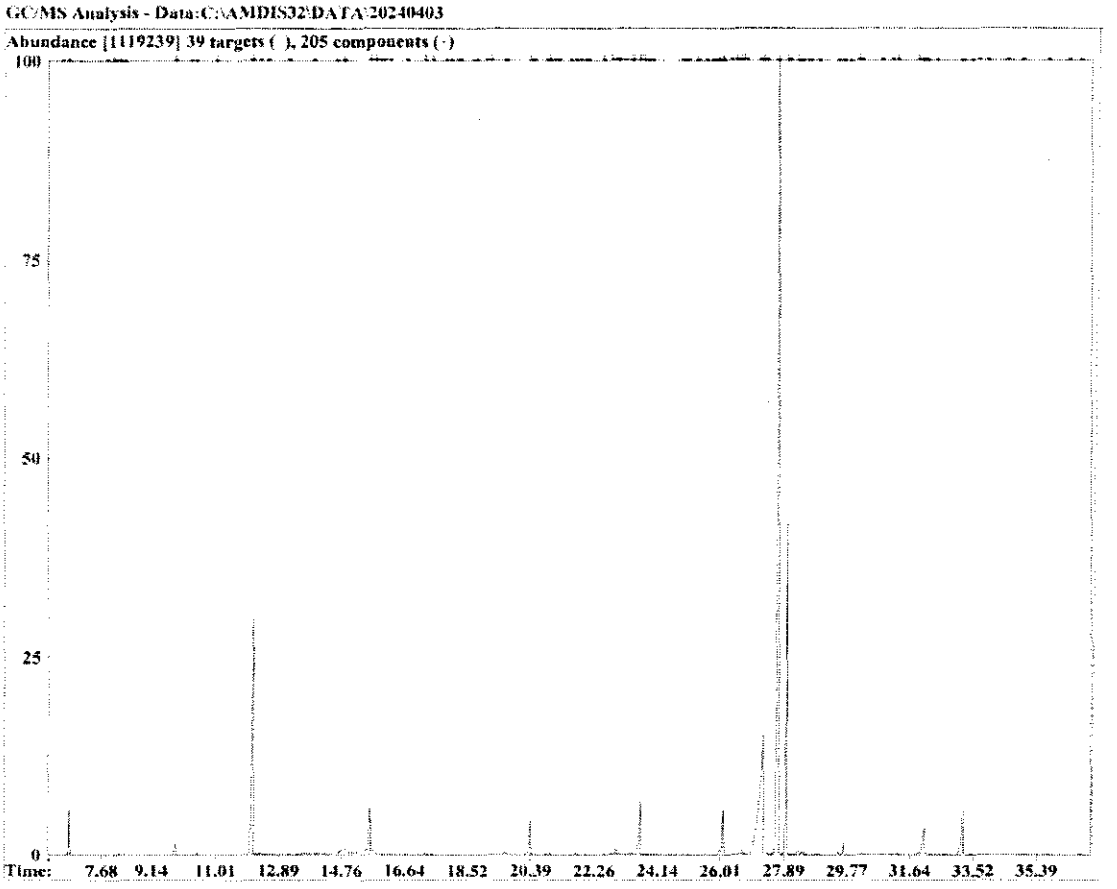

GC/MS Analysis - Data:C:\AMDIS32\DATA\0717013H
Abundance |5531981| 87 targets ( ), 617 components (-)
TIC
100
75
50
25
0
Time: 7.68 9.14 11.01 12.89 14.76 16.64 18.52 20.39 22.27 24.14 26.01 27.89 29.77 31.64 33.52 35.39

GC/MS Analysis - Data:C:\AMDIS32\DATA\03120503



GC/MS Analysis - Data:C:\AMDIS32\DATA\06140403




GC/MS Analysis - Data:C:\AMDIS32\DATA\20240403
Abundance [1119239] 39 targets ( ), 205 components (-)
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50
25
0
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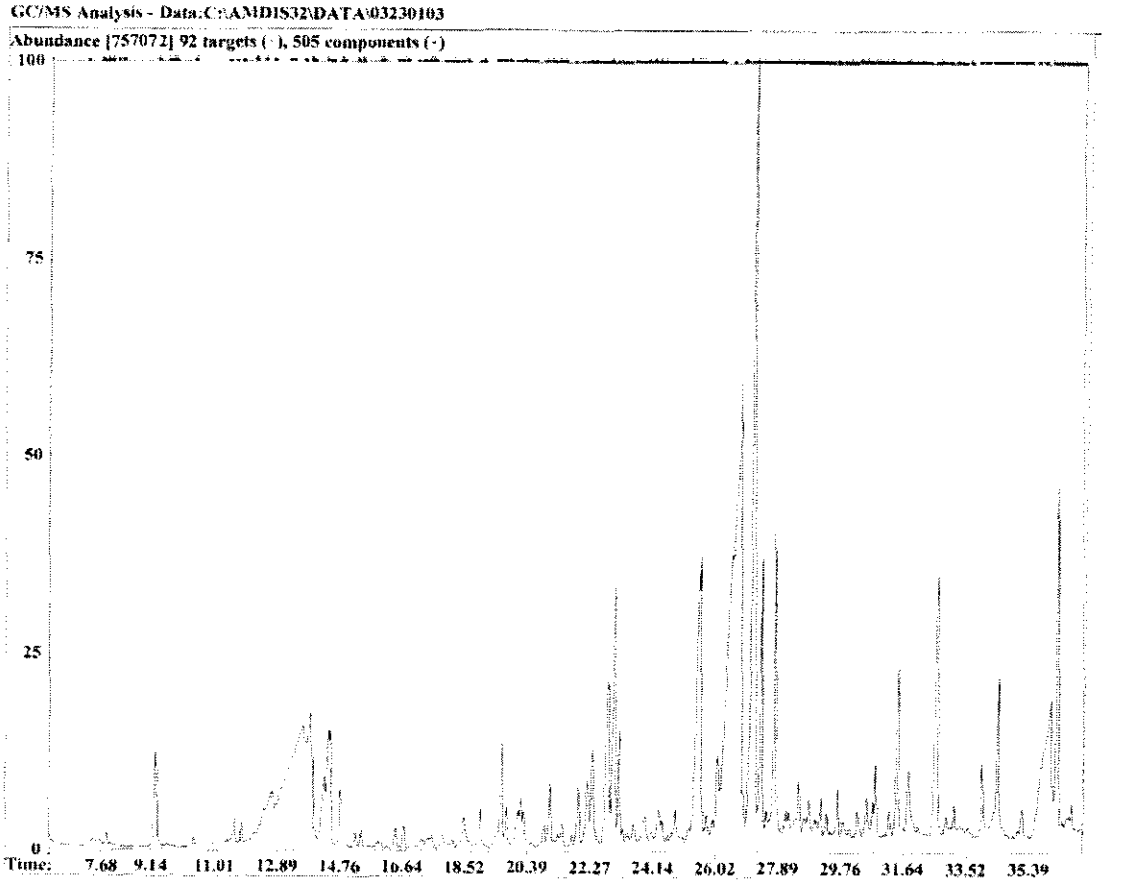
GC/MS Analysis - Data:C:\AMDIS32\DATA\03230103
Abundance [757072] 92 targets ( ), 505 components (-)
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50
25
0
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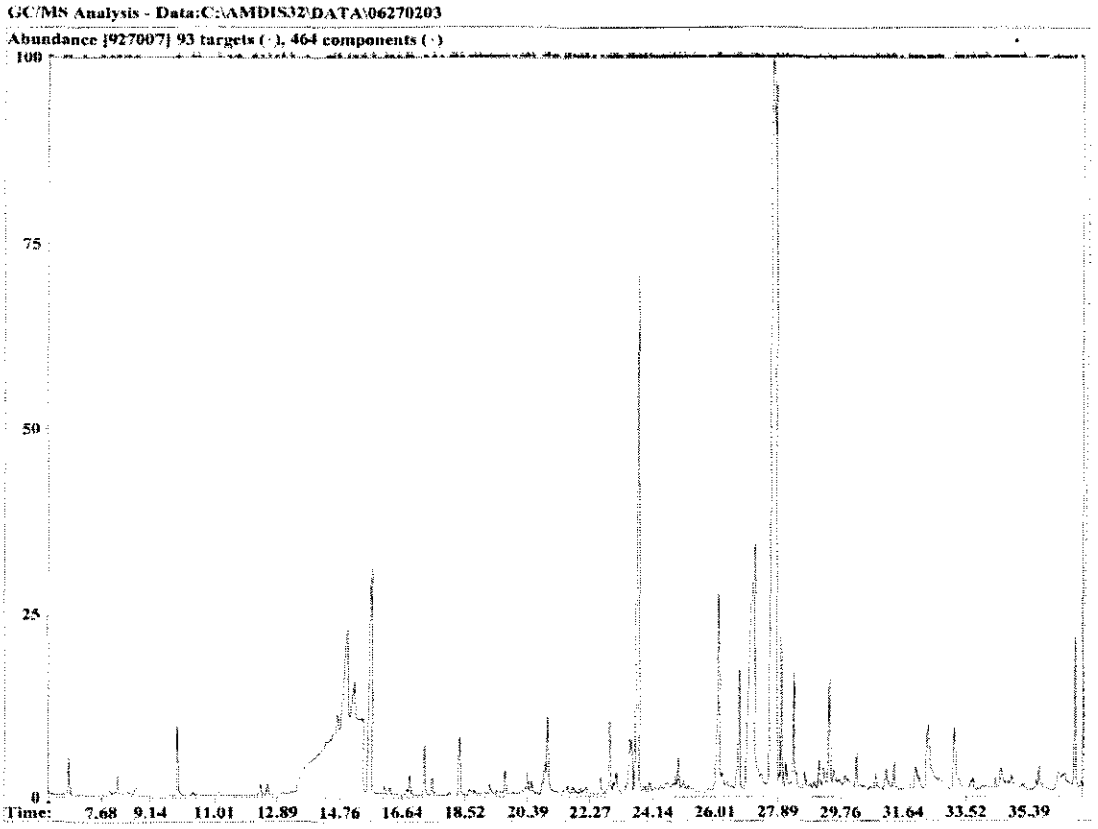
GC/MS Analysis - Data:C:\AMDIS32\DATA\06270203



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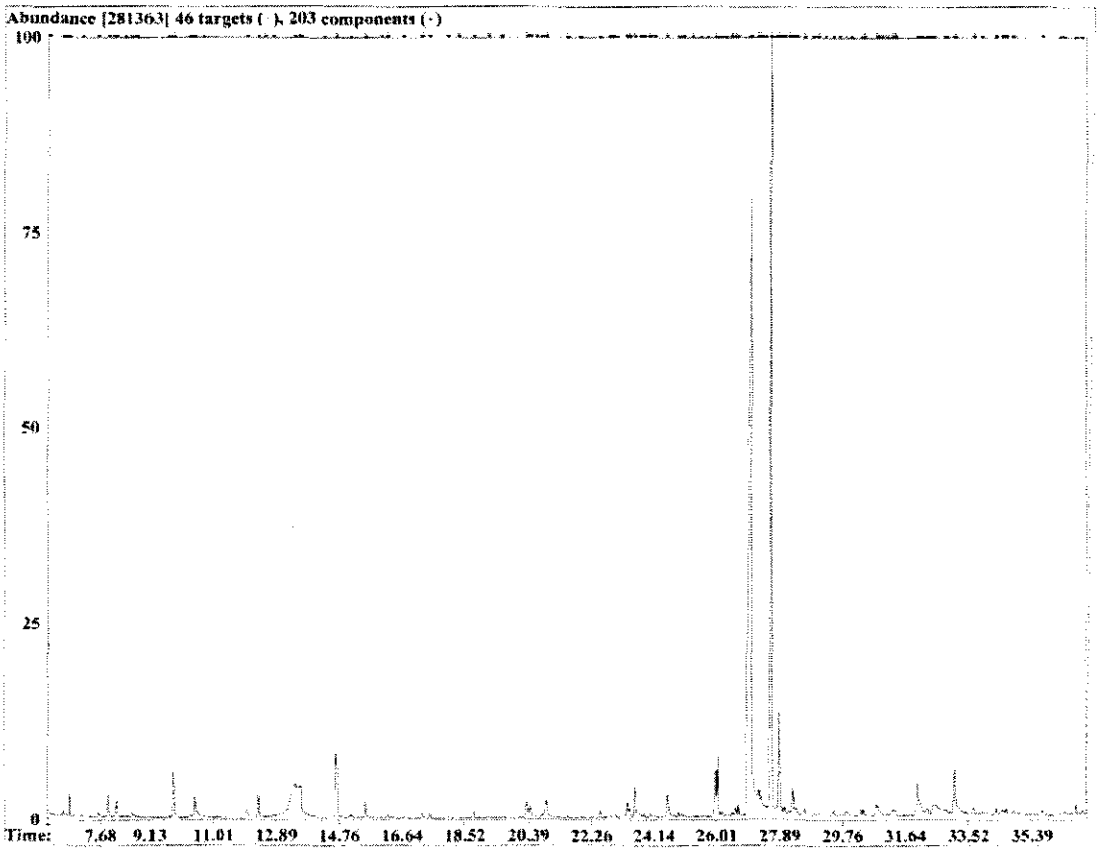

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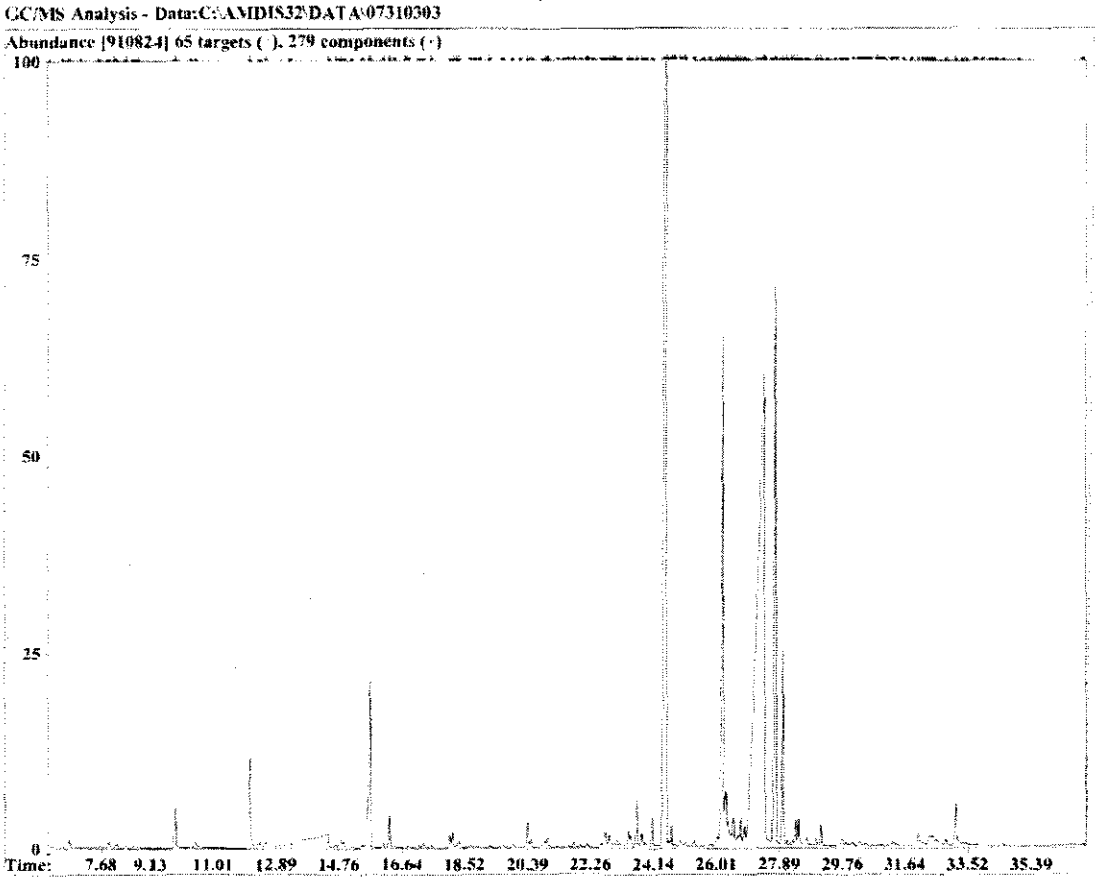

