Amorphism and polymorphism of azithromycin

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2 KOR. 12:9

“My genade is vir jou genoeg.
My krag kom juis tot volle werking
wanneer jy swak is.”

Dedicated to my Heavenly Father

AND

my parents, W.T. and Antoinette Odendaal

AND

my late grandparents,

Roelf & Petronella Odendaal

Hans & Lea van Dyk

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LIST OF ABBREVIATIONS

> - greater than

≥ - greater than or equal to

≤ - less than or equal to

< - less than

˚ - degrees

˚C - degrees Celsius

Ω - ohm

% - percentage

% WL - percentage weight loss

θ - Theta

µg - microgram

µL - micro litre

µm - micro metre

A - surface area

API - active pharmaceutical ingredient

ATP - adenosine triphosphate

AVG - average

AZM - azithromycin

AZM-DH - azithromycin dihydrate

AZM-G - azithromycin glass

BET - Brunauer-Emmett-Teller theory

BP - British Pharmacopeia

C - carbon

C_0 - initial concentration

cm - centimetre

conc. - concentration

DCM - dichloromethane

DL - limit of detection

DRIFTS - diffuse reflectance infrared Fourier transform spectroscopy

DSC - differential scanning calorimetry

e.g. - *exempli gratia* (for example)

EP - European Pharmacopeia

FTIR - Fourier transform infra-red spectroscopy

g - gram

h - hour

H - hydrogen

H_2O - water

HCl - hydrochloric acid

HPLC - high performance liquid chromatography

HSM - hot stage microscopy

ICH - International Conference on Harmonisation

i.e. - *id est* (that is)

IR - infrared spectroscopy

IUPAC - International Union of Pure and Applied Chemistry

J - Joule

K - Kelvin

KBr - potassium bromide

KCl - potassium chloride

kg - kilogram

KFT - Karl Fischer titration

L - litre

M - molar

m - metre

MAC - *Mycobacterium avium* complex

MDCK - Madin-Darby canine kidney

mg - milligram

MIC - minimum inhibitory concentration

min - minutes

mL - millilitre

mΩ - milli-ohm

MM - molecular mass

mm - millimetre

N - nitrogen

N - newton

NaOH - sodium hydroxide

nm - nanometre

O - oxygen

PAMPA - Parallel artificial membrane permeability assay

P_{app} - apparent permeability coefficient

P-gp - P-glycoprotein

ppm - parts per million

QL - limit of quantification

r^2 - correlation coefficient

RH - relative humidity

RNA - ribonucleic acid

rpm - revolutions per minute

RSD - relative standard deviation

s - second

SA - South Africa

SEM - scanning electron microscopy

STDEV (SD) - standard deviation

TAM - Thermal Activity Monitor

TEER - Trans-epithelial electrical resistance

T_g - glass transition temperature

TGA - thermogravimetric analysis

T_m - melting temperature (point)

UFLC - Ultra fast liquid chromatography

USA - United States of America

USP - United States Pharmacopeia

UV - ultraviolet

vs - versus

w/w - weight per weight

XRPD - X-ray powder diffraction

LIST OF EQUATIONS

CHAPTER 5: AZITHROMYCIN GLASS

$R_L = 1 / (1+b_L.C_0)$ ..... *(5.1)*

$T_{gmix} = w_1T_{g1} + w_2T_{g2}$ ..... *(5.2)*

$$T_{gmix} = \frac{(w1Tg1 + kw2Tg2)}{(w1 + kw2)} \quad \text{..... (5.3)}$$

Where $k = \frac{(\rho 1Tg1)}{(\rho 2Tg2)}$

$$\frac{1}{Tgmix} = \frac{w1}{Tg1} + \frac{w2}{Tg2} \quad \text{..... (5.4)}$$

CHAPTER 6: PRODUCT DEVELOPMENT

$P_B = w/v$ ..... *(6.1)*

$P_T = w/v$ ..... *(6.2)*

% compressibility = $((P_T-P_B)/P_T) \times 100$ ..... *(6.3)*

Hausner ratio = P_T/P_B ..... *(6.4)*

CHAPTER 7: PERMEABILITY

$P_{app} = (dC/dt) \times (1/A \bullet C_0 \bullet 60)$ ……. *(7.1)*

$\%_{AZM\text{-}G} = (\text{AZM-G } P_{app}/\text{AZM-DH } P_{app}) \times 100\ \%_{AZM\text{-}DH}$ …… *(7.2)*

