CHAPTER 2
SOLID STATE CHARACTERISATION

2.1 Introduction

A better understanding of the different aspects of polymorphism and amorphism can be acquired when investigating the physico-chemical properties of active pharmaceutical ingredients (API's). Different analytical methods that characterise the solid state forms of API's must be employed, in order to ultimately provide a complete profile of the physical and chemical solid state properties of pharmaceutical compounds. Analytical methods that fulfil the qualitative and quantitative aspects for optimum characterisation of solids include the following.

X-ray powder diffraction (XRPD) is the principle technique used for solid state characterisation, as it indicates the existence of different forms of a single compound. Other methods of importance include spectroscopy, thermal analysis, microscopy, microcalorimetry, moisture sorption analysis and Karl Fischer titration. High performance liquid chromatography (HPLC) is the principal analytical method used for the identification and quantification of compounds, whilst pharmaceutical properties, such as solubility, stability and dissolution rate, are also important considerations. It is always important during the discovery and development of new polymorphic or amorphous forms whether the new/developed forms display improved physical and chemical properties. Therefore, the constant need for new and improved methods to characterise solid state compounds cannot be emphasised enough (Bernstein, 2002:94; Chieng *et al*., 2011:619; Threlfall, 1995:2438; West, 1999:167; Yu *et al*., 1998:119).

This chapter describes the methods used for characterising the polymorphic and amorphous forms of azithromycin, prepared during this study. The development and validation of a robust HPLC method is described, whilst the methods for establishing the solubility, stability and dissolution rate are also presented in this chapter.

2.2 Materials

Azithromycin dihydrate (AZM-DH), having a purity of 96.5 % (on a dried basis), was purchased from DB Fine Chemicals, South Africa (Batch number: IF-AZ080506). AZM-DH

was used to prepare the various forms of azithromycin (AZM), as described in this study, i.e. the anhydrous forms (Chapter 4) and recrystallisations of AZM (Chapter 4), as well as the amorphous glass (AZM-G) (Chapter 5). The organic solvents being utilised for recrystallisation included acetone, acetonitrile, 1-butanol, dichloromethane, diethyl ether, 1,4-dioxane, ethyl acetate and methanol, all purchased from ACE, Merck and Sigma-Aldrich (South Africa). Potassium dihydrogen orthophosphate, sodium hydroxide, glacial acetic acid, hydrochloric acid (HCl) and chromatographic grade acetonitrile were acquired from Merck, South Africa and sodium acetate trihydrate from Fluka. Milli-Q water (prepared in-house by a Milli-Q Gradient water system, Millipore®), with a resistivity of 18.2 MΩ•cm at 25°C, was used. For specificity tests during HPLC method validation, commercially available tablets (available in South Africa), containing AZM-DH, were also used.

2.3 Methods for solid state characterisation

2.3.1 Infrared spectroscopy (IR)

Infrared spectroscopy (IR) is known to be the principal method for studying molecular properties of a compound in its solid state. Although IR has its limitations and experimental difficulties, it is still regarded as one of the easiest and most effective analytical methods for the characterisation of different polymorphic forms (Bernstein, 2002:129). The analysis of different polymorphs with IR is complementary to other techniques being used to characterise polymorphic properties (Bugay & Williams, 1995:61). IR is also becoming increasingly efficient in the quantitative analysis of polymorphs and is therefore utilised to establish polymorphic purity. The basic principle of IR relates to measuring the vibrational modes of the bound atoms of the solid particles, and therefore the molecules' vibrational motions are fully analysed and measured. The energy coupled with IR can be categorised into three regions, i.e. the near-IR (20,000 – 4,000 cm^{-1}), mid-IR (4,000 - 400 cm^{-1}) and far-IR (400 - 50 cm^{-1}) regions. The absorption is generally measured within the range of 4,000 - 400 cm^{-1}. Absorption bands in the mid-IR region between 3,500-3,000 cm^{-1} (also referred to as the OH stretching region), are representative of OH-groups, which may result from the presence of water molecules, or hydrogen bonds. The presence of these bands is instrumental in the identification of hydrates. Since azithromycin mostly occurs in the dihydrate form, the bands showing in the region of 3,500 - 3,000 cm^{-1} are important in identifying changes in the hydration state

of the solid form. IR spectroscopy should thus be sensitive for studying interconversions between the anhydrate, monohydrate and dihydrate forms of azithromycin (Bernstein, 2002:125; Brittain, 1999:256; Bugay & Williams, 1995:60; West, 1999:180).

2.3.1.1 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) has become the primary method for the characterisation and determination of molecular properties. FTIR has the advantage of monitoring the bonds and bound atoms, as well as disturbances in vibrations. Vibrational disturbances are caused by conformational factors (or environmental) between different polymorphic forms and will hence result in spectral differences. Polymorphic forms of a specific compound may only show slight differences at distinct bands, which provide the basis for the characterisation of different polymorphic forms. With FTIR it is possible to determine the exact wavenumber (cm^{-1}) of the absorption bands, which are presented to the analyst in the form of a spectral image. A spectrum represents absorption as a function of wavenumber. The spectra of the different polymorphs are studied and peaks are compared to determine the peak differences at their respected wavenumbers. FTIR additionally provides the opportunity for single crystal and bulk material analysis (Bernstein, 2002:125; Brittain, 1999:258; Bugay & Williams, 1995:60; West, 1999:179).

2.3.1.2 Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS)

Since the IR method is generally used to compare spectral differences of polymorphs, it must be emphasised that sample preparation is of utmost importance. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) is a technique in which a polymorph can be investigated as is, or dispersed in a non-absorbing matrix of powdered alkali halide (usually KCl or KBr) prior to analysis. The sample is ultimately irradiated by the infrared beam for absorption, reflection and diffraction by the sample molecules to occur. With DRIFTS, the risk of polymorph transforming or desolvating is minimised, compared to other techniques, through direct analysis of the sample. This means that the sample is also fully recoverable and can therefore be used for analysis with other analytical methods. The spectral regions that can be associated with DRIFTS are the near-IR and mid-IR regions. According to Threlfall (1995:2440), DRIFTS is recommended as the main technique for initial IR analysis of different polymorphic forms. This technique is ideal for studies on different polymorphs, due to the fact that it is non-invasive, quantitative, whilst

limited sample handling means that the polymorphic nature of the molecules stays intact (Bernstein, 2002:129; Bugay & Williams, 1995:68).

The one limitation of the DRIFTS technique is particle size dependency. The particle size of both the polymorph (sample) and the non-absorbing matrix must comply with a specific range, before analysis can commence. This particle size range may be achieved by using a mortar and a pestle to mix the sample and the non-absorbing matrix (KCl or KBr) (Bugay & Williams, 1995:69).

A Shimadzu IRPrestige-21 (Shimadzu, Japan), using a Pike Multi-Reflectance ATR accessory, with Shimadzu IRSolution Version 1.40 software, was utilised during this study. The spectral region of 4000 - 400 cm^{-1} (mid-IR) was applied. Potassium bromide (KBr) was used as background for sample measurements. Each sample was dispersed in a matrix of powdered KBr, whilst DRIFTS was used to measure the IR spectrum in a reflectance cell. The vertical axis represents the transmittance (%), whereas the horizontal axis reflects the wavenumber (cm^{-1}).

2.3.2 X-ray powder diffraction (XRPD)

X-ray powder diffraction (XRPD) is believed to be the single, most effective method for revealing the existence of different polymorphic forms of a drug compound. In essence, XRPD is based on the conditions described by Bragg (also known as Bragg's law). According to Brittain (1995:14), Bragg's law "describes the diffraction of a monochromatic X-ray beam impinging on a plane of atoms". The crystal planes diffract the incident rays at various angels upon impact. These angles relate to the spaces between individual parallel planes within the crystal lattice. A sample is powdered in order to expose all the planes that exist in the sample. The angle at which scattering occurs is determined through slow rotation of the sample, during which the angles of the diffracted X-rays (relevant to the incident beam's angle) are measured. The sample may also be kept in a fixed position, meaning that the detector (Geiger counter or scintillation counter) has to move in order to detect and measure the angles of the diffracted X-rays. The detector is connected to a diffractometer. Therefore, a powder diffractometer has a moving detector which, at a constant angular velocity, detects the 2θ values. For the range of measurement to be sufficiently indicative of the powder pattern, it generally is between $10^{\circ}2\theta$ and $80^{\circ}2\theta$. The measured values are presented as an XRPD pattern, where intensity is given as a function of degrees-2θ. The intensities can be representative of the height or the areas of the

peaks. Most XRPD studies are performed at room temperature, although the powder analysis can be performed at increasing/decreasing temperatures, to determine the effect of temperature (heated or cooled) on polymorphic forms (Brittain, 1995:14; Suryanarayanan, 1995:188; West, 1999:138).