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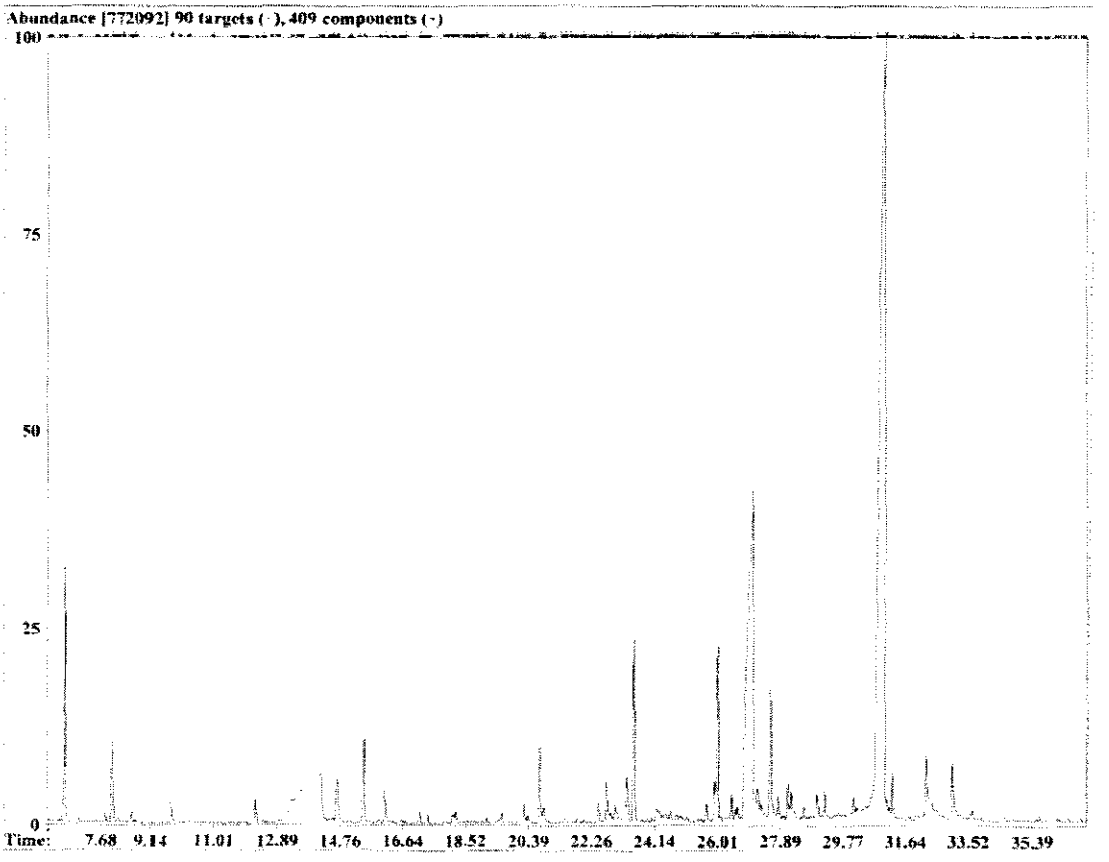

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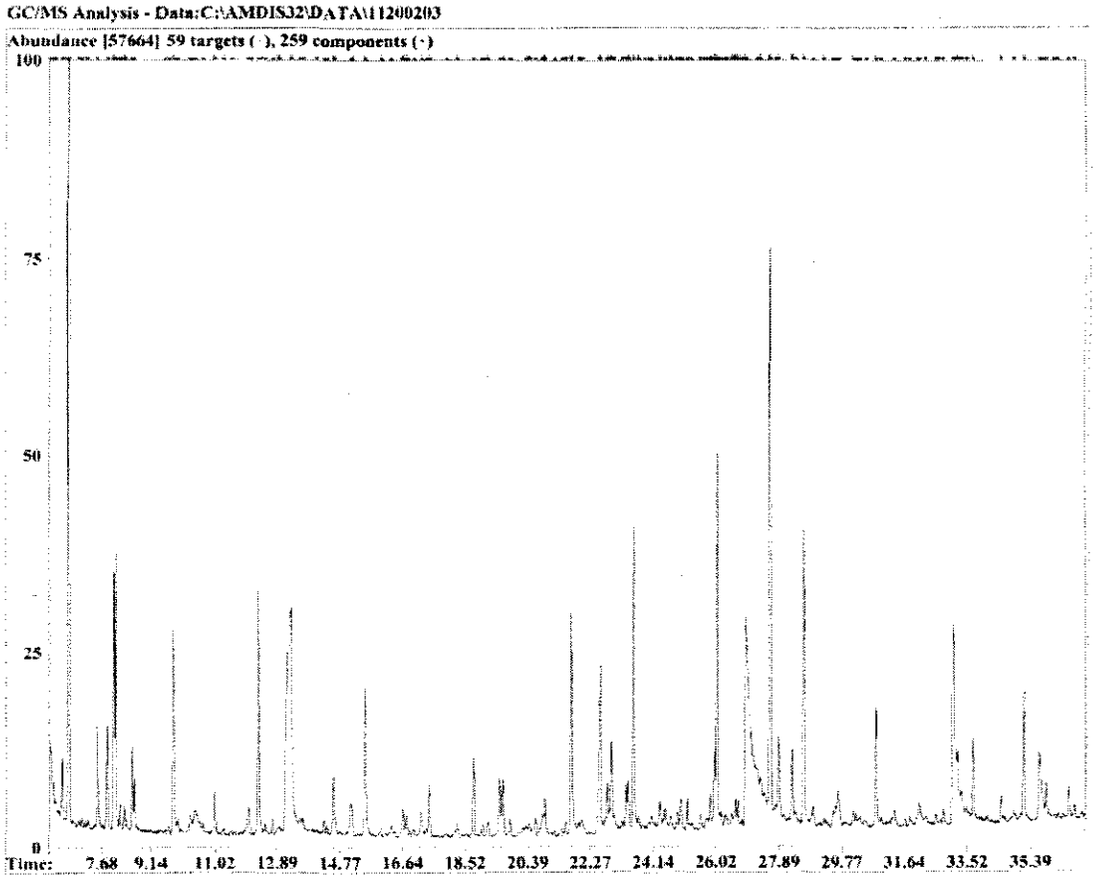

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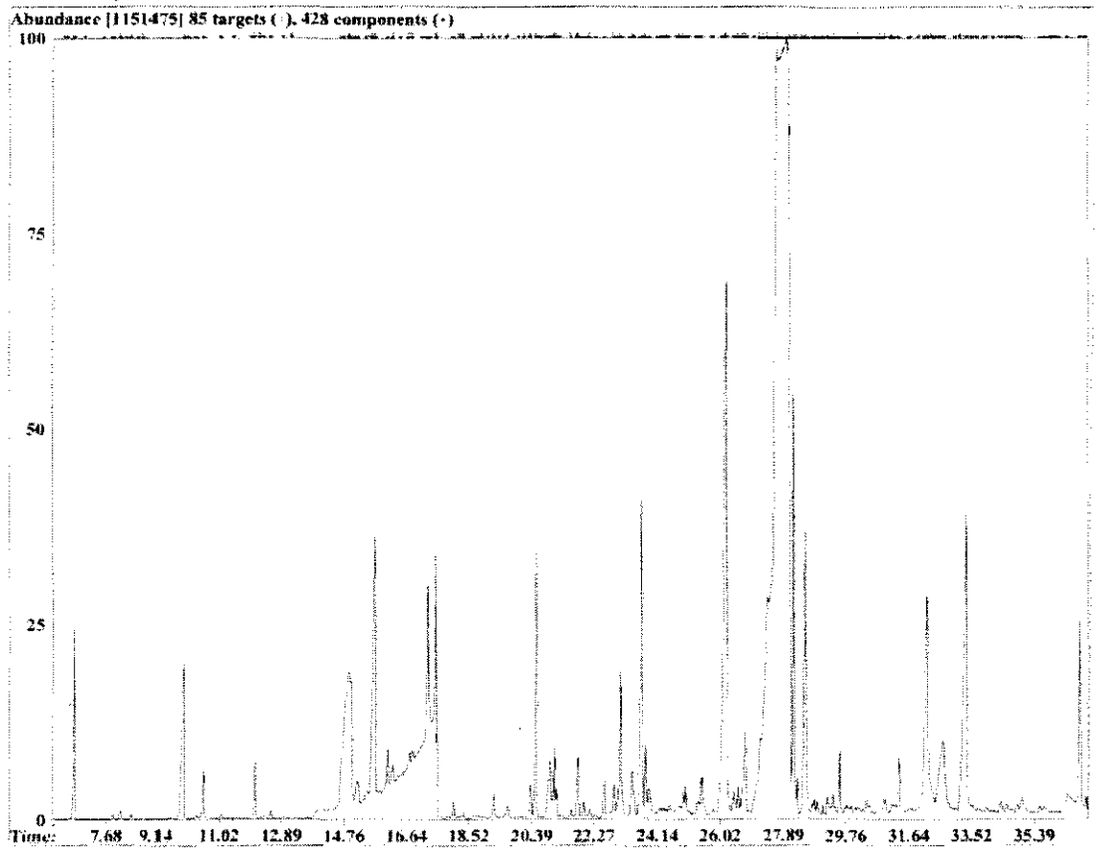
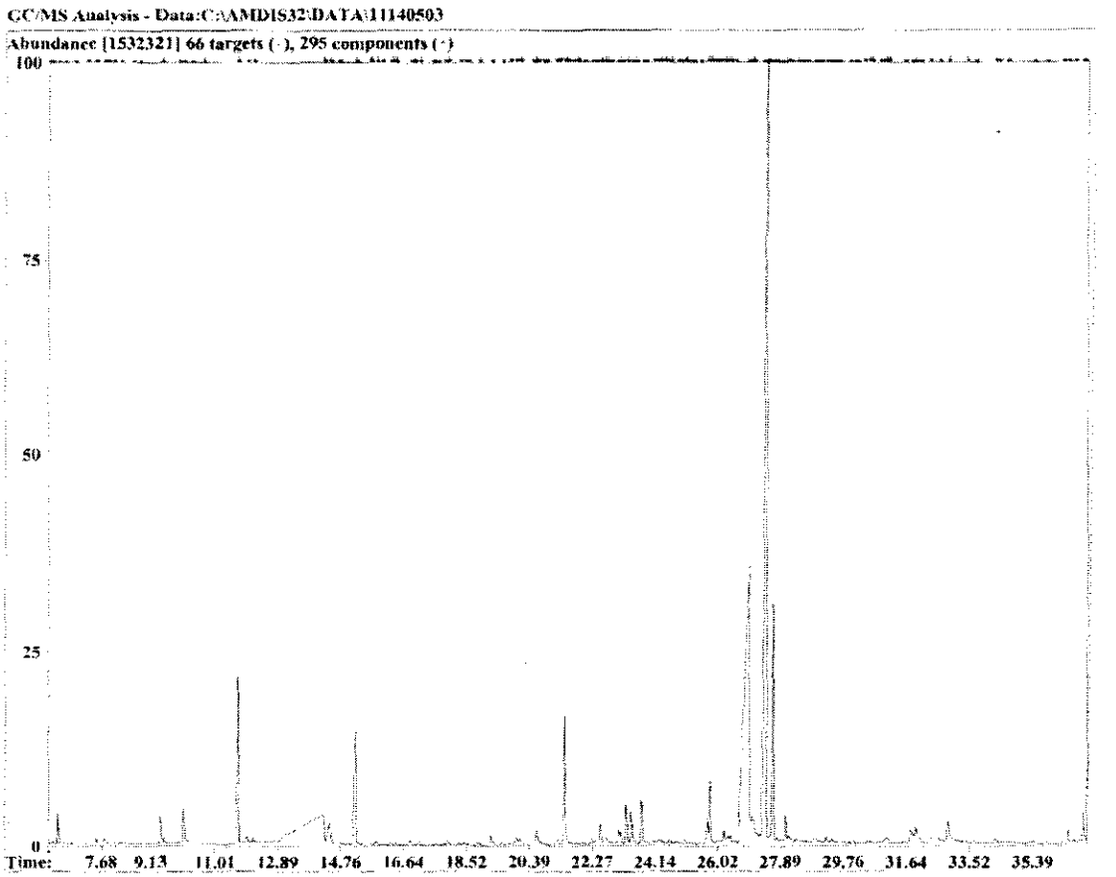
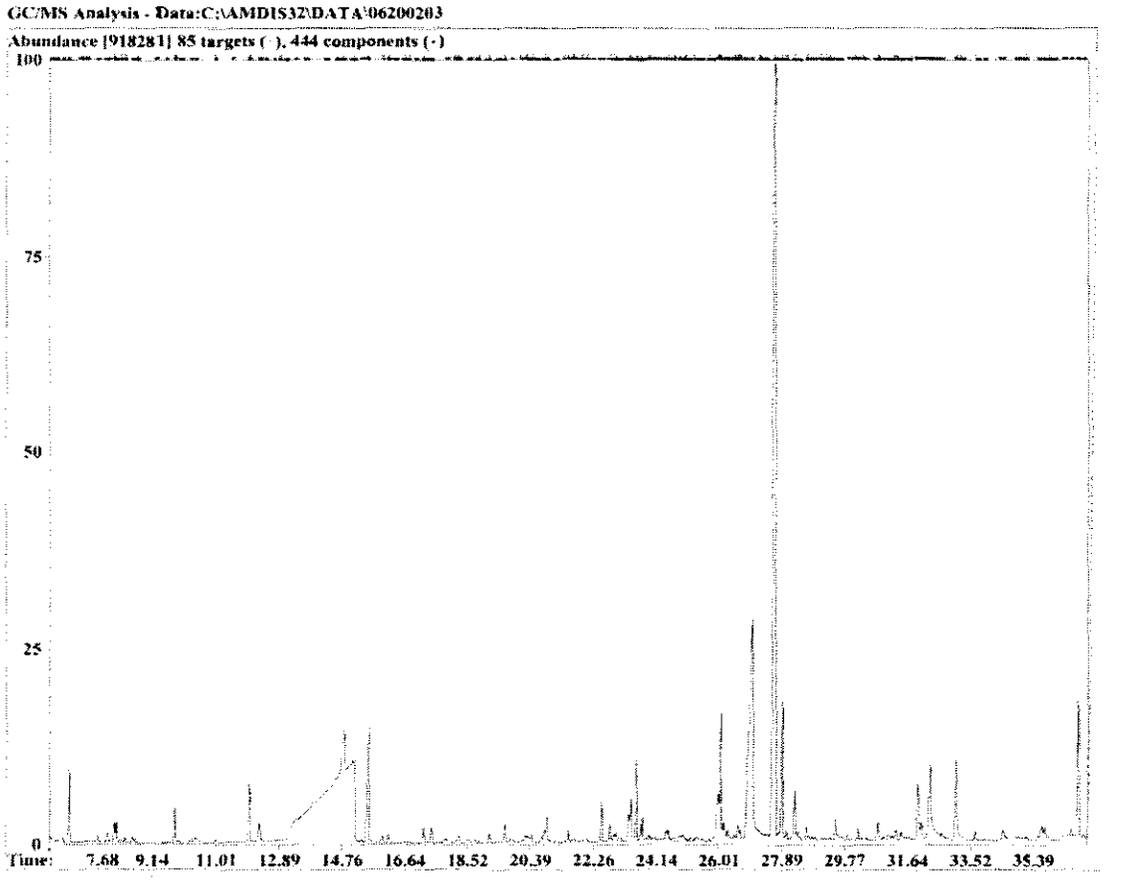
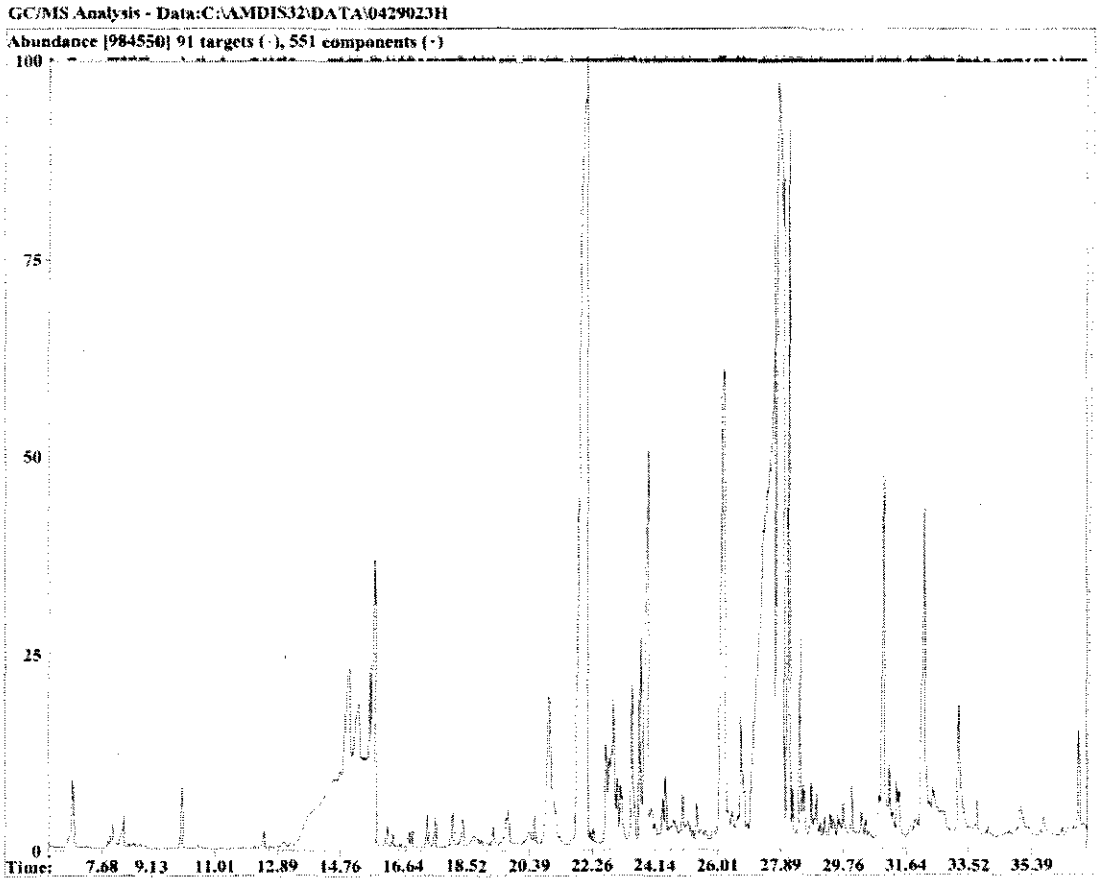


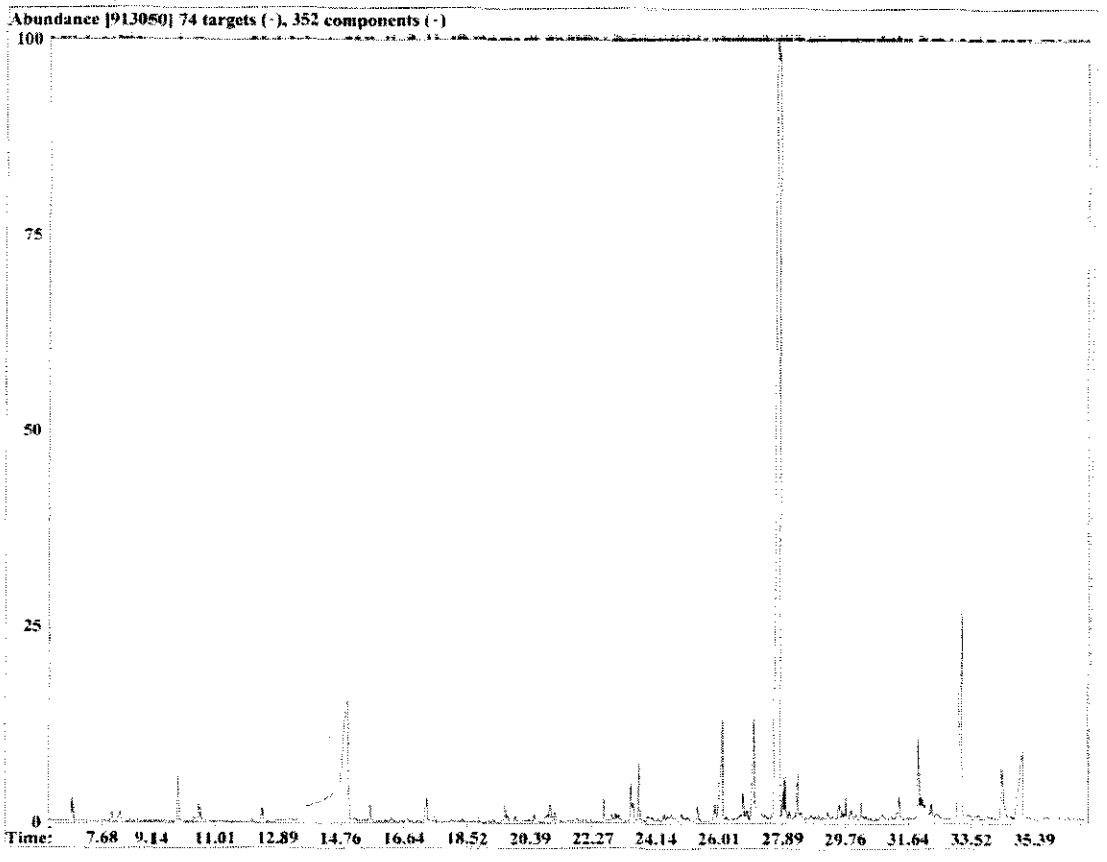

GC/MS Analysis - Data:C:\AMDIS32\DATA\11140503
Abundance [1532321] 66 targets (·), 295 components (·)
100
75
50
25
0
Time: 7.68 9.13 11.01 12.89 14.76 16.64 18.52 20.39 22.27 24.14 26.02 27.89 29.76 31.64 33.52 35.39

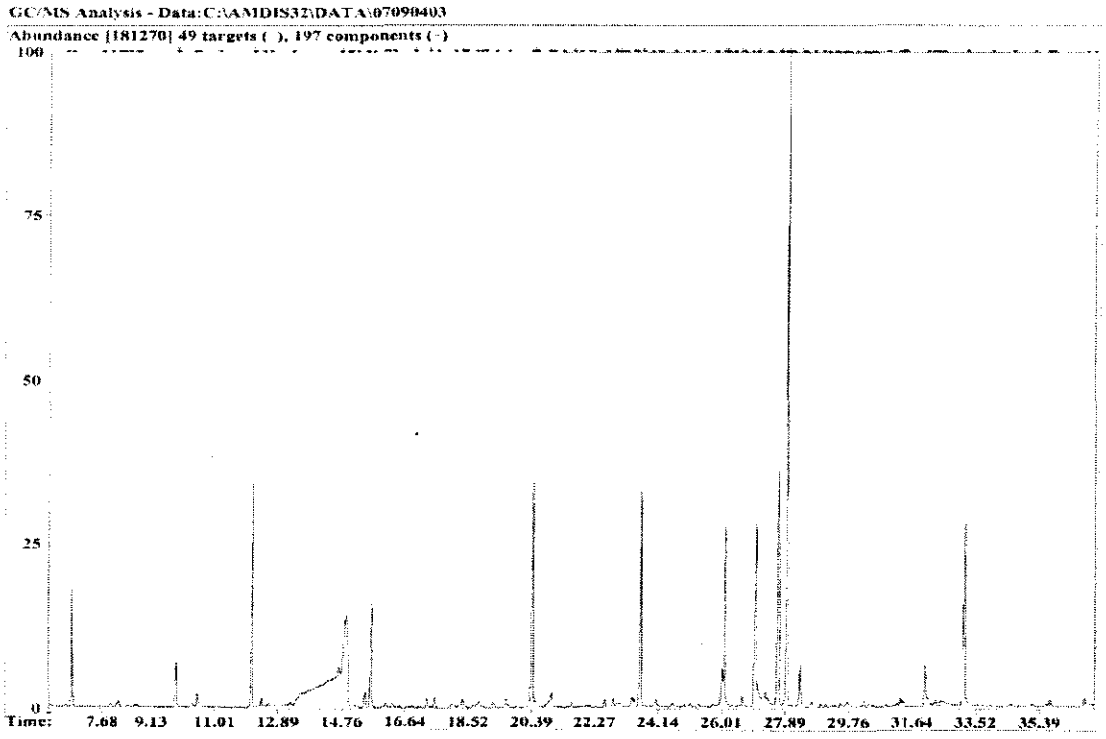

GC/MS Analysis - Data:C:\AMDIS32\DATA\06200203
Abundance [918281] 85 targets (·), 444 components (·)
100
75
50
25
0
Time: 7.68 9.14 11.01 12.89 14.76 16.64 18.52 20.39 22.26 24.14 26.01 27.89 29.77 31.64 33.52 35.39

GC/MS Analysis - Data:C:\AMDIS32\DATA\0429023H

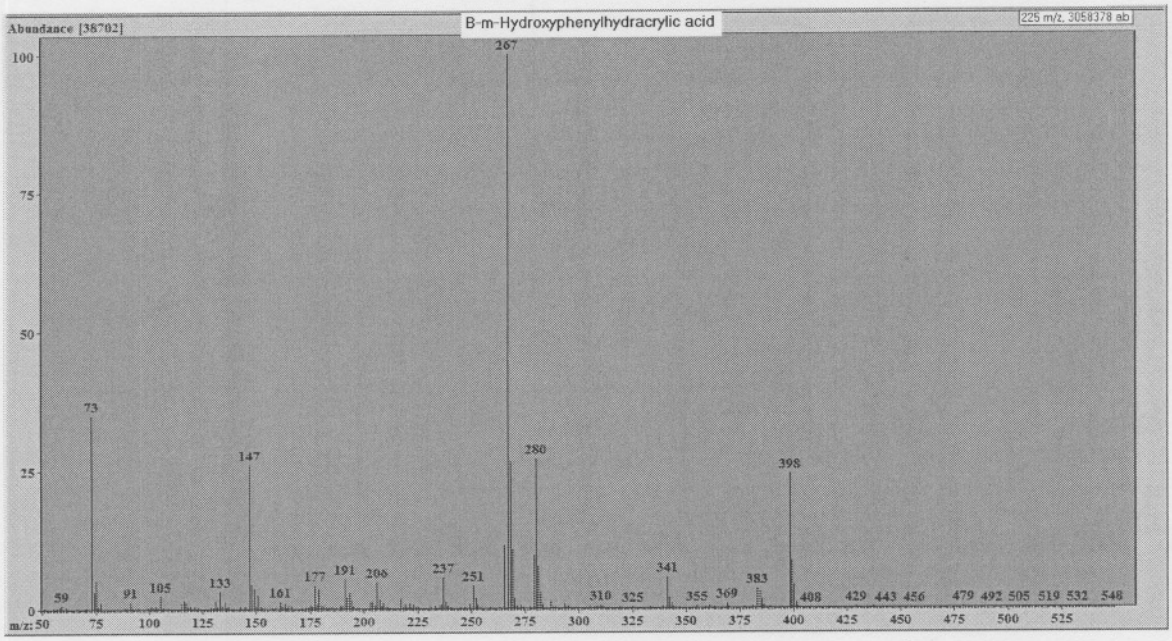


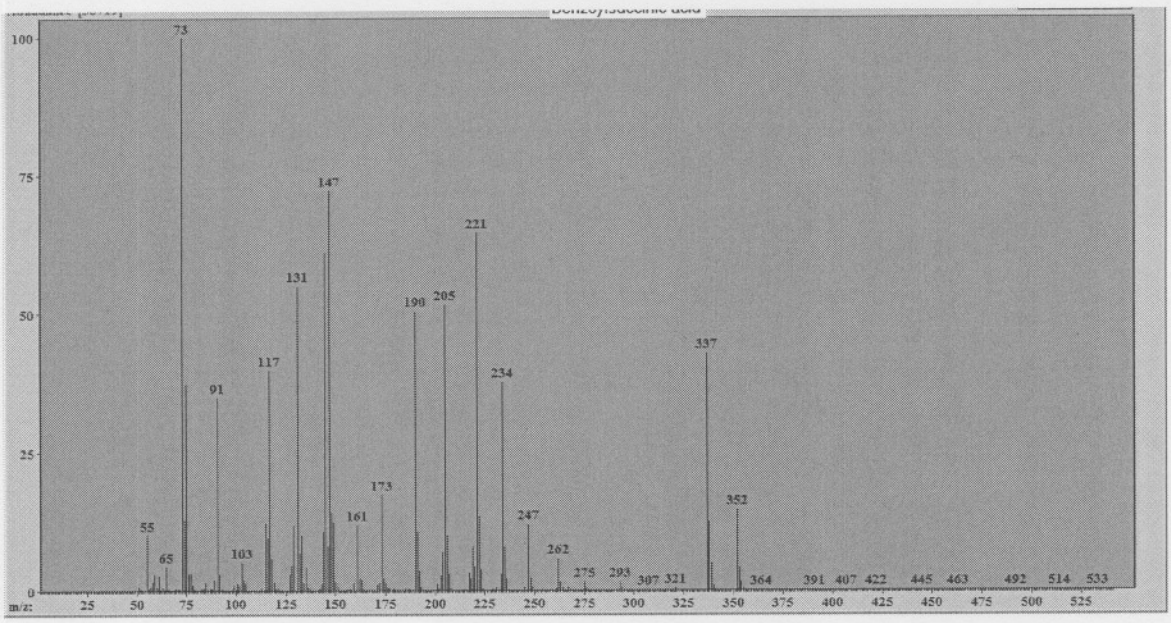
GC/MS Analysis - Data:C:\AMDIS32\DATA\12200303