Every polymorphic (crystalline) form has its own unique and characteristic powder pattern (Chieng *et al*., 2011:619) and can be interpreted as a unique fingerprint of the polymorph. Differences in powder patterns of polymorphs can be examined according to their peak intensities, or the position of peaks, based on their degrees-2θ values (Brittain, 1995:14; Chieng *et al*., 2011:619; Suryanarayanan, 1995:188; West, 1999:138).

Amorphous powders cause the scattering of X-rays to be incoherent, which ultimately cause the powder pattern to present as a diffuse halo (i.e. background scatter in the form of a broad diffuse maxima). This halo pattern is therefore conclusive of a powder being amorphous. Because of this halo pattern, XRPD is limited to qualitative analysis regarding amorphous materials (Byrn *et al.*, 1999:63; Chieng *et al*., 2011:620; Suryanarayanan, 1995:189; Yu *et al*., 1998:124).

XRPD can be used as a quantitative and qualitative method for the characterisation of different polymorphic forms. Peak heights are utilised for quantitative application of XRPD, but a reference pattern of the pure standard of the compound is required. According to the reference powder pattern, the similar peak heights can be compared to establish the quantity of the original compound. Qualitative analyses with XRPD are used for the identification of different polymorphic forms of a single compound and for determining the degree of crystallinity of said polymorphs (Bernstein, 2002:122; Suryanarayanan, 1995:188).

XRPD patterns for this study were generated using a PANalytical Xpert-Pro (PANalytical, Netherlands). The measurement conditions were fixed as summarised in Table 2.1.

Table 2.1 XRPD settings for the measurement of azithromycin samples

Minimum step size °2Theta:	0.001
Divergence Slit Type:	Fixed
Divergence Slit Size [°]:	0.9570
Specimen Length [mm]:	10
Measurement Temperature [°C]:	25
Anode Material:	Cu
K-Alpha1 [Å]:	1.54060
K-Alpha2 [Å]:	1.54443
K-Beta [Å]:	1.39225
K-A2 / K-A1 Ratio:	0.50000
Generator Settings:	45 mA, 40 kV
Diffractometer Type:	0000000011018023
Diffractometer Number:	0
Goniometer Radius [mm]:	240
Dist. Focus-Diverg. Slit [mm]:	91
Incident Beam Monochromator:	No
Spinning:	Yes

2.3.3 Thermal methods

Thermal methods are used, in contrast to microscopy (Section 2.3.4), for generating quantitative information on the physico-chemical properties of solid state materials. The quantitative information being gathered through thermal methods includes the relative stability of the different polymorphs, phase changes and the energy associated with such changes. The measured physico-chemical properties are given as a function of temperature (McCauley & Brittain, 1995:224). During phase transformation, the physical state may change along with the absorption, or release of heat. The enthalpy and heat flow of the transformation reactions can be established, which in essence describe the basic principle of thermal methods. The two general methods being utilised for thermal analysis are differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) (Bernstein, 2002:104; Brittain, 1999:245; McCauley & Brittain, 1995:224; West, 1999:203).

2.3.3.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is regarded as the most effective and widely used thermal method for the analysis of pharmaceutical compounds (Brittain, 1999:252). The use of a DSC instrument allows for the individual heating of a sample and a reference, therefore enforcing the principle of null balance. This implies that the heat flow associated with any phase transformation will be compensated for in the reference, by preserving the temperature of the sample at the exact temperature of the reference, thus maintaining temperature equality with heat flow changes. The heat flow is recorded as a function of temperature (or time). The peak that represents the phase transformation can be used to quantify the enthalpy (yielded by the area of the peak) of the transformation (Bernstein, 2002:104; Brittain, 1999:252; McCauley & Brittain, 1995:235; West, 1999:206).

Features that are typically revealed through DSC analysis include the glass transition temperature (T_g) of glasses (amorphous solids), the crystallisation of the amorphous form into a crystalline form, exothermic solid-solid transformation, the melting point of a crystalline form, recrystallisation of the melt and ultimately the melting point of the recrystallised crystals. These transformations are represented by either endothermic (melting, boiling, sublimation, vaporisation, desolvation, solid-solid transformation and chemical degradation), or exothermic (crystallisation, oxidative decomposition) events on the DSC trace. The heating rate is a very significant experimental parameter, since different traces will be achieved with different heating rates. This means that some of the phase transformations may not be exhibited on the DSC trace, due to the specific heating rate used. It is therefore important to determine the most suited and practical heating rate (usually achieved through trial and error) prior to experimental DSC analysis. Other factors that may result in variations in results and the integrity of the measured results include sample mass, particle size and shape, impurities present in the sample, and the presence of seeds or nuclei of the polymorphic forms. The type of sample holder (aluminium pans are most commonly used) used for DSC analysis may further turn out to be a determining factor in the quality of the analysis being performed (Bernstein, 2002:105; Brittain, 1999:245; McCauley & Brittain, 1995:235; West, 1999:208).

DSC traces for this study were generated on a Shimadzu DSC-60A (Shimadzu, Japan) with TA60 Version 2.11 software. The DSC was calibrated (using indium samples) on a weekly basis. Approximately 2.0 - 4.0 mg of each sample was weighed and heated in aluminium crucibles. Each sample crucible had a pin hole in the lid to allow for the

evaporation of solvents during desolvation. Samples were heated from 25°C to 150°C at 2°C/min in an inert nitrogen atmosphere.

2.3.3.2 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) is a thermal method, allowing for the measurement of sample mass change with an increase in temperature (or time) in the furnace at a controlled heating rate. The atmosphere is controlled through the constant flow of gas (nitrogen). The resulting mass change being recorded by the microbalance is given as a function of temperature. Factors that may influence the quality of the TGA can be instrumental (furnace heating rate, atmosphere), or related to the sample (sample size, particle size) (Bernstein, 2002:109; Brittain, 1999:247; McCauley & Brittain, 1995:243; West, 1999:203).

TGA (in combination with DSC) is especially valuable for the characterisation of polymorphic solvates and hydrates which contain volatile solvents, or water. These volatile solvents (or water) are removed from the chemical structure of the solvate (or hydrate), as the temperature increases during the analysis. The sample mass loss being recorded by the TGA is used to determine the theoretical amount of solvent molecules lost during desolvation, or dehydration. This information can then be used to establish the solid molecule:solvent molecule ratio of the solvate or hydrate. For anhydrous forms, the TGA will result in no significant weight loss, whereas hydrates will result in a weight loss according to the amount of water molecules included in the chemical structure. TGA of hydrates are very useful to determine the exact water content, when performed in conjunction with Karl Fischer titrations (KFT) (Bernstein, 2002:109; Brittain, 1999:246; McCauley & Brittain, 1995:246; West, 1999:203).

TGA traces for this study were obtained using a Shimadzu DTG-60 (Shimadzu, Japan) with TA60 Version 2.11 software. Samples were heated from 25°C to 150°C at a rate of 2°C/min in open aluminium crucibles. Nitrogen gas was used as purge gas.