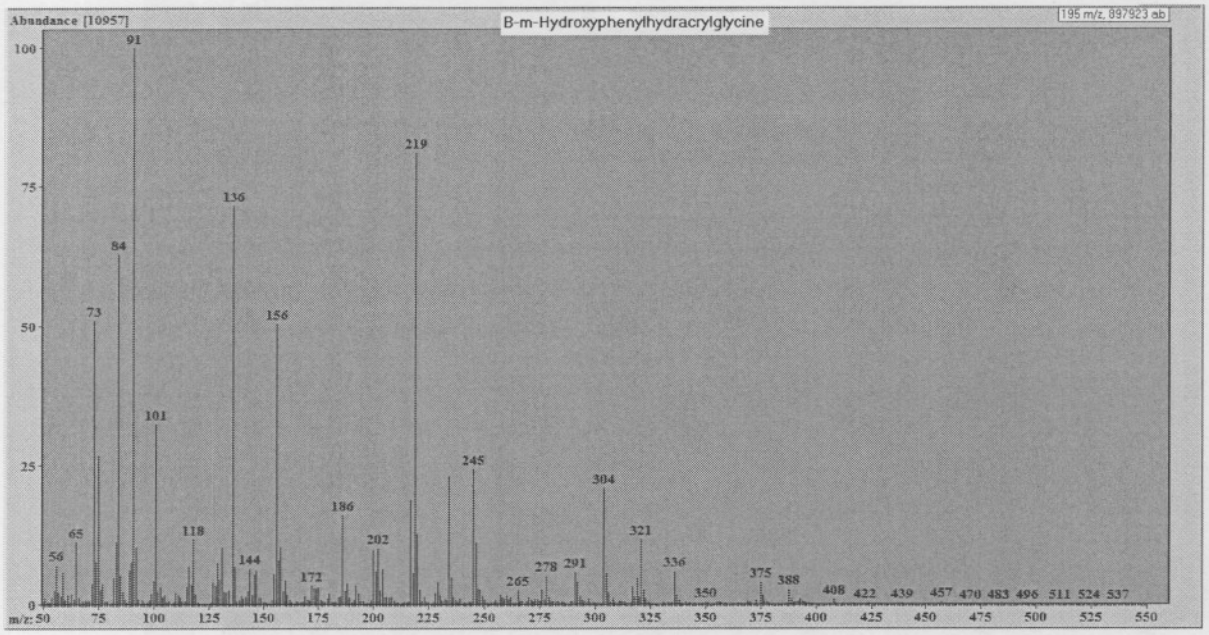


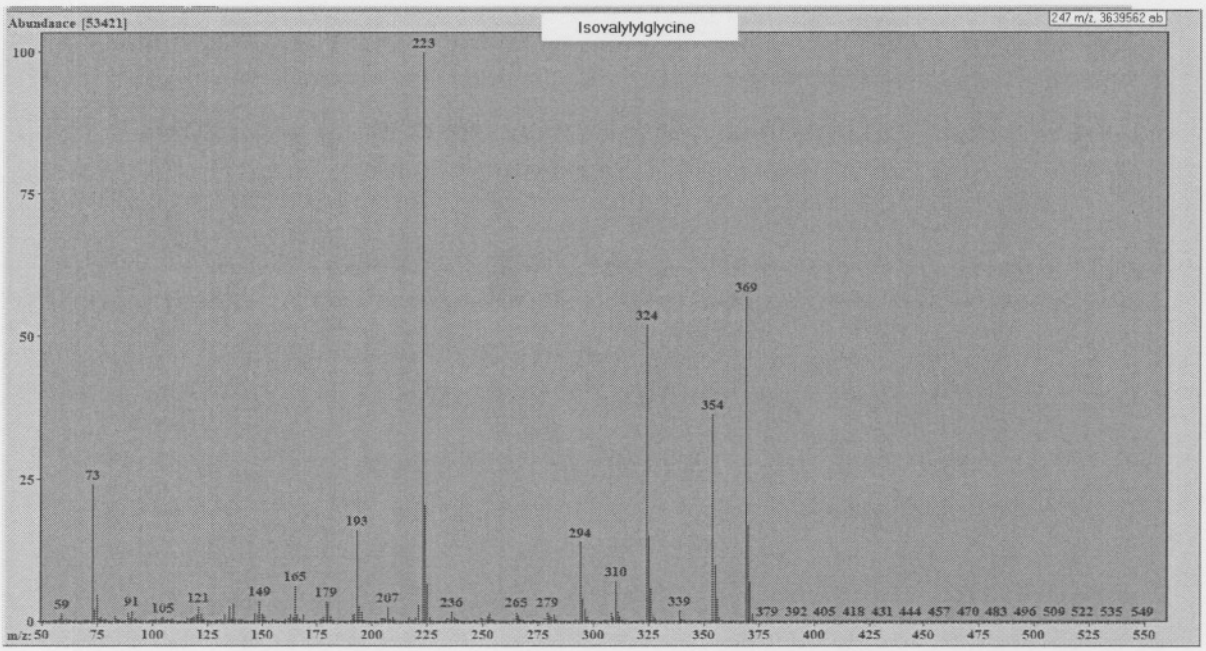

GC/MS Analysis - Data:C:\AMDIS32\DATA\07090403
Abundance [181270] 49 targets ( ), 197 components ( )
100
75
50
25
0
Time:
7.68
9.13
11.01
12.89
14.76
16.64
18.52
20.39
22.27
24.14
26.01
27.89
29.76
31.64
33.52
35.39

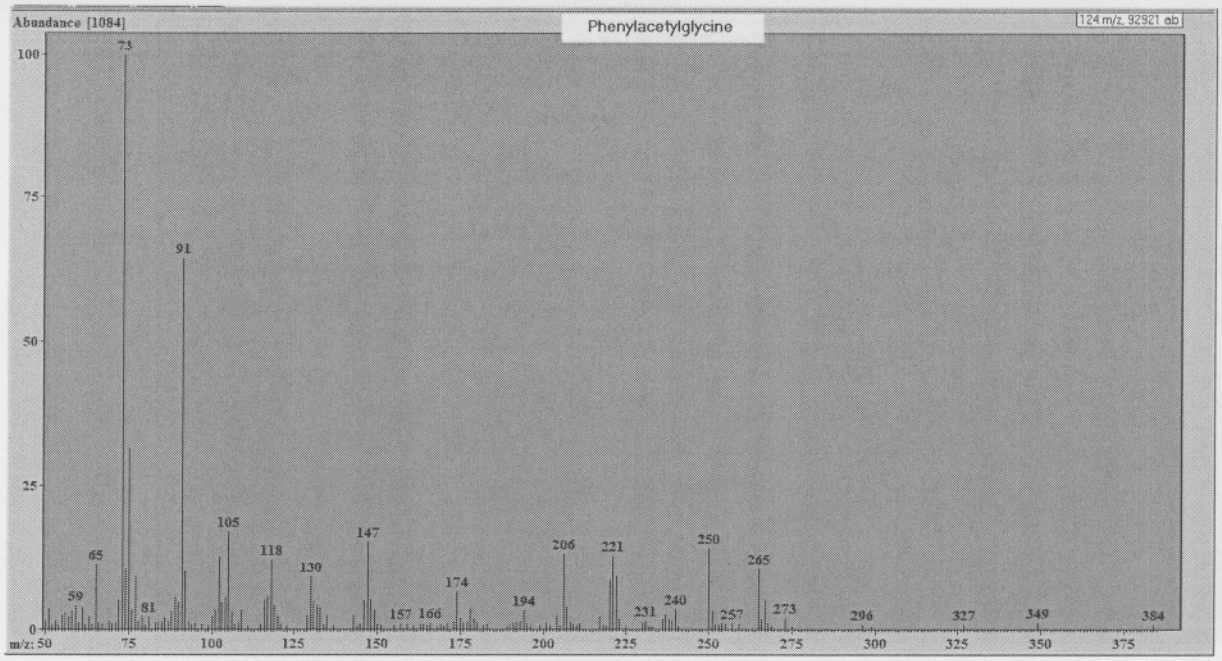
APPENDIX III






Abundance [10957]
B-m-Hydroxyphenylhydracrylglycine
195 m/z, 897923 ab
m/z:


Abundance [53421]
Isovalylylglycine
247 m/z, 3639562 ab
m/z:


Abundance [1084]
Phenylacetylglycine
124 m/z, 92921 ab
100
75
50
25
0
m/z:
50
75
100
125
150
175
200
225
250
275
300
325
350
375
73
91
105
118
130
147
157
166
174
194
206
221
231
240
250
257
265
273
296
327
349
384
65
59
81

APPENDIX IV

Patient 12/09/05/03

3,4-Dihydroxybenzeneacetic acid	128.135
3-Methoxy-4-hydroxy benzeneacetic acid	137.829
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	14.052
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	326.443
4-HYDROXY-3-METHOXYPHENYLLACTIC-TRITMS	19.475
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	65.277
ACETYLTYROSINE-DITMS	19.395
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	753.034
HIPPURIC-TMS	2176.966
ISOVANILGLYCOLIC-TRITMS	1077.517
MANDELIC-DITMS	15.837
m-HYDROXYBENZOIC-DITMS	450.049
m-HYDROXYPHENYLPROPIONIC-DITMS	19.822
o-HYDROXYHIPPURIC-DITMS	39.527
o-HYDROXYPHENYLACETIC-DITMS	36.894
p-HYDROXYHIPPURIC-DITMS	535.640
p-HYDROXYMANDELIC-TRITMS	269.477
p-HYDROXYPHENYLACETIC-DITMS	1606.585
p-HYDROXYPHENYLLACTIC-TRITMS	213.580
VANILLYLMANDELIC-TRITMS	1077.517
VANYLHYDRACRYLIC-TRITMS	97.376
PHOSPHORIC-TRITMS	880.056
GLYCERIC-TRITMS	43.300
GLYCERIN-TRITMS	25.583
GLYCOLIC-DITMS	20.895
LACTIC-DITMS2	56.603
HYDANTOINPROPIONIC-TRITMS	87.481
5-INDOLE-CARBOXYLIC-DITMS	6.412
INDOL-3-ACETIC-DITMS	157.015
3-HYDROXYBUTYRIC-DITMS	130.573
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	7.896
ERYTHRITOL	15.686
ERYTHRONIC-TETRATMS	130.968
GLUCURONIC-PENTATMS	17.535
THREITOL-TETRATMS	16.694
2-HYDROXYGLUTARIC-TRITMS	120.421
ACONITIC-TRITMS	1533.136

CITRIC-TETRATMS	2353.219
FUMARIC-DITMS	58.247
ISOCITRICLACTON-DITMS	33.597
ISOCITRIC-TETRATMS	2198.432
MALIC-TRITMS	170.298
SUCCINIC-DITMS	19.976
3-METHYLGLUTARIC-DITMS	16.571
GLUTARIC-DITMS	39.590
3,4-DIHYDROXYBUTYRIC-TRITMS	101.531
5-PYROLIDON-2-CARBOXYLIC-DITMS	252.057
DIOCTYLPHTALATE	172.207
FERULIC-DITMS	20.542
ISOFERULIC-DITMS	24.622
QUINOLINIC-DITMS	50.423
RESORCYLIC-TRITMS	304.229
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	145.963
1-METHYLZANTHINE-DITMS	6.791
2-HYDROXYISOBUTYRIC-DITMS	10.440
URACIL-DITMS	22.279
URIC-TETRATMS	46.660
METHYLCITRIC-TETRATMS	75.081
METHYLMALONIC-DITMS	1137.625
2-HYDROXY-ADIPIC-TRITMS	25.798
2-HYDROXYSEBACIC-TRITMS	16.477
2-METHYLSUCCINIC-DITMS	90.612
3-HYDROXYSEBACIC-TRITMS	34.363
3-METHYLADIPIC-DITMS	53.941
3-METHYLPIMELIC-DITMS	39.428
ADIPIC-DITMS	31.448
AZELAIC-DITMS	114.108
ETHYLMALONIC-DITMS	370.328
PALMITIC-TMS	77.631
STEARIC-TMS	63.361
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	114.883
3-METHYLGLUTACONIC-DITMS	98.161
18.76 min Internal standard	262.500
CITRAMALIC-TRITMS	70.133
OXALIC-DITMS	1173.535

Patient 01/24/01/03

2,3-DIHYDROXYBENZOIC-TRITMS	8.948
3,4-Dihydroxybenzeneacetic acid	22.814

3,4-DIHYDROXYMANDELIC-TETRATMS	11.747
3-HYDROXYPHENYLACETIC-DITMS	25.090
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	10.882
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	152.756
ACETYLTYROSINE-DITMS	3.239
BENZOIC-TMS	9.163
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	725.336
HIPPURIC-TMS	881.346
MANDELIC-DITMS	10.595
m-HYDROXYBENZOIC-DITMS	49.797
m-HYDROXYPHENYLPROPIONIC-DITMS	16.209
o-HYDROXYPHENYLACETIC-DITMS	8.009
P-AMINOBEMZOIC-TMS	29.631
p-COUMARIC-DITMS	7.060
p-HYDROXYHIPPURIC-DITMS	401.027
p-HYDROXYMANDELIC-TRITMS	172.602
p-HYDROXYPHENYLACETIC-DITMS	440.544
p-HYDROXYPHENYLLACTIC-TRITMS	72.479
p-HYDROXYPHENYLPYRUVIC-TRITMS	3.579
VANILLIC-DITMS	23.322
VANILLYLMANDELIC-TRITMS	449.985
VANYLHYDRACRYLIC-TRITMS	353.346
PHOSPHORIC-TRITMS	181.544
GLYCERIC-TRITMS	11.951
GLYCOLIC-DITMS	17.078
LACTIC-DITMS2	26.716
PYRUVIC-TMS	27.371
5-HYDROXYINDOLACETIC-DITMS	44.170
5-HYDROXYINDOLACETIC-DITMS	20.818
5-INDOLE-CARBOXYLIC-DITMS	15.853
INDOL-3-ACETIC-DITMS	42.163
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	29.109
ERYTHRONIC-TETRATMS	24.842
FURAN-2,5-DICARBOXYLIC-DITMS	12.366
THREITOL-TETRATMS	12.164
2-HYDROXYGLUTARIC-TRITMS	125.246
2-KETOGLUTARIC-TRITMS	44.377
ACONITIC-TRITMS	441.568
CITRIC-TETRATMS	888.468
FUMARIC-DITMS	44.750
ISOCITRICLACTON-DITMS	11.267
ISOCITRIC-TETRATMS	883.743
SUCCINIC-DITMS	444.715
3-METHYLGLUTARIC-DITMS	12.851
GLUTARIC-DITMS	121.619
CAFFEIC-TRITMS	4.466
DIPROPYLACETIC-TMS	2.714
PROPANETRICARBOXYLIC-TRITMS	5.081