2.3.4 Microscopy

Microscopy is a visual technique that allows for a morphological investigation of the solid state. Due to the increased demand to accurately quantify the observed solid state materials/compounds, the use of microscopy has decreased to a large extent. The microscope, however, has a unique role to play in solid state studies, as it is essential in

establishing the visual homogeneity, or variations among polymorphic samples. An approximation of the crystallinity of the solid can be made with microscopy, whereas differences in crystal size, shape and colour may also be established. The presence of crystalline traces in amorphous (glass) samples can also be studied and observed with microscopy. The use of microscopy can therefore signify that polymorphic forms exist and that further solid state characterisation is necessary for the complete elucidation of the said polymorphic forms. The two microscopic techniques that are most widely utilised for morphological characterisation are optical microscopy (which includes hot stage microscopy) and electron microscopy (Bernstein, 2002:95; Brittain, 1999:238; Newman & Brittain, 1995:128; West, 1999:167).

2.3.4.1 Hot stage microscopy (HSM)

Hot stage microscopy (HSM), also referred to as thermomicroscopy is a technique used to observe any thermally induced changes in the solid state of a material. The thermal conditions are therefore monitored and controlled at all times. The hot stage can account for temperatures ranging from 25˚C up to 350˚C, or even higher, if necessary. The changes in optical properties during polymorphic phase transformations make it possible to allocate the exact temperature to the relevant transformations. The process of transformation is kinetically controlled, which emphasises the importance of a slow heating rate during the hot stage technique. The addition of a hot stage to polarised light microscopy enables the determination of the melting point of the sample, whilst observing the actual melting thereof (Bernstein, 2002:95). The melting point is a very significant characteristic of a polymorph, since polymorphic forms may exhibit different melting points, although the melting points of various polymorphs may also be similar. The melted products of polymorphic forms all conclude into the same liquid, which in essence would confirm the existence of polymorphic forms. Insight into the nature of phase transformations is provided by HSM. Processes, such as nucleation (crystal growth), dehydration and desolvation can be observed with thermomicroscopy. The desolvation of a solvate can be more accurately observed, when the sample is covered with silicone gel prior to HSM analysis. HSM has also been used to observe crystallisation at different temperatures and samples are prepared on microscopic slides (Bernstein, 2002:98; Brittain, 1999:244; Newman & Brittain, 1995:137).

Hot stage microscopy was used to compare the morphology of different solid forms of azithromycin. HSM analyses for this study were performed using a Nikon Eclipse E4000

microscope, fitted with a Nikon DS-Fi1 camera (Nikon, Japan). A Metratherm 1200d temperature regulator (Metratherm, Germany), coupled with a Leitz regulator (Leitz, Germany), were connected to the microscope to enable heating of the hot stage. Samples on the microscopic slide were coated with silicone oil prior to HSM analysis. Examination of samples was mostly done at 10x magnification. Images were taken at increasing temperatures, starting at 25˚C up to 150˚C. Each prepared sample was individually observed and screened under the above conditions, whilst taking images and recording the corresponding temperatures.

2.3.4.2 Scanning electron microscopy (SEM)

The use of scanning electron microscopy (SEM) to observe the morphological properties of pharmaceutical compounds is an efficient method for supplying information for the characterisation of compounds. The functionality of SEM as a characterisation tool is not quantitative and is therefore limited to produce visual characteristics. This means that SEM must be utilised in combination with other characterisation methods to ultimately attain the best possible solid state profile for pharmaceutical compounds (Brittain, 1999:239; Newman & Brittain, 1995:140; West, 1999:172).

A scanning electron microscope generates illumination (an electron beam) through an electron gun. The beam is focused on the sample via a column of lenses. The sample occupies the sample chamber that is connected to a detector. The sample chamber and electron gun require high vacuum conditions for analysis. Sample preparation requires that the sample is mounted with a double-sided tab on a sample mount. Samples that are non-conductive must be coated with a conductive metal coating prior to analysis, of which gold, or gold-palladium are the most commonly used. The main function of the coating is to prevent the accumulation of charges on the surface of the sample and thus allows for more clear and stable imaging of the sample (Newman & Brittain, 1995:141; West, 1999:172).

SEM is often used to investigate the morphological properties of active pharmaceutical ingredients, excipients, bulk drugs and the compression of pharmaceutical solids. The surface characteristics of pharmaceutical materials can also be investigated with SEM (West, 1999:175). In light of the characterisation of pharmaceutical materials, it is evident that SEM is a valuable technique when used in conjunction with other characterisation methods (Newman & Brittain, 1995:152; West, 1999:172).

An FEI Quanta 200 ESEM & Oxford INCA 400 EDS system (FEI, USA) was used to obtain photomicrographs of the various crystal and amorphous forms prepared during this study. In preparation, samples were adhered to a small piece of carbon tape, mounted onto a metal stub and coated with a gold-palladium film (Eiko Engineering ion Coater IB-2, USA).

2.3.5 Microcalorimetry

Russel *et al*. (2009:194) state that all reactions (biological, chemical, or physical) either produce or consume heat. Through microcalorimetry (which is ultimately more sensitive than DSC), the extent of these reactions can be accurately measured and can then be interpreted and described according to the heat, the heat flow and the heat capacity (Buckton, 1995:117). This technique can be utilised in the pharmaceutical industry to establish the chemical and physical stability of an API and to characterise its physical form (Phipps & Mackin, 2000:9). The identification of amorphous content in a crystalline product is an important application of microcalorimetry and has been used as part of the quality control processes of solid dosage forms (Briggner *et al*., 1994:125; Gaisford, 2012:432; Phipps & Mackin, 2000:13). Physical transformation between crystal forms, as well as recrystallisation from the amorphous into the crystalline state can be detected and studied by using microcalorimetry (Buckton, 1995:122). The degradation rate and the predicted shelf-life are also determined from the data obtained (Wilson *et al*., 1996:45).

An TAM III (TA Instruments, United States) instrument studied azithromycin modifications in terms of heat flows during aqueous dissolution as well as under controlled storage.

For the heat of solution determinations the water bath temperature was set to 35˚C. Approximately 43.75 mg and 42.88 mg of AZM-DH and AZM-G were weighed, respectively, into glass crushing ampoules. The crushing ampoules were sealed with inert wax and attached to a gold stirrer. In both instances, 20 mL of distilled water was pipetted into the reaction vessel that was then fixed to the calorimeter unit. This experimental unit was then lowered into the calorimeter and the stirrer set at 300 rpm. The solution was allowed to equilibrate at 35˚C, prior to each determination. The heat of solution test involved two electric calibration steps, followed by the break of the ampoule. After the breaking, two electric calibration steps concluded the heat of solution determination. The heat of solution data was calculated by taking the four calibration steps into account.

For the relative humidity test, approximately 100 mg of each AZM-DH and AZM-G was accurately weighed into two separate glass vials. Micro hygrostats were added to the vials

containing distilled water and the vials sealed with aluminium crimp caps. A hook and pin were attached to both the vials, as well as to an empty, sealed reference vial. All the sealed vials were attached to the hooks of the multicalorimeter and lowered into the calorimeter unit. The water bath temperature was set at 55˚C. After equilibration of the calorimeters, the samples and reference vial were lowered into the calorimeter unit. The heat flow was measured over a period of three days.

2.3.6 Moisture sorption analysis

Many pharmaceutical compounds are to a large extent affected by the presence of moisture. Not only is the amount of moisture of value to pharmaceutical researchers, but also the chemical behaviour of the compound in the presence of moisture. These behavioural changes as a result of moisture can be determined by their adsorption and desorption isotherms, which can be obtained with a vapour sorption analyser. This analytical instrument regulates conditions (temperature and humidity) in two individual chambers, of which the one contains the sample and the other a reference. The resulting weight change of the sample in the sample chamber represents the moisture uptake by the sample (Mansour & Zografi, 2007:382).

Different temperature and % RH (relative humidity percentage) profiles can be generated with a vapour sorption analyser. The temperature for experimental conditions can be varied between 5˚C to 60˚C at ambient pressure. The % RH can effectively be controlled within the range of 2 % to 98 % RH. Most moisture sorption studies consist of increasing the % RH over the desired RH range at a fixed temperature. The changes in sample weight are continuously measured by the microbalance, monitored and recorded. The percentage weight change is given as a function of % RH, thus producing an isotherm that displays the relationship between the amount of adsorbed moisture (at a fixed temperature) and the equilibrium pressure (concentration) (Rouquerol *et al*., 1999:6). The adsorption isotherm can now be classified according to the International Union of Pure and Applied Chemistry (IUPAC, 1985) classification guideline for isotherms. The type of isotherm describes the likelihood of the process of adsorption to occur for a sample at conditions of increased humidity at constant temperature (Hassel, 2006:1; Rouquerol *et al*., 1999:19; Swaminathan & Kildsig, 2001:1).

The vapour sorption analyser used during this study was a VTI-SA from TA Instruments (TA, USA). An RH range of 0 % to 95 % was selected and analyses performed at a

constant temperature of 25˚C (VTI-SA). The individual samples were dried at 50˚C (heating rate at 5˚C/minute) for 60 minutes as a standard pre-treatment, before the actual analysis was initiated. The vertical axis represented the weight (%) of the sample, whereas the horizontal axis expressed the % RH.

2.3.7 Karl Fischer titration (KFT)

Karl Fischer titration (KFT) is an analytical technique commonly used to determine the exact water content (usually given in % (w/w), or g/kg) for a range of different compounds. KFT is especially useful to determine the water content of hydrates in solid state studies. The KFT method is very effective when used in conjunction with TGA. The percentage mass change recorded by the TGA can be correlated with the water content achieved with KFT. Therefore, the hydrated state of a solid can be accurately determined. The basic principle of KFT lies within the reaction of water with iodine in an alcohol environment. The reaction of water and iodine occurs at a ratio of 1:1. The titration end point is signified by the presence of excess iodine in the alcohol solution. The alcohol that is generally used is methanol. KFT can be categorised into volumetric and coulometric titrations (De Caro *et al*., 2001:432; Grünke, 2003:100; Ronkart *et al*., 2006:1006).

2.3.7.1 Volumetric KFT

Volumetric KFT relates to the addition of iodine (in the form of a reagent) to the alcohol solution, by means of a mechanical device. The Karl Fischer reagent contains the iodine, which will ultimately react with the water content of the sample being analysed. The quantification of the water content is based on the volume of Karl Fischer reagent that is added to the alcohol solution, as well as the amount of iodine present in the solution. Volumetric KFT is the titration method of choice for higher water contents (up to 100 %), but can be used to determine water content as low as 100 ppm (De Caro *et al*., 2001:432; Grünke, 2003:100; Ronkart *et al*., 2006:1006).

Volumetric KFT analysis was performed on a Metrohm 870 KF Titrino Plus, connected to an 803 Ti Stand, hosting the titration vessel (Metrohm, Switzerland). The system was managed by Metrohm KF software Version 5.870.0014. Methanol (HPLC grade) was used as solvent in the titration vessel, whereas Hydranal® Composite-5 (Batch number: 7078B) was utilised as Karl Fischer reagent for the supply of iodine. A minimum of 100 mg sample was required for each KFT analysis.

2.3.7.2 Coulometric KFT

Coulometric KFT involves the electrochemical generation of iodine during the titration process. Ultimately, the water content in the sample is quantified by the total charge being passed through the solution. The measured current (ampéres) is given as a function of time, which concludes to the total charge passed. Coulometric KFT is ideal for establishing water content below 50 - 100 ppm (De Caro *et al*., 2001:432; Grünke, 2003:100; Ronkart *et al*., 2006:1006).

2.4 High-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) is an analytical method that is based on the separation of a compound and its relevant analytes in a constituted liquid phase (mobile phase), which is pumped at high pressure through a stationary phase (column). The use of HPLC has become increasingly dominant in the analytical role of drug discovery and development (Dong, 2006:2). HPLC assays are used to determine the purity of a drug compound, to establish potency, for the detection of impurities and are also useful during dissolution studies. Therefore, HPLC analysis fulfils an important aspect of quality control of pharmaceutical compounds and products. Dong (2006:136) stated the following functions for use of HPLC in research, development and quality control of pharmaceutical compounds and products:

- Identifying entities that may result in newly discovered drug candidates.
- Assists in chemical development (synthesis routes and scale-up) of pharmaceutical compounds.
- Pharmaceutical development, involving dosage form design through optimisation of stability and drug delivery profiles.
- Assists in assessing the metabolic and pharmacokinetic studies conducted on animals and humans.
- The assessment of the quality of manufactured products and the conformity of these products to the specifications, as recommended for each of them.

Reversed-phase chromatography is the most common analysis method, used by 70 % of analysts, of HPLC. This method is the exact opposite of normal-phase chromatography.

Normal-phase chromatography is based on the separation of the analytes, during which the polar analytes move slower through the stationary phase (column) than the non-polar analytes. Reversed-phase chromatography allows for the principle of "polar first and non-polar last", thus meaning that the polar analytes elute first, while the non-polar analytes move slower through the column, due to stronger interactions with the hydrophobic (C_{18}) groups present in the column. The mobile phase used for reversed-phase chromatography typically consists of a mixture of either methanol or acetonitrile with water, or an aqueous buffer (Dong, 2006:7; Kanfer *et al*., 1998:259).

2.4.1 HPLC method development for the identification and quantification of azithromycin

Published HPLC methods for the identification and/or quantification of azithromycin include electrochemical, or ultraviolet (UV) detection (Kanfer *et al*., 1998:270; Miguel & Barbas, 2003:213; Shaikh *et al*., 2008:1482). Methods using electrochemical (coulometric and amperometric) detection of azithromycin in biological samples have been illustrated by Shepard *et al.* (1991:323). The United States Pharmacopoeia (USP) describes an assay method, using an amperometric method for detection (USP, 2010). Zubata *et al.* (2002:833) describe an HPLC method, using UV detection to identify azithromycin and its impurities present in azithromycin tablets. Since all of these methods utilise equipment not readily available in many laboratories, an HPLC method, using UV detection, was developed during this study. Kanfer *et al* (1998:270) reported that the poor UV absorptivity of azithromycin posed a limitation to its detection but that this can be negated by the specificity and clear separation of this novel method.

Known published HPLC methods (Kanfer *et al*., 1998:270; Miguel & Barbas, 2003:213; Shaikh *et al*., 2008:1482; Shepard *et al*., 1991:323; USP, 2010; Zubata *et al*., 2002:833) were investigated as quantification tool for azithromycin from solubility and stability studies. All of these methods used different constituents for the mobile phases at variable pH, ranging from 3 - 9. The columns used ranged from 125 to 250 mm in length, and 2.1 to 4.6 mm (5 µm) in internal diameter. Detection wavelengths ranged from 205 to 235 nm. These HPLC methods, however, caused difficulty in quantifying azithromycin, as a result of peak interference (poor separation), poor retention and peak tailing.

The need for the development and validation of a reliable HPLC method that would accurately identify and/or quantify azithromycin during product assays, solubility, stability

and dissolution studies, was hence identified. The following paragraphs describe the method development and validation processes being followed.

2.4.2 Experimental

2.4.2.1 Materials

Materials used during this HPLC method development were as described in Section 2.2.

2.4.2.2 Instrumentation and software

A Shimadzu (Shimadzu, Japan) UFLC (LC-20AD) chromatographic system was utilised. The system consisted of a SIL-20AC autosampler, fitted with a sampler cooler, a UV/VIS photodiode array detector (SPD-M20A) and a LC-20AD solvent delivery module. The software used was LabSolutions, LCSolution®, Release 1.21 SP1.

2.4.2.3 Chromatographic conditions

A Phenomenex® Luna C_{18} (5 µm) 150 mm x 4.6 mm column was used as stationary phase. The mobile phase was carefully selected to achieve symmetrical chromatographic peaks, acceptable separation from tablet excipients and solvents, and optimum selectivity for azithromycin (Figure 2.1). The chosen mobile phase consisted of a combination of 0.06 M phosphate buffer at pH 6.0 (pH adjusted with 1.0 M sodium hydroxide solution) and acetonitrile (700/300). Isocratic elution was used. The flow rate was set at 1.0 mL/min. Photodiode array detection showed that two wavelengths, i.e. 205 nm and 210 nm, were most favourable for azithromycin detection. The injection volume for each sample was 15 µL.