3,4-DIHYDROXYBUTYRIC-TRITMS	41.929
DEHYDROABIETIC ACID	16.318
FERULIC-DITMS	45.820
HYDROCHINON-DITMS	74.058
ISOFERULIC-DITMS	27.296
QUINOLINIC-DITMS	34.889
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	2.938
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	63.841
OROTIC-TRITMS	29.058
THYMINE-DITMS	5.901
URACIL-DITMS	99.026
METHYLCITRIC-TETRATMS	35.205
METHYLMALONIC-DITMS	49.587
2-HYDROXY-ADIPIC-TRITMS	11.045
2-HYDROXYSEBACIC-TRITMS	29.038
2-METHYLSUCCINIC-DITMS	81.265
3-HYDROXYADIPYLLACTONE-TMS	13.480
3-HYDROXYSEBACIC-TRITMS	37.153
3-METHYLADIPIC-DITMS	33.235
3-METHYLPIMELIC-DITMS	14.088
ADIPIC-DITMS	26.012
AZELAIC-DITMS	42.359
ETHYLMALONIC-DITMS	69.324
METHYLPIMELIC-DITMS	19.637
PALMITIC-TMS	25.186
SEBACIC-DITMS	26.732
STEARIC-TMS	21.056
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	77.620
3-HYDROXYISOBUTYRIC-DITMS	11.763
3-METHYLGLUTACONIC-DITMS	41.304
18.76 min Internal standard	262.500
ACETYLASPARTIC-DITMS	6.523
CITRAMALIC-TRITMS	39.235
HOMOCITRIC-TERATMS	11.048
OXALIC-DITMS	465.896

Patient 06/21/01/03

3,4-Dihydroxybenzeneacetic acid	33.934
3-Methoxy-4-hydroxy benzeneacetic acid	76.493
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	6.696
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	80.332
ACETYLTYROSINE-DITMS	3.485
BENZOIC-TMS	69.595
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	728.213

HIPPURIC-TMS	850.629
MANDELIC-DITMS	40.219
m-HYDROXYBENZOIC-DITMS	110.635
m-HYDROXYMANDELIC-TRITMS	81.347
m-HYDROXYPHENYLPROPIONIC-DITMS	23.678
o-HYDROXYPHENYLACETIC-DITMS	4.976
p-HYDROXYHIPPURIC-DITMS	396.911
p-HYDROXYMANDELIC-TRITMS	81.192
p-HYDROXYPHENYLACETIC-DITMS	200.201
p-HYDROXYPHENYLLACTIC-TRITMS	24.448
VANILGLYCOLIC-TRITMS	236.007
VANILLIC-DITMS	15.459
VANILLYLMANDELIC-TRITMS	238.627
PHOSPHORIC-TRITMS	198.258
GLYCERIC-TRITMS	3.179
GLYCERIN-TRITMS	4.797
GLYCOLIC-DITMS	10.282
PYRUVIC-TMS	10.487
INDOL-3-ACETIC-DITMS	6.449
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	10.998
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	74.178
FURAN-2,5-DICARBOXYLIC-DITMS	106.675
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	164.394
GLUCURONIC-EO	7.156
GLUCURONIC-PENTATMS	9.394
mannonic-1,4-lactone	4.170
THREITOL-TETRATMS	6.534
2-HYDROXYGLUTARIC-TRITMS	23.634
ACONITIC-TRITMS	240.372
CITRIC-TETRATMS	100.168
FUMARIC-DITMS	10.090
ISOCITRICLACTON-DITMS	19.225
ISOCITRIC-TETRATMS	578.564
MALIC-TRITMS	7.334
SUCCINIC-DITMS	267.607
3-METHYLGLUTARIC-DITMS	2.756
GLUTARIC-DITMS	4.125
PROPANETRICARBOXYLIC-TRITMS	9.319
3,4-DIHYDROXYBUTYRIC-TRITMS	14.319
5-PYROLIDON-2-CARBOXYLIC-DITMS	69.121

a-RESORCYLIC	41.441
DIOCTYLPHTALATE	72.989
FERULIC-DITMS	37.982
ISOFERULIC-DITMS	3.033
LACTYLLACTATE	2.484
RESORCYLIC-TRITMS	1.563
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	8.364
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	27.551
1-METHYLZANTHINE-DITMS	1.838
URACIL-DITMS	10.544
METHYLCITRIC-TETRATMS	8.638
ACETYLTRHEONINE-DITMS	3.922
2-HYDROXY-ADIPIC-TRITMS	3.218
2-METHYLSUCCINIC-DITMS	21.698
3-METHYLADIPIC-DITMS	6.317
4-METHYLSUBERIC-DITMS	57.233
ADIPIC-DITMS	6.565
AZELAIC-DITMS	56.712
ETHYLMALONIC-DITMS	7.265
PALMITIC-TMS	6.882
STEARIC-TMS	6.553
SUBERIC-DITMS	12.559
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	47.442
3-HYDROXYISOBUTYRIC-DITMS	3.915
3-METHYLGLUTACONIC-DITMS	14.247
18.76 min Internal standard	262.500
4-HYDROXYBUTYRIC-DITMS	3.576
ACETYLASPARTIC-DITMS	4.854
CITRAMALIC-TRITMS	23.461
MESACONIC-DITMS	4.079
OXALIC-DITMS	170.091

Patient 07/17/01/03

2,3-DIHYDROXYBENZOIC-TRITMS	13.492
3,4-Dihydroxybenzeneacetic acid	22.487
3,4-DIHYDROXYPHENYLACETIC-TRITMS	22.681
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	10.179
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	154.186
4-HYDROXY-3-METHOXYPHENYLLACTIC-TRITMS	12.130
ACETYLTYROSINE-DITMS	2.972

BENZOIC-TMS	8.171
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	715.129
HIPPURIC-TMS	609.662
m-HYDROXYBENZOIC-DITMS	167.772
m-HYDROXYMANDELIC-TRITMS	106.958
m-HYDROXYPHENYLPROPIONIC-DITMS	22.897
o-HYDROXYPHENYLACETIC-DITMS	12.578
p-COUMARIC-DITMS	5.987
p-HYDROXYHIPPURIC-DITMS	591.612
p-HYDROXYMANDELIC-TRITMS	106.679
p-HYDROXYPHENYLACETIC-DITMS	399.493
p-HYDROXYPHENYLLACTIC-TRITMS	49.637
VANILLIC-DITMS	27.728
VANILLYLMANDELIC-TRITMS	446.830
PHOSPHORIC-TRITMS	250.270
GLYCERIC-TRITMS	13.049
GLYCERIN-TRITMS	3.512
GLYCOLIC-DITMS	13.910
LACTIC-DITMS2	18.450
5-INDOLE-CARBOXYLIC-DITMS	5.917
INDOL-3-ACETIC-DITMS	36.064
1,6-ANHYDRO-B-d-GLUCOPYRANOSE-TRITMS	50.346
2-FUROYLGLYCINE-TMS	62.889
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	698.029
ERYTHRO-2,3-DIHYDROXYBUTYRIC-TRITMS	50.648
FURAN-2,5-DICARBOXYLIC-DITMS	278.207
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	247.358
THREITOL-TETRATMS	8.088
2-HYDROXYGLUTARIC-TRITMS	32.475
ACONITIC-TRITMS	162.983
CITRIC-TETRATMS	620.079
FUMARIC-DITMS	11.332
ISOCITRICLACTON-DITMS	33.994
SUCCINIC-DITMS	349.155
3-METHYLGLUTARIC-DITMS	12.249
GLUTARIC-DITMS	35.837
PIPECOLIC-DITMS	182.672
NICOTINIC-TMS	1.969
paracetamol (bis TMS)	182.954
PROPANETRICARBOXYLIC-TRITMS	6.247

TARTARIC-TETRATMS	10.464
3,4-DIHYDROXYBUTYRIC-TRITMS	14.695
5-PYROLIDON-2-CARBOXYLIC-DITMS	183.877
a-RESORCYLIC	287.187
DIOCTYLPHTALATE	68.784
FERULIC-DITMS	76.230
ISOFERULIC-DITMS	2.963
QUINOLINIC-DITMS	47.067
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	30.977
1-METHYLZANTHINE-DITMS	1.787
THYMINE-DITMS	7.097
URACIL-DITMS	82.302
URIC-TETRATMS	22.571
XANTHURENIC-TRITMS	20.756
METHYLCITRIC-TETRATMS	21.213
METHYLMALONIC-DITMS	20.764
2-HYDROXYSEBACIC-TRITMS	31.937
2-METHYLSUCCINIC-DITMS	49.189
3-HYDROXYADIPYLLACTONE-TMS	8.547
3-HYDROXYSEBACIC-TRITMS	18.609
3-METHYLADIPIC-DITMS	8.048
3-METHYLPIMELIC-DITMS	15.406
4-METHYLSUBERIC-DITMS	49.451
ADIPIC-DITMS	20.904
ETHYLMALONIC-DITMS	54.521
STEARIC-TMS	13.413
SUBERIC-DITMS	19.209
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	63.022
3-HYDROXYISOBUTYRIC-DITMS	4.273
3-METHYLGLUTACONIC-DITMS	31.709
18.76 min Internal standard	262.500
ACETYLASPARTIC-DITMS	13.336
CITRAMALIC-TRITMS	26.014
OXALIC-DITMS	444.390

Patient 07/09/04/03

2,3-DIHYDROXYBENZOIC-TRITMS	3.290
3,4-Dihydroxybenzeneacetic acid	8.896
3-Methoxy-4-hydroxy benzeneacetic acid	216.799
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	24.597

B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	762.291
HIPPURIC-TMS	202.189
m-HYDROXYBENZOIC-DITMS	5.943
m-HYDROXYHIPPURIC-DITMS	236.245
p-COUMARIC-DITMS	2.872
p-HYDROXYHIPPURIC-DITMS	226.520
p-HYDROXYMANDELIC-TRITMS	18.498
p-HYDROXYPHENYLACETIC-DITMS	300.532
p-HYDROXYPHENYLLACTIC-TRITMS	6.683
VANILLYLMANDELIC-TRITMS	56.599
PHOSPHORIC-TRITMS	33.337
4-DEOXYTETRONIC-TRITMS	15.065
ERYTHRONIC-TETRATMS	5.429
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	35.384
GLUCURONIC-PENTATMS	8.245
2-HYDROXYGLUTARIC-TRITMS	11.929
ACONITIC-TRITMS	201.140
CITRIC-TETRATMS	247.358
FUMARIC-DITMS	1.503
ISOCITRICLACTON-DITMS	4.226
ISOCITRIC-TETRATMS	115.908
SUCCINIC-DITMS	163.086
GLUTARIC-DITMS	7.567
1,2-DIHYDROXYBUTANE-DI-TMS	2.117
4-HYDROXY-2-METHYLVALERIC-DITMS	4.075
a-RESORCYLIC	7.421
FERULIC-DITMS	3.897
ISOFERULIC-DITMS	4.844
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	3.988
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	13.755
2-HYDROXYISOBUTYRIC-DITMS	2.351
URACIL-DITMS	3.269
PYROGLUTAMIC-DITMS	15.568
3-METHYLADIPIC-DITMS	6.653
ADIPIC-DITMS	4.987
ETHYLMALONIC-DITMS	9.429
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	14.037
3-HYDROXYISOBUTYRIC-DITMS	2.226
3-METHYLGLUTACONIC-DITMS	5.462
18.76 min Internal standard	262.500

CITRAMALIC-TRITMS	7.088
OXALIC-DITMS	79.589

Patient 03/12/05/03

3,4-DIHYDROXYBENZALDEHYDE-DITMS	4.975
3,4-Dihydroxybenzeneacetic acid	17.537
3-HYDROXYPHENYLACETIC-DITMS	2.661
3-Methoxy-4-hydroxy benzeneacetic acid	154.341
3-METHOXY-4-HYDROXYBENZYLALCOHOL-DITMS	1.997
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	37.443
4-HYDROXY-3-METHOXYPHENYLLACTIC-TRITMS	11.200
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	862.134
HIPPURIC-TMS	408.069
ISOVANILHYDRACRYLIC-TRITMS	8.680
MANDELIC-DITMS	4.084
m-HYDROXYBENZOIC-DITMS	44.359
m-HYDROXYPHENYLPROPIONIC-DITMS	15.300
o-HYDROXYPHENYLACETIC-DITMS	715.115
p-COUMARIC-DITMS	8.960
p-HYDROXYHIPPURIC-DITMS	273.339
p-HYDROXYMANDELIC-TRITMS	59.571
p-HYDROXYPHENYLACETIC-DITMS	717.085
p-HYDROXYPHENYLLACTIC-TRITMS	40.266
VANILGLYCOL-TRITMS	211.016
VANILLIC-DITMS	271.798
VANILLYLMANDELIC-TRITMS	262.258
VANYLHYDRACRYLIC-TRITMS	107.867
PHOSPHORIC-TRITMS	97.540
2-GLYCEROPHOSPHATE-TERATMS	3.500
GLYCERIC-TRITMS	2.547
GLYCERIN-TRITMS	5.712
LACTIC-DITMS2	41.361
5-INDOLE-CARBOXYLIC-DITMS	10.024
3-HYDROXYBUTYRIC-DITMS	7.753
ARABINONIC-g-LACTONE-TRITMS	5.519
ERYTHRO-2,3-DIHYDROXYBUTYRIC-TRITMS	7.502
ERYTHRONIC-TETRATMS	16.184
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	51.217
THREITOL-TETRATMS	6.446
2-HYDROXYGLUTARIC-TRITMS	6.506
CITRIC-TETRATMS	18.958

FUMARIC-DITMS	5.568
SUCCINIC-DITMS	118.082
GLUTARIC-DITMS	6.427
DEPAKINE	4.223
3,4-DIHYDROXYBUTYRIC-TRITMS	24.907
DEHYDROABIETIC ACID	37.457
DIOCTYLPHTALATE	57.349
FERULIC-DITMS	106.888
QUINOLINIC-DITMS	6.387
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	2.382
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	7.718
URACIL-DITMS	21.470
METHYLCITRIC-TETRATMS	5.763
METHYLMALONIC-DITMS	15.802
3-HYDROXYSEBACIC-TRITMS	5.786
3-METHYLADIPIC-DITMS	7.065
3-METHYLPIMELIC-DITMS	12.919
ADIPIC-DITMS	7.425
AZELAIC-DITMS	37.583
ETHYLMALONIC-DITMS	21.916
METHYLPIMELIC-DITMS	13.112
PALMITIC-TMS	39.106
PIMELIC-DITMS	7.326
STEARIC-TMS	34.191
3-METHYLGLUTACONIC-DITMS	9.704
18.76 min Internal standard	262.500
OXALIC-DITMS	286.334

Patient 06/14/04/03

3(4-HYDROXYPHENYL)PROPIONIC-TMS	2.892
3,4-Dihydroxybenzeneacetic acid	29.337
3,4-DIHYDROXYBENZOIC-TRITMS	34.136
3-Methoxy-4-hydroxy benzeneacetic acid	187.138
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	77.887
4-HYDROXY-3-METHOXYPHENYLLACTIC-TRITMS	32.996
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	859.482
m-HYDROXYBENZOIC-DITMS	222.977
m-HYDROXYPHENYLPROPIONIC-DITMS	7.309
p-COUMARIC-DITMS	13.482
p-HYDROXYMANDELIC-TRITMS	39.676
p-HYDROXYPHENYLACETIC-DITMS	1404.632

p-HYDROXYPHENYLLACTIC-TRITMS	39.371
VANILGLYCOLIC-TRITMS	286.287
VANILLYLMANDELIC-TRITMS	276.122
PHOSPHORIC-TRITMS	37.769
LACTIC-DITMS2	9.716
PYRUVIC-TMS	2.827
5-HYDROXYINDOLACETIC-DITMS	4.227
5-INDOLE-CARBOXYLIC-DITMS	8.323
INDOL-3-ACETIC-DITMS	10.861
INDOL-3-ACETIC-DITMS	11.349
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	4.667
2-HYDROXYGLUTARIC-TRITMS	85.341
CITRIC-TETRATMS	27.879
FUMARIC-DITMS	9.496
3-METHYLGLUTARIC-DITMS	14.441
GLUTARIC-DITMS	47.318
PIPECOLIC-DITMS	5.218
CAFFEIC-TRITMS	2.418
PROPANETRICARBOXYLIC-TRITMS	61.142
3,4-DIHYDROXYBUTYRIC-TRITMS	27.808
4-HYDROXY-2-METHYLVALERIC-DITMS	13.256
FERULIC-DITMS	27.530
ISOFERULIC-DITMS	8.186
QUINOLINIC-DITMS	47.544
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	63.857
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	6.874
DIHYDROURACIL-DITMS	2.205
URACIL-DITMS	66.850
URIC-TETRATMS	72.019
PYROGLUTAMIC-DITMS	5.218
METHYLCITRIC-TETRATMS	21.390
METHYLMALONIC-DITMS	1655.681
2-HYDROXY-ADIPIC-TRITMS	34.039
2-HYDROXYSEBACIC-TRITMS	19.610
3-HYDROXYSEBACIC-TRITMS	95.367
3-METHYLADIPIC-DITMS	11.878
ADIPIC-DITMS	57.478
AZELAIC-DITMS	36.878
ETHYLMALONIC-DITMS	2.439
PIMELIC-DITMS	12.275
SUBERIC-DITMS	30.526
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	289.068

3-HYDROXYISOBUTYRIC-DITMS	2.770
3-METHYLGLUTACONIC-DITMS	9.975
18.76 min Internal standard	262.500
CITRAMALIC-TRITMS	39.305
MALONIC-DITMS	2.816
OXALIC-DITMS	343.046

Patient 03/23/01/03

2,3-DIHYDROXYBENZOIC-TRITMS	9.494
3,4-Dihydroxybenzeneacetic acid	70.552
3-HYDROXYPHENYLACETIC-DITMS	204.825
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	213.419
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	7.831
ACETYLTYROSINE-DITMS	21.269
BENZOIC-TMS	9.929
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	1121.135
HIPPURIC-TMS	2216.393
m-HYDROXYBENZOIC-DITMS	488.920
m-HYDROXYPHENYLPROPIONIC-DITMS	21.406
o-HYDROXYHIPPURIC-DITMS	17.118
o-HYDROXYPHENYLACETIC-DITMS	46.755
p-COUMARIC-DITMS	9.168
p-HYDROXYHIPPURIC-DITMS	303.025
p-HYDROXYMANDELIC-TRITMS	229.914
p-HYDROXYPHENYLACETIC-DITMS	807.686
p-HYDROXYPHENYLLACTIC-TRITMS	72.770
p-HYDROXYPHENYLPYRUVIC-TRITMS	5.602
VANILLIC-DITMS	170.866
VANILLYLMANDELIC-TRITMS	522.330
PHOSPHORIC-TRITMS	1474.076
GLYCERIC-TRITMS	16.000
GLYCERIN-TRITMS	26.959
LACTIC-DITMS2	242.859
5-HYDROXYINDOLACETIC-DITMS	10.922
5-INDOLE-CARBOXYLIC-DITMS	6.886
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	120.614
FURAN-2,5-DICARBOXYLIC-DITMS	64.912
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	1133.307
THREITOL-TETRATMS	22.347
2-HYDROXYGLUTARIC-TRITMS	131.000
ACONITIC-TRITMS	463.381

CITRIC-TETRATMS	1983.101
ISOCITRICLACTON-DITMS	28.117
MALIC-TRITMS	45.922
SUCCINIC-DITMS	906.114
3-METHYLGLUTARIC-DITMS	13.740
GLUTARIC-DITMS	49.364
CAFFEIC-TRITMS	19.939
PROPANETRICARBOXYLIC-TRITMS	26.990
3,4-DIHYDROXYBUTYRIC-TRITMS	96.022
a-RESORCYLIC	62.407
FERULIC-DITMS	50.714
HYDROCHINON-DITMS	8.930
RESORCYLIC-TRITMS	81.525
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	58.858
URACIL-DITMS	44.814
METHYLCITRIC-TETRATMS	23.130
2-HYDROXY-ADIPIC-TRITMS	11.114
2-HYDROXYSEBACIC-TRITMS	14.358
2-METHYLSUCCINIC-DITMS	62.120
3-HYDROXYSEBACIC-TRITMS	89.582
3-METHYLADIPIC-DITMS	33.870
3-METHYLPIMELIC-DITMS	35.767
ADIPIC-DITMS	46.507
AZELAIC-DITMS	48.700
ETHYLMALONIC-DITMS	96.586
LCHAD (27.59)	15.689
OCTADECENOIC-TMS	25.291
OLEIC-TMS	33.025
PALMITIC-TMS	210.024
PENTADECANOIC-TMS	12.875
STEARIC-TMS	109.144
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	84.879
3-METHYLGLUTACONIC-DITMS	46.420
18.76 min Internal standard	262.500
BCHBIB entry 3	527.717
CITRAMALIC-TRITMS	82.043
HOMOCITRIC-TERATMS	25.302
OXALIC-DITMS	115.570

Patient 06/27/02/03

2,3-DIHYDROXYBENZOIC-TRITMS	45.486

3,4-Dihydroxybenzeneacetic acid	58.039
3-Methoxy-4-hydroxy benzeneacetic acid	914.771
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	512.018
ACETYLTYROSINE-DITMS	34.815
BENZOIC-TMS	22.022
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	1981.953
HIPPURIC-TMS	2554.545
MANDELIC-DITMS	20.584
m-HYDROXYBENZOIC-DITMS	536.423
m-HYDROXYPHENYLPROPIONIC-DITMS	26.504
o-HYDROXYHIPPURIC-DITMS	29.387
o-HYDROXYPHENYLACETIC-DITMS	57.491
P-CRESOL-tms	10.857
PHENYLACETIC-TMS	42.243
p-HYDROXYHIPPURIC-DITMS	859.555
p-HYDROXYMANDELIC-TRITMS	372.029
p-HYDROXYPHENYLACETIC-DITMS	2958.127
p-HYDROXYPHENYLLACTIC-TRITMS	294.527
VANILLYLMANDELIC-TRITMS	2391.744
PHOSPHORIC-TRITMS	767.562
GLYCERIC-TRITMS	54.633
GLYCOLIC-DITMS	53.762
LACTIC-DITMS2	133.671
PYRUVIC-TMS	25.913
1H-Indole-1-acetic acid, trimethylsilyl ester (	133.639
5-HYDROXYINDOLACETIC-DITMS	72.616
5-INDOLE-CARBOXYLIC-DITMS	20.336
INDOL-3-ACETIC-DITMS	499.394
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	66.055
ERYTHRITOL	53.439
ERYTHRONIC-TETRATMS	72.133
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	562.520
mannonic-1,4-lactone	25.756
RIBONIC-PENTATMS	14.132
XYLONIC-PENTATMS	16.009
2-HYDROXYGLUTARIC-TRITMS	578.294
2-KETOGLUTARIC-TRITMS	402.964
ACONITIC-TRITMS	1365.618
CITRIC-TETRATMS	3365.253
FUMARIC-DITMS	378.850
ISOCITRICLACTON-DITMS	67.238
ISOCITRIC-TETRATMS	2835.433

MALIC-TRITMS	827.519
SUCCINIC-DITMS	3178.991
3-METHYLGLUTARIC-DITMS	15.277
GLUTARIC-DITMS	683.594
TARTARIC-TETRATMS	17.485
3,4-DIHYDROXYBUTYRIC-TRITMS	311.877
4-HYDROXY-2-METHYLVALERIC-DITMS	16.556
5-PYROLIDON-2-CARBOXYLIC-DITMS	511.859
DEHYDROABIETIC ACID	91.562
ETHYLENGLYCOL-DITMS	50.009
ISOFERULIC-DITMS	12.910
QUINOLINIC-DITMS	104.436
RESORCYLIC-TRITMS	3.645
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	12.046
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	216.344
THYMINE-DITMS	22.825
URACIL-DITMS	72.664
3-HYDROXYPROPIONIC-DITMS	17.260
METHYLCITRIC-TETRATMS	178.145
METHYLMALONIC-DITMS	237.874
ACETYLTRHEONINE-DITMS	44.147
2-HYDROXY-ADIPIC-TRITMS	44.045
2-HYDROXYSEBACIC-TRITMS	91.696
2-METHYLSUCCINIC-DITMS	149.694
3-ETHYLHYDRACRYLIC-DITMS	23.587
3-HYDROXYSEBACIC-TRITMS	235.370
3-METHYLADIPIC-DITMS	65.424
3-METHYLPIMELIC-DITMS	213.037
ADIPIC-DITMS	117.603
AZELAIC-DITMS	725.550
ETHYLMALONIC-DITMS	317.347
LAURIC-DITMS	30.917
LCHAD (34.8)	19.653
PALMITIC-TMS	148.285
PIMELIC-DITMS	109.963
STEARIC-TMS	104.733
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	139.109
3-HYDROXYISOBUTYRIC-DITMS	58.217
3-METHYLGLUTACONIC-DITMS	90.709
18.76 min Internal standard	262.500
ACETYLASPARTIC-DITMS	71.420
CITRACONIC-DITMS	12.971

CITRAMALIC-TRITMS	125.632
HOMOCITRIC-TERATMS	16.492
MALONIC-DITMS	11.691
OXALIC-DITMS	1375.699

Patient 02/25/03/03

2,3-DIHYDROXYBENZOIC-TRITMS	74.474
3,4-Dihydroxybenzeneacetic acid	125.454
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	23.903
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	1495.186
HIPPURIC-TMS	9769.799
ISOVANILHYDRACRYLIC-TRITMS	74.038
m-HYDROXYBENZOIC-DITMS	118.298
m-HYDROXYPHENYLPROPIONIC-DITMS	32.931
o-HYDROXYHIPPURIC-DITMS	112.020
p-HYDROXYHIPPURIC-DITMS	781.850
p-HYDROXYMANDELIC-TRITMS	202.161
p-HYDROXYPHENYLACETIC-DITMS	509.662
p-HYDROXYPHENYLLACTIC-TRITMS	106.241
VANILLYLMANDELIC-TRITMS	454.592
PHOSPHORIC-TRITMS	1390.124
INDOL-3-ACETIC-DITMS	51.883
4-DEOXYTETRONIC-TRITMS	85.474
ARABINONIC-g-LACTONE-TRITMS	44.388
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	551.697
GLUCURONIC-PENTATMS	48.528
CITRIC-TETRATMS	8075.359
ISOCITRICLACTON-DITMS	313.104
SUCCINIC-DITMS	386.618
3-HYDROXYGLUTARIC-TRITMS	48.745
GLUTARIC-DITMS	17.969
5-PYROLIDON-2-CARBOXYLIC-DITMS	364.657
a-RESORCYLIC	101.019
FERULIC-DITMS	62.953
ISOFERULIC-DITMS	59.217
RESORCYLIC-TRITMS	77.414
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	67.351
URACIL-DITMS	55.846
METHYLCITRIC-TETRATMS	78.689
METHYLMALONIC-DITMS	30.203
3-METHYLADIPIC-DITMS	29.528

AZELAIC-DITMS	103.610
PALMITIC-TMS	128.517
PIMELIC-DITMS	37.373
STEARIC-TMS	68.182
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	199.032
3-METHYLGLUTACONIC-DITMS	27.780
18.76 min Internal standard	262.500
ACETYLASPARTIC-DITMS	30.424
CITRAMALIC-TRITMS	164.462
OXALIC-DITMS	1164.699

Patient 07/31/03/03

2,3-DIHYDROXYBENZOIC-TRITMS	6.434
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	21.353
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	51.158
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	1901.912
HIPPURIC-TMS	3317.992
m-HYDROXYBENZOIC-DITMS	28.978
m-HYDROXYPHENYLPROPIONIC-DITMS	54.634
o-HYDROXYPHENYLACETIC-DITMS	24.923
p-COUMARIC-DITMS	39.873
p-HYDROXYHIPPURIC-DITMS	446.070
p-HYDROXYMANDELIC-TRITMS	108.692
p-HYDROXYPHENYLACETIC-DITMS	544.554
VANILLYLMANDELIC-TRITMS	363.014
PHOSPHORIC-TRITMS	83.713
GLYCERIC-TRITMS	9.372
GLYCOLIC-DITMS	13.995
LACTIC-DITMS2	28.558
PYRUVIC-TMS	11.761
1H-Indole-2-carboxylic acid, 1-(trimethylsilyl)	209.292
5-INDOLE-CARBOXYLIC-DITMS	22.226
INDOL-3-ACETIC-DITMS	303.133
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	36.282
ERYTHRONIC-TETRATMS	11.380
FUCONO-g-LACTONE-PENTATMS	23.946
2-HYDROXYGLUTARIC-TRITMS	73.871
ACONITIC-TRITMS	2655.537
CITRIC-TETRATMS	2897.398
FUMARIC-DITMS	28.363
ISOCITRICLACTON-DITMS	181.862

MALIC-TRITMS	16.089
SUCCINIC-DITMS	2553.861
3-METHYLGLUTARIC-DITMS	4.026
GLUTARIC-DITMS	53.575
PIPECOLIC-DITMS	3.482
PROPANETRICARBOXYLIC-TRITMS	20.292
TARTARIC-TETRATMS	3191.243
4-HYDROXY-2-METHYLVALERIC-DITMS	39.513
5-PYROLIDON-2-CARBOXYLIC-DITMS	112.841
a-RESORCYLIC	124.857
FERULIC-DITMS	23.661
LACTYLLACTATE	143.730
PYROLCARBOXYLIC-DITMS	164.528
QUINOLINIC-DITMS	27.569
SUCCINYLLACTATE-DITMS	109.108
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	26.437
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	35.875
URACIL-DITMS	297.364
METHYLCITRIC-TETRATMS	42.950
METHYLMALONIC-DITMS	53.970
2-HYDROXY-ADIPIC-TRITMS	17.021
3-METHYLADIPIC-DITMS	20.775
3-METHYLPIMELIC-DITMS	50.149
ADIPIC-DITMS	41.050
AZELAIC-DITMS	152.861
ETHYLMALONIC-DITMS	38.012
PALMITIC-TMS	6.416
PIMELIC-DITMS	36.171
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	121.988
3-HYDROXY-ISO-VALERIC-DITMS	19.454
3-METHYLGLUTACONIC-DITMS	14.529
18.76 min Internal standard	262.500
CITRAMALIC-TRITMS	87.451
OXALIC-DITMS	702.271