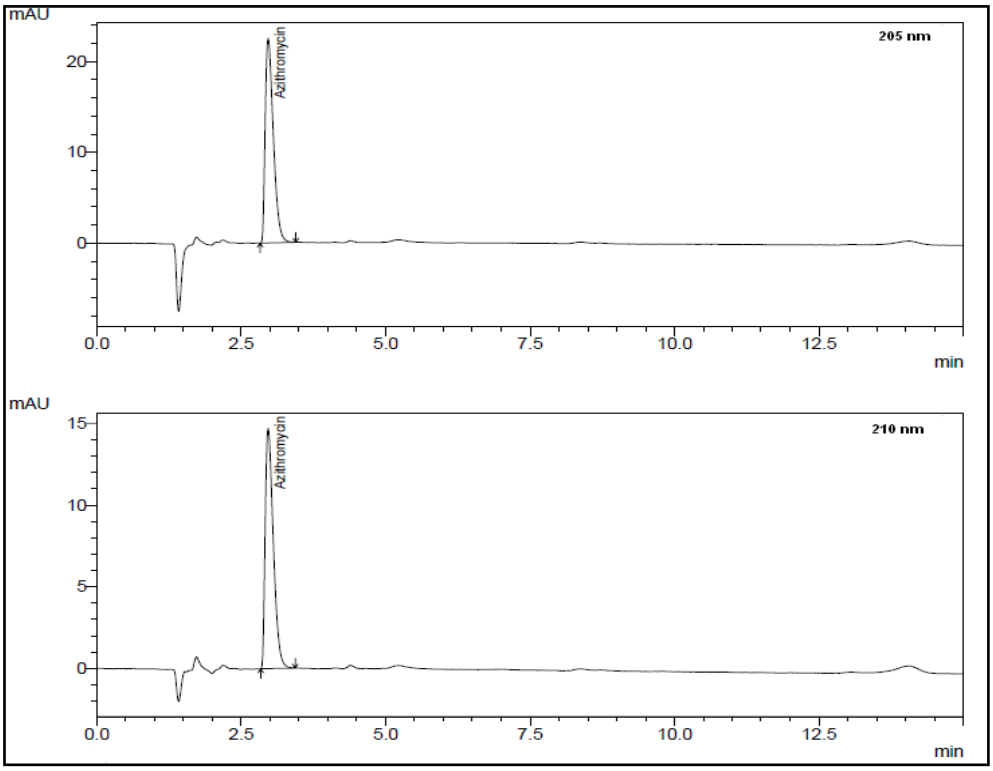

Figure 2.1 HPLC chromatograms of AZM-DH reference material dissolved in mobile phase.

2.4.3 Preparation of solutions

2.4.3.1 Calibration standard solutions

Five calibration solutions of AZM-DH were prepared for the validation process. The azithromycin dihydrate powder was individually weighed and dissolved in mobile phase. The concentrations of the calibration solutions ranged from 0.1 - 5.0 mg/mL. All solutions were each filtered through a 0.45 µm Millipore® filter.

For the purpose of this validation, the calibration solution, having a concentration of 1.0 mg/mL, was indicated as the theoretical 100% drug concentration. The control solution (1.0 mg/mL) was also prepared to establish the accuracy and recovery of azithromycin with this proposed reversed-phase method.

2.4.3.2 Preparation of test solutions

Commercially purchased AZM-DH tablets were weighed and carefully powdered, using a mortar and pestle. The resulting powder was accurately weighed and dissolved in mobile phase to attain a solution with a concentration of 5.0 mg/mL, then sonicated for 10 minutes and filtered through a 0.45 µm Millipore® filter.

2.4.3.3 Preparation of test solutions in solubility media

The four media being tested for application in this method in the solubility and dissolution studies were phosphate buffer (pH 6.8), acetate buffer (pH 4.5), 0.1 M HCl (HCl) (pH 1.2) and distilled water. The three buffers were prepared according to BP specifications (BP, 2011). Solutions with AZM-DH concentrations of 1.0 mg/mL (acetate buffer and 0.1 M HCl), 0.5 mg/mL (phosphate buffer) and 0.1 mg/mL (water) were prepared and filtered through 0.45 µm Millipore® filters.

2.4.4 Method validation

Validation of the developed method was established at two wavelengths, i.e. at 205 nm and at 210 nm. Five calibration standard solutions with concentrations ranging between 0.1 - 5.0 mg/mL (10 - 500 % of the theoretical 100 % concentration) were prepared to determine linearity. These solutions consisted of AZM-DH dissolved in mobile phase. Each solution was injected in duplicate. Accuracy was determined by injecting duplicate samples of the calibration control solutions (100 % theoretical concentration). The recovered concentration was compared to the initial theoretical concentration of the control solutions. To determine specificity, a commercial AZM-DH tablet was dissolved in mobile phase. Specificity was also resolved by dissolving AZM-DH at a known concentration in the four general solubility media (acetate buffer, phosphate buffer, 0.1 M HCl and water). Precision of this method was assessed on two levels, i.e. repeatability and intermediate precision. Repeatability was measured through five injections of the calibration standard solutions that represented 100 % of the theoretical concentration. To establish intermediate precision, this method was performed by two different analysts in the same laboratory on consecutive days. The chromatographic columns and the HPLC systems used were different. Analysts used variable methods for preparing the calibration standard and control solutions for the validation process. One analyst prepared the solutions with dilutions to attain the set concentrations, whereas the other accurately weighed and dissolved the AZM-DH powder to achieve the required concentrations.

2.4.5 Results and discussion

2.4.5.1 Method optimisation

During the method development stage it became evident that the pH and composition of the mobile phase were critical factors. For pH < 6 (Figure 2.2), the azithromycin peak overlapped significantly with the mobile phase peak appearing at approximately 2.6 minutes, causing poor separation of the azithromycin and the mobile phase peaks.

The retention of azithromycin was also very short, which was undesirable, especially for the detection of azithromycin in solubility or dissolution media. The interference of the aqueous media caused very poor peak identification, separation and unreliable peak area integration. For pH > 6 (Figure 2.3), the symmetry of the azithromycin peak was weak. The absence of symmetrical peaks was due to peak tailing, which made peak area integration inaccurate and quantitative repeatability impossible (Figure 2.3).

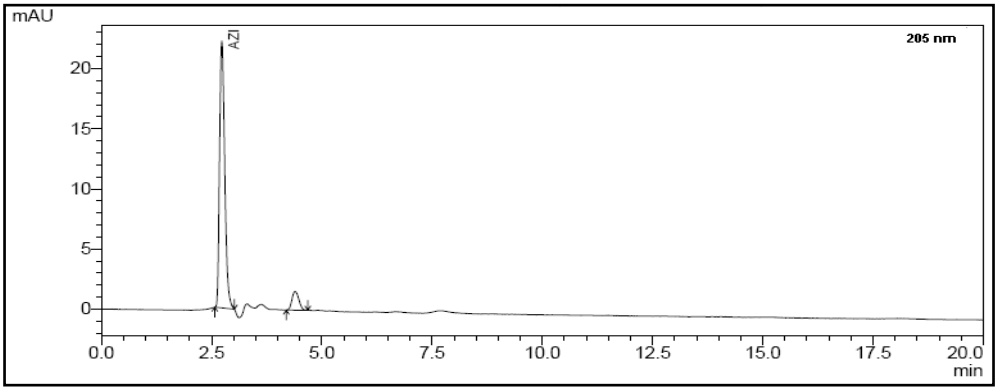

Figure 2.2 HPLC chromatogram of AZM-DH dissolved in mobile phase (1 mg/mL) at 205 nm. Mobile phase: phosphate buffer adjusted with phosphoric acid (pH 3.0)/acetonitrile (600/400), Flow rate 1.0 mL/min, Injection volume 15 µL. UV detection 205 nm.

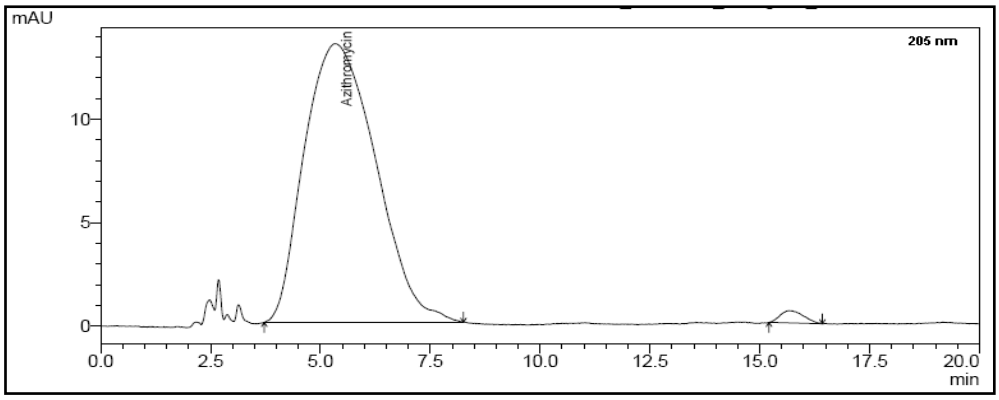

Figure 2.3 HPLC Chromatogram of AZM-DH dissolved in mobile phase (1 mg/mL), at 205 nm. Mobile phase: phosphate buffer adjusted with 1.0 M sodium hydroxide (pH 8.4)/acetonitrile (500/500), Flow rate 1.0 mL/min, Injection volume 15 µL. UV detection 205 nm.

2.4.6 Method validation

2.4.6.1 Linearity and range

Linearity of this method concluded to a regression curve (using Analysis ToolPak, Excel™) having a correlation coefficient of r^2 = 0.9999 and r^2 = 0.9999 at wavelengths 205 nm and 210 nm, respectively. The linearity parameters obtained for azithromycin at both wavelengths are listed in Table 2.2. The calculated p-values at a confidence level of 95 % for the curves obtained at 205 nm and 210 nm were 0.293 and 0.424, respectively.

Table 2.2 Equations for regression obtained during method validation to compare linearity at 205 nm and 210 nm

Wavelength	Range ($\mu g.ml^{-1}$)	Slope (± SD)	Intercept (± SD)	Correlation coefficient (r^2)
205 nm	100 – 5000	232.37 (± 0.37)	-1068.59 (± 884.13)	0.9999
210 nm	100 – 5000	139.60 (± 0.28)	-594.57 (± 668.41)	0.9999

2.4.6.2 Accuracy

Accuracy was determined by comparing the established azithromycin value of the control solution to the theoretical (accepted reference) value, as summarised in Table 2.3.

Table 2.3 Accuracy as a result of azithromycin recovered from the 100 % calibration solution

Wavelength	Theoretical AZM concentration (mg/mL)	Recovered AZM concentration (mg/mL)	Recovery (%)	RSD (%)
205 nm	1.072	1.079	100.68	0.06
210 nm	1.072	1.08	100.74	0.06

2.4.6.3 Specificity

The inactive ingredients in the commercial AZM-DH tablet did not show any interference with the azithromycin chromatographic peaks at both wavelengths (Figure 2.4). Unknown peaks were visible at retention times ranging between 7.0 - 15.0 minutes (Figure 2.4). A blank mobile phase sample was injected to conclude the presence, but not interference, of small peaks, due to the buffer and solvents being used in the mobile phase. From Figure 2.4 it is clear that these unknown substances (tablet excipients) caused no interference with the azithromycin peak, but the azithromycin peak was clearly identifiable with acceptable separation. This underlined the true significance of the method's specificity for azithromycin in the presence of inactive ingredients (tablet excipients). Specificity regarding the detection of azithromycin and the application of this method in the various solubility (and dissolution) media are further discussed in Section 2.4.7.

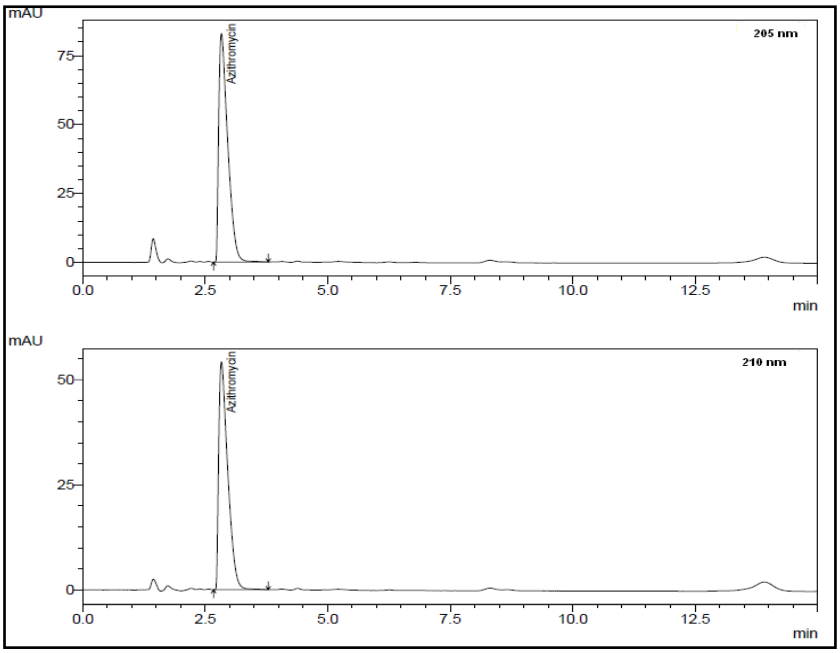

Figure 2.4 HPLC chromatogram of AZM-DH tablet (5 mg/mL) dissolved in mobile phase at 205 nm and 210 nm. Mobile phase: phosphate buffer (adjusted with 1.0 M sodium hydroxide to pH 6.0)/acetonitrile (700/300), Flow rate 1.0 mL/min, Injection volume 15 µL. UV detection 205 nm and 210 nm.

2.4.6.4 Precision

Repeatability

Data recovered from the five replicate injections of the 100% solution (1.0 mg/mL) at both wavelengths (205 nm and 210 nm) showed relative standard deviation (RSD) values of 0.16 % and 0.17 %, respectively.

Intermediate precision and robustness

Results obtained by the second analyst to establish intermediate precision and the robustness of this analytical method are given in Table 2.4.

Table 2.4 Summary of intermediate validation results at 205 nm and 210 nm, as obtained by the second analyst

Wavelength	Equation for regression	Correlation coefficient (r^2)	% RSD	% Recovery
205 nm	y = 218.03x + 4213.21	0.9983	0.3	104.95
210 nm	y = 139.89x + 3948.10	0.9970	0.3	106.66

Despite using a different HPLC system and column by another analyst, the method proved to be very accurate in retaining azithromycin without peak interference. Accuracy and precision was also established by the second analyst from 0.1 mg/mL to 5.0 mg/mL. This indicated that the method was in fact robust and could be repeated with success under similar conditions, using similar equipment. Concentrations exceeding the range of the HPLC method were calculated by using extrapolation.

2.4.6.5 Limit of detection and quantitation

The smallest concentration of analyte in a sample that the method is able to detect, but not essentially quantified, can be described as the limit of detection (DL) (Sarrio & Silvestri, 1996:22). The detection limit (DL) may be expressed as:

$$DL = \frac{3.3\sigma}{S}$$

Where: σ is the standard deviation of the response, and S is the slope of the calibration curve. The quantitation limit (QL) can be expressed as the smallest analyte concentration in a sample that the method is capable of quantifying with apt accuracy and precision (Sarrio & Silvestri, 1996:23). The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\sigma}{S}$$

Where: σ = the standard deviation of the response, and S is the slope of the calibration curve. An Excel™ spreadsheet (Analysis ToolPak), using linear regression, was utilised to calculate the DL and QL values, as summarised in Table 2.5.

Table 2.5 DL and QL values achieved by the HPLC method at 205 nm and 210 nm.