Patient 09/04/02/03

2-PHENYLLACTIC	1729.003
3,4-DIHYDROXYBUTYRIC	955.852
3,4-DIHYDROXYBENZOIC-TRITMS	1677.069
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	1889.069
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	1328.144

BENZOIC-TMS	527.729
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	2454.275
HIPPURIC-TMS	57542.470
m-HYDROXYBENZOIC-DITMS	4198.843
o-HYDROXYHIPPURIC-DITMS	68482.720
o-HYDROXYPHENYLACETIC-DITMS	135.027
p-COUMARIC-DITMS	199.491
PHENYLACETIC-TMS	290.168
p-HYDROXYHIPPURIC-DITMS	7933.421
p-HYDROXYMANDELIC-TRITMS	2431.156
p-HYDROXYPHENYLACETIC-DITMS	33088.480
p-HYDROXYPHENYLLACTIC-TRITMS	1865.475
VANILLIC-DITMS	589.246
VANILLYLMANDELIC-TRITMS	3216.569
VANYLHYDRACRYLIC-TRITMS	1345.409
UREA	8631.816
PHOSPHORIC-TRITMS	6174.703
ADIPIC	552.984
GLYCERIC-TRITMS	316.026
GLYCERIN-TRITMS	264.023
GLYCOLIC-DITMS	566.937
LACTIC-DITMS2	5230.935
PYRUVIC-TMS	726.122
PROPANETRICARBOXYLIC	1397.462
INDOL-3-ACETIC-DITMS	5499.319
1,9-DIMETHYLURIC	7300.300
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	234.555
ARABINONIC-g-LACTONE-TRITMS	473.155
ERYTHRONIC-TETRATMS	573.256
PALMITIC	29563.980
2-HYDROXYGLUTARIC-TRITMS	3360.032
ACONITIC-TRITMS	11539.960
FUMARIC-DITMS	169.360
ISOCITRICLACTON-DITMS	727.418
ISOCITRIC-TETRATMS	15141.050
SUCCINIC-DITMS	18930.390
3-METHYLGLUTARIC-DITMS	130.835
GLUTARIC-DITMS	1304.065
PIMELIC	259.978
HEXACOSANE	863.384
EPTADECANOIC	246.194
TARTARIC-TETRATMS	1140.767

PENTADECANOIC	427.134
5-PYROLIDON-2-CARBOXYLIC-DITMS	2975.804
FERULIC-DITMS	3766.993
ISOFERULIC-DITMS	1309.830
RESORCYLIC-TRITMS	3635.199
THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	1074.187
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	735.388
MANDELIC	191.609
METHYLPIMELIC	424.523
METHYLCITRIC-TETRATMS	534.157
o-PHTALIC	607.628
MYRISTIC	5541.315
3-METHYLADIPIC-DITMS	300.828
AZELAIC-DITMS	1082.494
ETHYLMALONIC-DITMS	491.003
URACIL	4916.827
3-METHYLGLUTACONIC-DITMS	404.499
7-METHYLZANTHINE	409.641
MARGARIC	1093.430
OLEIC	11368.730
STEARIC-TMS	45185.770
LINOLEIC	780.321
DEHYDROABIETIC	56195.930
EICOSANOIC	386.086
LAURIC	1187.312
18.76 min Internal standard	262.500
CITRACONIC-DITMS	117.258
CITRAMALIC-TRITMS	889.233
OXALIC-DITMS	8451.302

Patient 11/20/02/03

BENZOIC-TMS	208.107
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	2634.311
m-HYDROXYBENZOIC-DITMS	1411.192
PHENYLLACTIC-DITMS	4672.684
p-HYDROXYHIPPURIC	6671.385
p-HYDROXYPHENYLACETIC-DITMS	10516.190
p-HYDROXYPHENYLLACTIC-TRITMS	11830.490
P-HYDROXYMANDELIC	1432.126
2-HYDROIXYGLUTARIC	801.025
PHOSPHORIC-TRITMS	2483.244

ISOVANYLHYDRACRYLIC	873.236
VANYLHYDRACRYLIC	914.947
VANILGLYCOLIC	3199.532
LACTIC	9389.785
GLYCOLIC	913.948
3-HYDROXYBUTYRIC	1420.385
SUCCINIC	6948.939
4-HYDROXY-2METHYLVALERIC	263.029
FUMARIC	562.654
4-DEOXYTETRONIC	665.698
3-METHYLGLUTACONIC	343.375
CITRAMALIC	248.936
ADIPIC	354.273
MALIC	867.258
3-METHYLADIPIC	249.250
GLUTARIC	351.255
HIPPURIC	7870.002
CITRIC	11991.620
p-COUMARIC	243.350
PENTADECANOIC	154.380
PALMITIC	2939.875
6-OCTADECENOIC	292.627
OCTADECENOIC	594.643
GLYCERIC-TRITMS	648.958
GLYCERIN-TRITMS	424.916
UREA	7955.894
5-HYDROXYMETHYL-2-FURANECARBOXYL	5555.427
ERYTHONIC	2055.231
3,4-DIHYDROXYBUTYRIC-TRITMS	1698.233
TARTARIC	664.561
3-KETOVALERIC	277.730
3-DEOXYHEXONIC	278.553
3(4-HYDROXYPHENYL)PROPONIC	186.192
GLYCOPYRORONONO-(6-1)LACTONE	1425.560
4-HYDROXY-3-METHOXYPHENYLACETIC	1293.408
C-ACONITIC	8333.062
2,3-DIHYDROXYBENZOIC	291.726
TECEPHALIC	220.847
AZELAIC-DITMS	396.573
RESORCYLIC-TRITMS	149.009
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	1374.342
FURAN-2,5-DICARBOXYLIC	346.589

ETHYLMALONIC	357.370
DEHYDROABIETIC ACID	6316.053
STEARIC-TMS	1873.782
HEXACOSANE	810.551
18.76 min Internal standard	262.500
OXALIC-DITMS	9797.922

Patient 11/20/02/03

BENZOIC-TMS	208.107
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	2634.311
m-HYDROXYBENZOIC-DITMS	1411.192
PHENYLLACTIC-DITMS	4672.684
p-HYDROXYHIPPURIC	6671.385
p-HYDROXYPHENYLACETIC-DITMS	10516.190
p-HYDROXYPHENYLLACTIC-TRITMS	11830.490
P-HYDROXYMANDELIC	1432.126
2-HYDROIXYGLUTARIC	801.025
PHOSPHORIC-TRITMS	2483.244
ISOVANYLHYDRACRYLIC	873.236
VANYLHYDRACRYLIC	914.947
VANILGLYCOLIC	3199.532
LACTIC	9389.785
GLYCOLIC	913.948
3-HYDROXYBUTYRIC	1420.385
SUCCINIC	6948.939
4-HYDROXY-2METHYLVALERIC	263.029
FUMARIC	562.654
4-DEOXYTETRONIC	665.698
3-METHYLGLUTACONIC	343.375
CITRAMALIC	248.936
ADIPIC	354.273
MALIC	867.258
3-METHYLADIPIC	249.250
GLUTARIC	351.255
HIPPURIC	7870.002
CITRIC	11991.620
p-COUMARIC	243.350
PENTADECANOIC	154.380
PALMITIC	2939.875
6-OCTADECENOIC	292.627
OCTADECENOIC	594.643

GLYCERIC-TRITMS	648.958
GLYCERIN-TRITMS	424.916
UREA	7955.894
5-HYDROXYMETHYL-2-FURANECARBOXYL	5555.427
ERYTHONIC	2055.231
3,4-DIHYDROXYBUTYRIC-TRITMS	1698.233
TARTARIC	664.561
3-KETOVALERIC	277.730
3-DEOXYHEXONIC	278.553
3(4-HYDROXYPHENYL)PROPONIC	186.192
GLYCOPYRORONONO-(6-1)LACTONE	1425.560
4-HYDROXY-3-METHOXYPHENYLACETIC	1293.408
C-ACONITIC	8333.062
2,3-DIHYDROXYBENZOIC	291.726
TECEPHALIC	220.847
AZELAIC-DITMS	396.573
RESORCYLIC-TRITMS	149.009
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	1374.342
FURAN-2,5-DICARBOXYLIC	346.589
ETHYLMALONIC	357.370
DEHYDROABIETIC ACID	6316.053
STEARIC-TMS	1873.782
HEXACOSANE	810.551
18.76 min Internal standard	262.500
OXALIC-DITMS	9797.922

Patient 04/04/04/03

1,2-DIHYDROXYBENZENE-DITMS	7.06397
3,4-Dihydroxybenzeneacetic acid	329.258
3,4-DIHYDROXYBENZOIC-TRITMS	60.8036
3,4-DIHYDROXYMANDELIC-TETRATMS	30.361
3-HYDROXYPHENYLACETIC-DITMS	1140.28
3-Methoxy-4-hydroxy benzeneacetic acid	818.264
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	13.9226
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	734.374
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	162.216
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	2515.69
HIPPURIC-TMS	2830.42
ISOVANILHYDRACRYLIC-TRITMS	57.4773
MANDELIC-DITMS	10.088
m-HYDROXYBENZOIC-DITMS	153.691

m-HYDROXYPHENYLPROPIONIC-DITMS	48.019
o-HYDROXYHIPPURIC-DITMS	66.9799
o-HYDROXYPHENYLACETIC-DITMS	62.4553
p-COUMARIC-DITMS	83.255
p-HYDROXYHIPPURIC-DITMS	1737.96
p-HYDROXYMANDELIC-TRITMS	224.413
p-HYDROXYPHENYLACETIC-DITMS	2293.69
p-HYDROXYPHENYLLACTIC-TRITMS	123.069
VANILLYLMANDELIC-TRITMS	2565.27
PHOSPHORIC-TRITMS	127.756
GLYCERIC-TRITMS	147.157
GLYCOLIC-DITMS	60.3656
LACTIC-DITMS2	92.335
PYRUVIC-TMS	15.2326
HYDANTOINPROPIONIC-TRITMS	406.548
5-HYDROXYINDOLACETIC-DITMS	43.7584
5-METHYLINDOLE-2-CARBOXYL-DITMS	11.1438
INDOL-3-ACETIC-DITMS	146.038
3-KETOBUTYRIC-TMS	22.8248
1,6-ANHYDRO-B-d-GLUCOPYRANOSE-TRITMS	60.2836
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	65.4285
4-DEOXYTETRONIC-TRITMS	827.632
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	545.347
6-DEOXYGALACTONIC-TETRATMS	34.0415
ERYTHRONIC-TETRATMS	25.7481
FURAN-2,5-DICARBOXYLIC-DITMS	610.462
GLUCURONIC-PENTATMS	74.0149
MANNITOL-6TMS	119.655
THREITOL-TETRATMS	50.8712
2-HYDROXYGLUTARIC-TRITMS	109.098
ACONITIC-TRITMS	2159.56
CITRIC-TETRATMS	1214.76
FUMARIC-DITMS	115.37
ISOCITRIC-TETRATMS	2537.77
SUCCINIC-DITMS	2213.67
3-METHYLGLUTARIC-DITMS	25.4089
GLUTARIC-DITMS	43.3689
CAFFEIC-TRITMS	54.6506
3,4-DIHYDROXYBUTYRIC-TRITMS	84.5221
5-PYROLIDON-2-CARBOXYLIC-DITMS	969.246
FERULIC-DITMS	129.758
RESORCYLIC-TRITMS	361.719

THREO-2,3-DIHYDROXY-2-METHYLBUTYRIC-TRITMS	15.6285
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	919.328
2-HYDROXYISOBUTYRIC-DITMS	5.42581
URACIL-DITMS	168.191
URIC-TETRATMS	23.5553
XANTHURENIC-TRITMS	29.2894
METHYLCITRIC-TETRATMS	265.858
METHYLMALONIC-DITMS	1829.19
2-HYDROXY-ADIPIC-TRITMS	29.0642
2-HYDROXYSEBACIC-TRITMS	64.7334
3-ETHYLHYDRACRYLIC-DITMS	18.8507
3-METHYLADIPIC-DITMS	73.6212
ADIPIC-DITMS	329.191
AZELAIC-DITMS	470.078
PALMITIC-TMS	15.8748
PIMELIC-DITMS	1170.36
SUBERIC-DITMS	125.356
2-METHYL-3-HYDROXYBUTYRIC-DITMS	5.91872
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	337.889
3-HYDROXYISOBUTYRIC-DITMS	57.9736
3-METHYLGLUTACONIC-DITMS	196.025
18.76 min Internal standard	262.5
CITRAMALIC-TRITMS	2087.37
HOMOCITRIC-TERATMS	14.8799
MESACONIC-DITMS	21.0483
OXALIC-DITMS	2187.39

Patient 11/14/05/03

3,4-Dihydroxybenzeneacetic acid	122.356
3,4-DIHYDROXYMANDELIC-TETRATMS	233.377
3-HYDROXYPHENYLACETIC-DITMS	159.101
3-Methoxy-4-hydroxy benzeneacetic acid	2305.814
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	323.761
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	6379.830
HIPPURIC-TMS	7071.617
m-HYDROXYBENZOIC-DITMS	413.659
m-HYDROXYPHENYLPROPIONIC-DITMS	76.915
o-HYDROXYHIPPURIC-DITMS	26.462
o-HYDROXYPHENYLACETIC-DITMS	41.826
p-HYDROXYHIPPURIC-DITMS	783.092
p-HYDROXYMANDELIC-TRITMS	271.595
p-HYDROXYPHENYLACETIC-DITMS	1748.992

p-HYDROXYPHENYLLACTIC-TRITMS	93.178
VANILLYLMANDELIC-TRITMS	1287.539
PHOSPHORIC-TRITMS	743.986
GLYCERIC-TRITMS	44.923
3-HYDROXYBUTYRIC-DITMS	32.302
1,6-ANHYDRO-B-d-GLUCOPYRANOSE-TRITMS	55.004
1,6-ANHYDRO-B-d-MANNOPYRANOSE-TRITMS	97.755
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	3570.320
ARABINONIC-g-LACTONE-TRITMS	138.587
ERYTHRONIC-TETRATMS	43.249
FURAN-2,5-DICARBOXYLIC-DITMS	1332.128
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	531.901
GLUCURONIC-PENTATMS	209.560
mannonic-1,4-lactone	87.674
2-HYDROXYGLUTARIC-TRITMS	553.496
ACONITIC-TRITMS	2092.764
CITRIC-TETRATMS	5298.622
FUMARIC-DITMS	53.766
ISOCITRICLACTON-DITMS	78.672
ISOCITRIC-TETRATMS	6827.524
SUCCINIC-DITMS	5499.706
GLUTARIC-DITMS	101.986
2-HYDROXY-2-METHYLMALONIC-TRITMS	23.970
4-HYDROXY-2-METHYLVALERIC-DITMS	109.417
5-PYROLIDON-2-CARBOXYLIC-DITMS	496.720
a-RESORCYLIC	280.368
DIOCTYLPHTALATE	243.231
FERULIC-DITMS	98.056
RESORCYLIC-TRITMS	232.107
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	183.984
2-HYDROXYISOBUTYRIC-DITMS	25.583
URACIL-DITMS	65.306
METHYLCITRIC-TETRATMS	139.759
METHYLMALONIC-DITMS	226.897
2-HYDROXY-ADIPIC-TRITMS	44.671
3-HYDROXYSEBACIC-TRITMS	111.006
3-METHYLADIPIC-DITMS	117.992
3-METHYLPIMELIC-DITMS	65.564
ADIPIC-DITMS	76.182
AZELAIC-DITMS	157.150
ETHYLMALONIC-DITMS	108.335
PALMITIC-TMS	45.139