Wavelength	Limit of detection	Limit of quantitation
205 nm	3.88 µg/mL	11.76 µg/mL
210 nm	6.87 µg/mL	20.80 µg/mL

These values were confirmed during intermediate precision and robustness analyses, as well as during subsequent solubility and dissolution studies.

2.4.7 Application of HPLC analyses in solubility and dissolution studies

Blank samples of the solvents were first injected to identify those peaks relating to the buffer contents in each solvent. Subsequently, samples, containing an AZM-DH concentration of 1.0 mg/mL in each solvent were injected. Chromatograms (Figures 2.5 - 2.8) showed the clear separation of the azithromycin peak from the solvent peaks. A blank water sample was also injected, followed by a sample of AZM-DH in water (0.1 mg/mL).

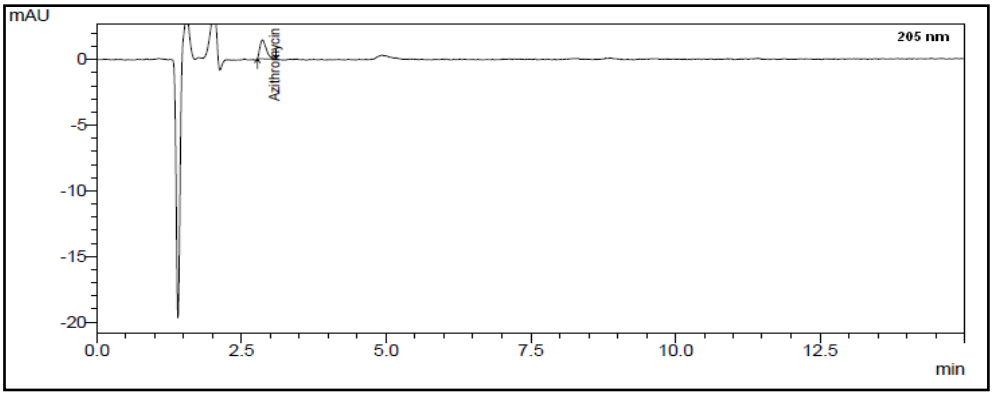

Figure 2.5 **HPLC chromatogram of AZM-DH dissolved in water (0.1 mg/mL) at 205 nm. Mobile phase: phosphate buffer (adjusted with 1.0 M sodium hydroxide to pH 6.0)/acetonitrile (700/300), Flow rate 1.0 mL/min, Injection volume 15 µL. UV detection 205 nm.**

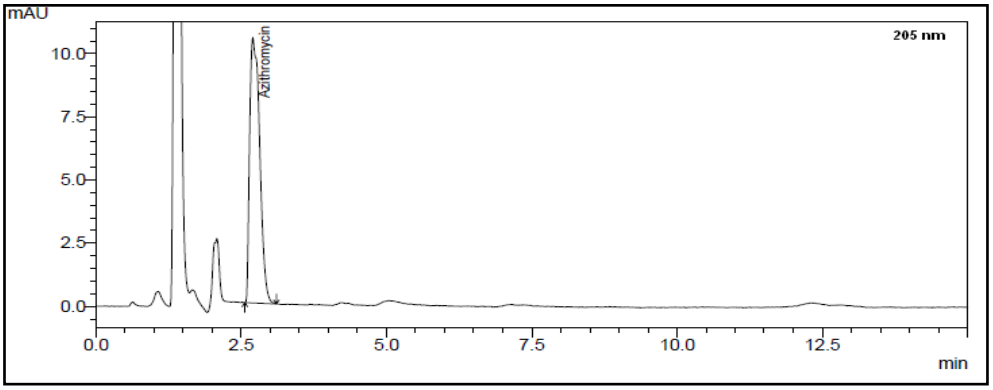

Figure 2.6 **HPLC chromatogram of AZM-DH dissolved in pH 4.5 acetate buffer (1.0 mg/mL) at 205 nm. Mobile phase: phosphate buffer (adjusted with 1.0 M sodium hydroxide to pH 6.0)/acetonitrile (700/300), Flow rate 1.0 mL/min, Injection volume 15 µL. UV detection 205 nm.**

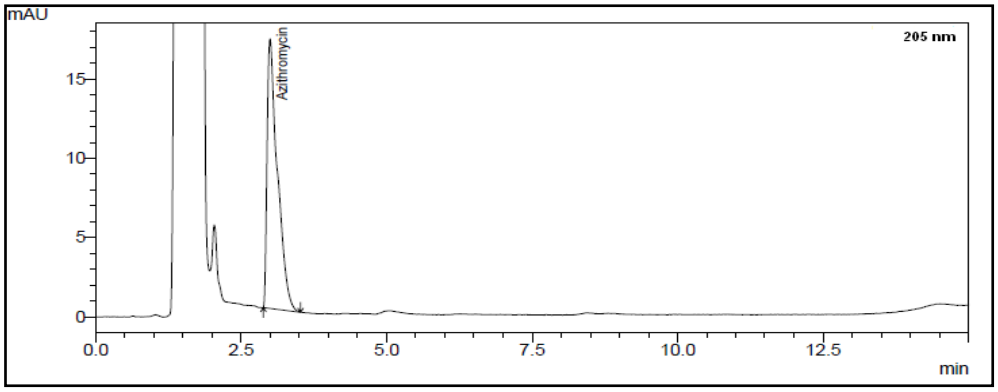

Figure 2.7 HPLC chromatogram of AZM-DH dissolved in pH 6.8 phosphate buffer (0.5 mg/mL) at 205 nm. Mobile phase: phosphate buffer (adjusted with 1.0 M sodium hydroxide to pH 6.0)/acetonitrile (700/300), Flow rate 1.0 mL/min, Injection volume 15 µL. UV detection 205 nm.

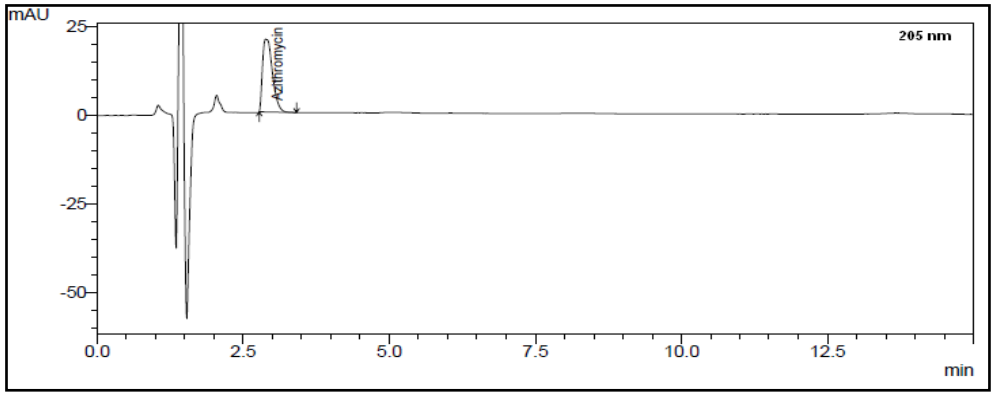

Figure 2.8 HPLC chromatogram of AZM-DH dissolved in 0.1 M HCl (pH 1.2) (1.0 mg/mL) at 205 nm. Mobile phase: phosphate buffer (adjusted with 1.0 M sodium hydroxide to pH 6.0)/acetonitrile (700/300), Flow rate 1.0 mL/min, Injection volume 15 µL. UV detection 205 nm.

Because of the poor water solubility of AZM-DH in water, the quantification of very low concentrations of this API was made difficult with the developed methods. However, as seen in Figure 2.5, there was sufficient separation of the azithromycin peak from the solvent peaks for accurate quantification (RSD = 0.06 %). The water samples were only integrated at 205 nm (Figures 2.5 - 2.8), since the validation achieved optimum detection at this wavelength with water as solvent. Calibration curves were set for each solvent used.

2.4.8 Conclusion

A novel, reversed-phase HPLC method, using UV detection, was developed as a multi-purpose method to identify and quantify azithromycin in drug samples. The rationale for developing this method was to establish an easy, reversed-phase HPLC method that could be applied for several analytical or detection purposes. This method showed to be adequately sensitive and robust for it to be successfully exploited during stability, solubility and dissolution studies of azithromycin. This HPLC method was further accepted for publication in Pharmazie (Annexure A). From the data obtained during method development, it became evident that the pH of the mobile phase used and the ratio of buffer to organic component was critical for the optimum detection of azithromycin.

2.5 Solubility

2.5.1 General aspects of solubility

The determination of the solubility of a solid in applicable solvents, as specified by monographs, is of importance, especially for future drug development. Solubility can be described as the amount of dissolved solid (API) that relates to a saturated solution in a specified solvent at a distinct temperature and pressure (Brittain & Grant, 1999:282). According to Grant and Brittain (1995:322), the factors playing a crucial role in the solubility of a solid are (1) physical form of the solid, (2) characteristics of the solvent, including its composition and nature, (3) the temperature and (4) the pressure of this closed system.

2.5.2 Method for solubility determination

Azithromycin dihydrate (AZM-DH) was used to prepare a sufficient amount of azithromycin glass (AZM-G), of which approximately half was powdered, thus giving equal amounts of AZM-G (glassy form) and powdered AZM-G. This was done to determine whether the milling process and the kinetic energy involved in milling AZM-G, would induce crystallisation into the more stable AZM-DH that would consequently influence the solubility of AZM-G. The milling would also result in increased surface areas, due to a change in particle size of the powder, compared to the glassy form and hence enable further investigation into the effect of the milling process on the solubility of AZM-G.

AZM-DH, AZM-G and AZM-G powder were individually weighed and transferred into 20 mL amber glass tubes with screw caps. 10 mL of the respective solvent (four different aqueous media) each was added to each glass tube to produce over-saturated solutions. Each tube was sealed with a small piece of Parafilm®, prior to attaching the cap tightly to avoid any leakage from the tubes. Six replicates of each form were prepared in each of the aqueous media. The glass tubes with the over-saturated solutions were placed on a fixed rotating axis (54 rpm) that was submerged in a water bath. The temperature of the water bath was set at 37°C ± 2°C and maintained throughout the 24 hours of rotation. Sufficient time (24 hours) was allowed for the solution to reach equilibrium. To remove any remaining solid particles, the solutions were individually filtered through 0.45 *µ*m Millipore® filters into HPLC vials. The validated HPLC method, as described in Section 2.3, was used to determine the concentration of the different filtrates. The four aqueous media utilised to ultimately determine the solubility of AZM-G were phosphate buffer (pH 6.8), acetate buffer (pH 4.5), 0.1 M HCl (pH 1.2) and distilled water (BP, 2011).

2.6 Stability

One of the main functions of stability tests is to establish a shelf-life for a given product. All pharmaceutical products are subjected to stability tests to ensure product stability up until the product expires. Through stability studies, optimal storage conditions of pharmaceutical products are determined by establishing thermal stability, as well as the sensitivity of the API to moisture. Products not stored at the prescribed storage conditions may be subject to chemical instability, whereas the quality of the product may be at risk. Stability testing is therefore important to establish the storage conditions for optimum product stability and product quality. These conditions include exposure to elevated temperatures and various levels of relative humidity (RH). Stability testing must be no less than two weeks of exposure to a fixed set of conditions (Byrn *et al*., 1999:38). According to ICH guidelines, general accelerated stability testing should occur over a period of six months at 40˚C ± 2˚C / 75 % ± 5 % RH. Conditions for long-term stability studies are at 25˚C ± 2˚C / 60 % ± 5% RH, or 30˚C ± 2˚C / 65 % ± 5% RH over a period of twelve months. The ICH prescribes intermediate stability testing performed at 30˚C ± 2˚C / 65 % ± 5 % RH over a period of six months (ICH, 2012).

2.6.1 Method for accelerated stability study

Azithromycin dihydrate (AZM-DH) powder was used to produce azithromycin glass (AZM-G). These glass samples, together with AZM-DH samples, were placed in a Labcon (Air & Vacuum Technologies, Instrulab, SA), having a temperature- and humidity controlled environment. These two sets of samples were stored at 40˚C ± 2˚C / 75 % ± 5% RH for a period of four weeks. All samples were prepared and stored in such a manner that weekly analysis was possible without disturbance of the other samples. The methods for analyses were as discussed in Section 2.2. Analyses of samples were performed each week to accurately determine the stability of AZM-G samples at the said conditions, while AZM-DH was used as reference throughout this stability trial.

2.6.2 Method for determining the acid stability at pH 1.2

Tests were done to determine the extent of AZM degradation in acidic conditions. AZM-DH and AZM-G were analysed to determine their stability in 0.1 M HCl (pH 1.2). This small stability indicating study aimed at establishing the percentage of degradation that AZM would undergo over a time of 120 minutes. The levels of degradation were determined by HPLC analyses of the samples at 15 minute intervals.

An accurate amount of each form of AZM was weighed and transferred into separate 20 mL volumetric flasks, where after 20 mL of 0.1 M HCl was added to each (sample concentration 1.0 mg/mL). The solutions were rapidly stirred and filtered through 0.45 μm Millipore® filters into HPLC vials. Two HPLC systems were prepared in order for the samples (AZM-DH and AZM-G) to be analysed simultaneously. The HPLC method, as described in Section 2.3 was used to determine the concentration of the different filtrates.

2.7 Dissolution study

2.7.1 Materials and methods

Tablets were manufactured from the azithromycin raw material (AZM-DH) and the glass (AZM-G) (Chapter 6, Sections 6.4.1 and 6.4.2) and subjected to dissolution studies. The four aqueous media (as described in the BP, 2011) being used as dissolution media consisted of acetate buffer (pH 4.5), phosphate buffer (pH 6.8), distilled water and 0.1 M HCl (pH 1.2).

The method used to perform the dissolution studies was based on the method, as described in the USP (2010). A VanKel VK700 dissolution apparatus (VanKel, USA), connected to a VanKel VK650A heater/circulator, was set up with rotating paddles and six glass vessels. The paddles were positioned 2.5 cm from the bottom of the glass vessels. 900 mL of medium was added to each vessel and allowed to heat to 37˚C, prior to commencement of the study. The rotation speed of the paddles was 75 rpm. Each dissolution study consisted of six replicates. Tablets were placed in the vessels, 2 minutes apart, to allow for sufficient time in between sampling. The withdrawal times were at 5, 10, 15, 30 and 45 minutes. The sampling volume was 5 mL. The sampling point was, according to USP specifications, at the point midway between the top of the paddle and the top of the fluid and not closer than 1 cm from the side of the glass vessel. All samples were filtered into HPLC vials through 0.45 µm Millipore® filters. Samples were analysed, using the validated HPLC method, as described in Section 2.3. The percentage of azithromycin content, being dissolved at each sampling interval, was calculated using an Excel® spreadsheet.

2.8 Conclusion

Studying the existence of different solid forms of pharmaceutical solids is an important objective of pharmaceutical research and development. The different solid forms of an API must be characterised to generate a complete profile of its pharmaceutical properties. To achieve this, various methods are utilised to characterise the pharmaceutical properties of the different solid forms. These methods make quantitative and qualitative characterisation possible. The use of XRPD is known to be the single most effective method in establishing the existence of polymorphic forms for APIs. However, a single method alone is not sufficient for gathering data regarding the profile of a pharmaceutical active. Other methods that contribute towards the pharmaceutical profile are IR, thermal methods (DSC and TGA), microscopy (HSM and SEM), moisture sorption analysis and KFT. Other significant aspects of the pharmaceutical profile that should also be quantified are solubility, dissolution rate, and the stability of said polymorphs or amorphous forms. To accurately identify and quantify azithromycin, a robust, reversed-phase HPLC method was developed and validated. During this study, this validated method was employed during the solubility, dissolution and stability studies.

In conclusion, various analytical methods are used to establish the characteristics and pharmaceutical properties of polymorphs. This is essential for improving existing pharmaceutical compounds and in discovering new drug entities with the potential of being registered as pharmaceutical compounds.

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