STEARIC-TMS	45.643
SUBERIC-DITMS	72.836
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	418.337
3-METHYLGLUTACONIC-DITMS	71.325
18.76 min Internal standard	262.500
CITRAMALIC-TRITMS	200.965
OXALIC-DITMS	1529.243

Patient 06/20/02/03

2,3-DIHYDROXYBENZOIC-TRITMS	287.078
3,4-DIHYDROXYBENZOIC-TRITMS	55.344
3,4-DIHYDROXYMANDELIC-TETRATMS	292.871
3-HYDROXYPHENYLACETIC-DITMS	399.553
3-Methoxy-4-hydroxy benzeneacetic acid	4956.330
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	1050.432
ACETYLTYROSINE-DITMS	70.353
BENZOIC-TMS	62.454
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	8352.801
HIPPURIC-TMS	11998.169
MANDELIC-DITMS	60.961
m-HYDROXYBENZOIC-DITMS	2510.093
m-HYDROXYPHENYLPROPIONIC-DITMS	102.515
o-HYDROXYHIPPURIC-DITMS	295.600
o-HYDROXYPHENYLACETIC-DITMS	176.262
p-COUMARIC-DITMS	39.792
p-HYDROXYHIPPURIC-DITMS	5156.137
p-HYDROXYMANDELIC-TRITMS	1484.249
p-HYDROXYPHENYLACETIC-DITMS	4893.495
p-HYDROXYPHENYLLACTIC-TRITMS	1005.370
VANILLYLMANDELIC-TRITMS	3412.637
VANILLYLMANDELIC-TRITMS	112.756
PHOSPHORIC-TRITMS	3345.015
GLYCERIC-TRITMS	50.604
GLYCOLIC-DITMS	401.246
LACTIC-DITMS2	1283.293
PYRUVIC-TMS	52.780
INDOL-3-ACETIC-DITMS	155.140
3-HYDROXYBUTYRIC-DITMS	253.630
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	81.864
ERYTHRITOL	86.068
ERYTHRONIC-TETRATMS	227.509

FURAN-2,5-DICARBOXYLIC-DITMS	122.311
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	2050.520
GLUCURONIC-PENTATMS	93.794
THREITOL-TETRATMS	78.736
2-HYDROXYGLUTARIC-TRITMS	618.057
ACONITIC-TRITMS	4167.167
CITRIC-TETRATMS	13781.511
FUMARIC-DITMS	129.725
ISOCITRICLACTON-DITMS	479.427
ISOCITRIC-TETRATMS	13683.351
SUCCINIC-DITMS	3585.726
3-METHYLGLUTARIC-DITMS	79.605
GLUTARIC-DITMS	346.545
PROPANETRICARBOXYLIC-TRITMS	120.446
1-BUTENE-1,4-DICARBOXYLIC-DITMS	96.869
3,4-DIHYDROXYBUTYRIC-TRITMS	1033.196
5-PYROLIDON-2-CARBOXYLIC-DITMS	2192.098
a-RESORCYLIC	44.189
DEHYDROABIETIC ACID	345.923
HEXACOSANE	143.661
HYDROCHINON-DITMS	14.707
ISOFERULIC-DITMS	70.219
QUINOLINIC-DITMS	113.585
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	753.891
2-HYDROXYISOBUTYRIC-DITMS	96.850
THYMINE-DITMS	61.792
URACIL-DITMS	494.120
URIC-TETRATMS	58.847
METHYLCITRIC-TETRATMS	315.001
ACETYLTRHEONINE-DITMS	76.827
2-HYDROXY-ADIPIC-TRITMS	84.965
2-METHYLSUCCINIC-DITMS	511.426
3-HYDROXYSEBACIC-TRITMS	345.458
3-METHYLADIPIC-DITMS	404.203
3-METHYLPIMELIC-DITMS	161.497
4-METHYLSUBERIC-DITMS	264.279
ADIPIC-DITMS	299.096
ETHYLMALONIC-DITMS	530.521
OLEIC-TMS	86.802
PALMITIC-TMS	681.245
PENTADECANOIC-TMS	33.920
STEARIC-TMS	435.722

3-HYDROXY-3-METHYLGLUTARIC-TRITMS	843.924
3-METHYLGLUTACONIC-DITMS	421.212
18.76 min Internal standard	262.500
ACETYLASPARTIC-DITMS	176.610
CITRAMALIC-TRITMS	326.703
HOMOCITRIC-TERATMS	41.966
OXALIC-DITMS	3059.616

Patient 04/29/02/03

2,3-DIHYDROXYBENZOIC-TRITMS	1009.261
3,4-Dihydroxybenzeneacetic acid	776.212
3,4-DIHYDROXYBENZOIC-TRITMS	1265.954
3-HYDROXYPHENYLACETIC-DITMS	1412.582
3-Methoxy-4-hydroxy benzeneacetic acid	3607.297
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	80.383
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	3134.589
4-HYDROXY-3-METHOXYPHENYLLACTIC-TRITMS	185.007
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC-DITMS	128.693
BENZOIC-TMS	190.448
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	9909.199
HIPPURIC-TMS	11083.407
MANDELIC-DITMS	111.475
m-HYDROXYBENZOIC-DITMS	1181.564
o-HYDROXYHIPPURIC-DITMS	8955.197
P-AMINOBEMZOIC-TMS	39.815
p-COUMARIC-DITMS	117.025
p-HYDROXYHIPPURIC-DITMS	5374.719
p-HYDROXYPHENYLACETIC-DITMS	6617.524
p-HYDROXYPHENYLLACTIC-TRITMS	1191.555
VANILGLYCOLIC-TRITMS	10697.769
VANILGLYCOL-TRITMS	378.218
VANILLIC-DITMS	908.260
VANILLYLMANDELIC-TRITMS	10570.851
PHOSPHORIC-TRITMS	5602.372
GLYCERIC-TRITMS	503.360
GLYCERIN-TRITMS	119.324
GLYCOLIC-DITMS	230.854
LACTIC-DITMS2	1291.774
PYRUVIC-TMS	90.687
INDOL-3-LACTIC-DITMS	257.380
3-HYDROXYBUTYRIC-DITMS	88.323

2-FUROYLGLYCINE-TMS	1632.467
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	11712.585
ERYTHRONIC-TETRATMS	1665.044
FURAN-2,5-DICARBOXYLIC-DITMS	10677.344
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	6946.636
GLUCURONIC-PENTATMS	415.013
LYXOSE-TETRATMS(i)	330.679
THREITOL-TETRATMS	1299.223
2-HYDROXYGLUTARIC-TRITMS	2361.252
ACONITIC-TRITMS	9451.010
CITRIC-TETRATMS	3444.135
FUMARIC-DITMS	1138.328
ISOCITRICLACTON-DITMS	286.816
ISOCITRIC-TETRATMS	11581.308
2-METHYLGLUTARIC-DITMS	221.411
3-HYDROXYGLUTARIC-TRITMS	1893.771
3-METHYLGLUTARIC-DITMS	139.066
GLUTARIC-DITMS	951.624
PIPECOLIC-DITMS	1493.072
acetylsalicylic acid (TMS)	1070.598
CAFFEIC-TRITMS	267.210
TARTARIC-TETRATMS	2211.178
3,4-DIHYDROXYBUTYRIC-TRITMS	1198.148
5-PYROLIDON-2-CARBOXYLIC-DITMS	1492.040
B-RESORCYLIC-TRITMS	1010.355
FERULIC-DITMS	1429.238
HYDROCHINON-DITMS	37.174
QUINOLINIC-DITMS	544.592
RESORCYLIC-TRITMS	1029.283
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	1137.952
OROTIC-TRITMS	324.618
THYMINE-DITMS	72.912
URACIL-DITMS	474.114
3-HYDROXYPROPIONIC-DITMS	68.722
METHYLCITRIC-TETRATMS	1175.168
METHYLMALONIC-DITMS	164.458
2-METHYLSUCCINIC-DITMS	1136.440
3-METHYLADIPIC-DITMS	140.357
3-METHYLPIMELIC-DITMS	570.239
4-METHYLSUBERIC-DITMS	705.007
6-OCTADECENOIC-TMS	50.379
6-Trimethylsilyloxyhexanoic acid, trimethylsil	385.480

ETHYLMALONIC-DITMS	443.302
PALMITIC-TMS	602.659
PIMELIC-DITMS	306.996
STEARIC-TMS	982.102
3-HYDROXY-3-METHYLGLUTARIC-TRITMS	3808.555
3-HYDROXYISOBUTYRIC-DITMS	93.465
3-METHYLGLUTACONIC-DITMS	619.429
18.76 min Internal standard	262.500
ACETYLASPARTIC-DITMS	230.886
CITRACONIC-DITMS	136.309
CITRAMALIC-TRITMS	992.166
ITACONIC-DITMS	130.092
OXALIC-DITMS	4079.714

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3,4-DIHYDROXYBENZOIC-TRITMS	328.221
3,4-DIHYDROXYBUTYRIC	2312.957
3,4-DIHYDROXYMANDELIC-TETRATMS	969.717
3-HYDROXY-3-METHYLGLUTARIC	11629.740
3-METHOXY-4-HYDROXYPHENYLPROPIONIC-DITMS	779.179
4-HYDROXY-3-METHOXYPHENYLACETIC-DITMS	8740.719
ACETYLTHREONINE	359.057
BENZEACETIC ACID, 3,4-BIS(TRIMETHYLSILYL)	1617.006
B-m-HYDROXYPHENYLHYDRACRYLIC-TRITMS	15662.730
HIPPURIC-TMS	39174.520
m-HYDROXYBENZOIC-DITMS	5760.390
m-HYDROXYPHENYLPROPIONIC-DITMS	549.371
o-HYDROXYHIPPURIC-DITMS	1452.567
o-HYDROXYPHENYLACETIC-DITMS	601.186
p-COUMARIC-DITMS	316.203
p-HYDROXYHIPPURIC-DITMS	5567.916
p-HYDROXYMANDELIC-TRITMS	2862.089
p-HYDROXYPHENYLACETIC-DITMS	24697.740
p-HYDROXYPHENYLLACTIC-TRITMS	1807.448
VANILLYLMANDELIC-TRITMS	26578.270
ASCORBIC	7901.132
PHOSPHORIC-TRITMS	43799.940
GLYCERIC-TRITMS	1401.072
GLYCERIN-TRITMS	450.510
GLYCOLIC-DITMS	701.280
LACTIC-DITMS2	2143.910

1,6-ANHYDRO-B-d-GLUCOPYRANOSE	4983.916
5-INDOLE-CARBOXYLIC-DITMS	337.822
INDOL-3-ACETIC-DITMS	1554.741
4-HYDROXYCYCLOHEXANE-1-CARBOXYLIC	2061.099
5-HYDROXYMETHYL-2-FURANECARBOXYLIC-DITMS	886.698
ERYTHRONIC-TETRATMS	2471.987
FURAN-2,5-DICARBOXYLIC-DITMS	503.205
GLUCOPYRORONO-(6-1)LACTONE-TRITMS	14591.910
ACETYLASPARTIC	811.855
RIBONIC-g-LACTONE	579.360
2-HYDROXYGLUTARIC-TRITMS	1457.512
2-HYDROXYSEBACIC	1014.971
3-HYDROXYSEBACIC	6182.224
FUMARIC-DITMS	407.032
CITRIC	512.365
ISOCITRIC-TETRATMS	256.258
METHYLPIMELIC	1585.641
PIMELIC	858.534
QUINOLINIC	1260.717
SUCCINIC-DITMS	10403.410
SUBERIC	1642.241
3-METHYLGLUTARIC-DITMS	116.304
GLUTARIC-DITMS	614.324
TARTARIC-TETRATMS	3412.228
5-PYROLIDON-2-CARBOXYLIC-DITMS	8073.683
FERULIC-DITMS	1586.618
THREO-2,3-DIHYDROXYBUTYRIC-TRITMS	1571.223
THYMINE	244.402
URACIL-DITMS	1473.034
METHYLCITRIC-TETRATMS	2717.605
METHYLMALONIC-DITMS	7036.853
2-HYDROXY-ADIPIC-TRITMS	704.113
3-METHYLADIPIC-DITMS	320.908
ADIPIC-DITMS	1488.060
AZELAIC-DITMS	5693.773
ETHYLMALONIC-DITMS	923.551
PALMITIC-TMS	1015.131
STEARIC-TMS	854.645
URIC	22623.950
UREA	28907.920
3-METHYLGLUTACONIC-DITMS	974.694
ERYTHRITOL	284.839

18.76 min Internal standard	262.500
c-ACONITIC	23234.370
ISOCITRICLACTON	4523.089
CITRAMALIC-TRITMS	3008.765
OXALIC-DITMS	26434.810

APPENDIX V

Patient 1 Amino acid profile

AMINO ACID	CONCENTRATION IN URINE (MMOL/MOL CREATININE)	REFERENCE VALUE (MMOL/MOL CREATININE)
Glycine	323.5	110-356
Alanine	60.3	41-162
Alfa-Aminobuturic acid	10.5	0-214
Serine	103.1	45-124
Threonine	31.6	15-63
Proline	13.5	0-61
Ornithine	12.1	0-8
Arginine	8.5	0-8
Valine	53.8	7-21
Leucine	40.7	3-36
Lycine- glutamine	269.8	78-234
Lycine	31.7	16-69
Homocystine	24.9	0.2-4
Methionine	12.2	7-29
Histidine	212.4	87-287
1&3-Methylhistidine	53.9	22-57
Citrulline (nr 215)	0.0	0-7
Citrulline (nr 232)	8.3	0-7
Alfa-Amino adipic acid and 3-OH-buterylglycine	168.8	<30.2
Aspartaric acid	7.9	3-10
Glutamic acid	14.0	0-11
Tryptophan	1.1	<16.5
Carnosine	36.1	<0

Patient 2 Amino acid profile

AMINO ACID	CONCENTRATION IN URINE (MMOL/MOL CREATININE)	REFERENCE VALUE (MMOL/MOL CREATININE)
Glycine	693.4	283-1097
Alanine	59.9	75-206
Alfa-Aminobuturic acid	17.7	2-107
Serine	159.1	80-282
Threonine	71.4	20-138
Proline	53.7	21-213
Ornithine	21.7	0-19
Arginine	23.9	0-14
Valine	11.1	3-26
Leucine	43.7	23-351
Lycine- glutamine	230.5	74-376
Lycine	132.4	22-171
Homocystine	25.9	0.2-4
Methionine	7.4	7-27
Histidine	67.0	80-295
1&3-Methylhistidine	30.5	20-39
Citrulline (nr 215)	0.00	0-11
Citrulline (nr 232)	8.2	0-11
Alfa-Amino adipic acid and 3-OH-buterylglycine	212.4	<30.2
Aspartaric acid	4.1	<30.2
Glutamic acid	5.0	0-30
Tryptophan	0.7	<16.5
Carnosine	24.8	<0