$\%\ \text{improvement} = \%_{AZM\text{-}G} - 100$ …….. *(7.3)*

ABSTRACT

Azithromycin, an azalide and member of the macrolide group, is a broad spectrum antimicrobial, representing one of the bestselling antimicrobials worldwide. It is derived from erythromycin and exhibits improved acidic stability as a result of its structural modifications. The stable solid form of azithromycin is its dihydrate, although it also naturally occurs in its metastable forms, i.e. the monohydrate and anhydrate. Because azithromycin is poorly soluble in water, its absorption from the gastro-intestinal tract is negatively influenced, which ultimately affects its bioavailability following oral administration (37 %).

Polymorphic (monohydrates and dihydrates) and anhydrous forms of azithromycin were screened and investigated. One anhydrous form also proved to be amorphous, which shifted the focus of this study from polymorphism to amorphism. An amorphous glassy azithromycin was subsequently prepared and fully characterised to present its solid state profile.

The stability of this amorphous glassy form was established at a high temperature and relative humidity over a period of four weeks. Exposure to increased relative humidity (up to 95 %) and increased water content (up to 50 %) also served as stability indicating tests. Its solubility in various aqueous media was determined. A solid dosage form (tablet), containing the azithromycin glass, was prepared, whereafter these tablets were subjected to dissolution studies in different aqueous media. The stability of azithromycin glass in tablet form was determined over a period of three months. The permeability of azithromycin glass across excised pig intestinal tissue was further established at various pH values.

This amorphous glassy form of azithromycin (AZM-G) proved to be very stable at high temperature and relative humidity, whilst also remaining stable after prolonged exposure to 95 % of relative humidity, as it only adsorbed moisture onto its surface. Water content (up to 50 %) had no plasticising effect on azithromycin glass. It demonstrated a significantly higher water solubility (339 % improvement) in comparison with the commercially available azithromycin dihydrate and was it also 39 % more soluble in phosphate buffer (pH 6.8) than its dihydrate counterpart.

The prepared azithromycin glass tablets showed a promising dissolution profile in water, due to the improved water solubility of this glass form. The transport of azithromycin glass at higher pH values (6.8 and 7.2) across the membrane proved to be significantly higher than that of azithromycin dihydrate, thus also illustrating its pH dependence for its transport across pig intestinal tissue.

The improved water solubility of the azithromycin glass, together with its faster dissolution rate, its superior stability and its increased permeability, may ultimately result in a higher azithromycin bioavailability following oral administration.

These research outcomes hence give rise to the need for investigating the effect of administering lower dosages of azithromycin and to determine whether the same antimicrobial efficacy would possibly be achieved, due to maintaining the same tissue concentration levels at these lower dosages.

Key words: Macrolide; Azithromycin; Amorphous; Solubility; Stability; Dissolution; Permeability.

UITTREKSEL

Asitromisien, ‘n asalied wat deel van die makroliedgroep vorm, is ‘n breë sprektrum antimikrobe geneesmiddel, wat wêreldwyd as een van die topverkopers van antimikrobiese geneesmiddels beskou word. Asitromisien is ‘n derivaat van eritromisien en toon verbeterde stabiliteit in suuromgewings, as gevolg van sy strukturele modifikasies. Die dihidraat word as die stabiele soliede vorm van asitromisien beskou, alhoewel dit ook natuurlik in sy monohidraat- en anhidraat vorms voorkom. Omdat asitromisien swak oplosbaar is in water, word sy absorpsie vanuit die gastroïntestinale kanaal negatief beïnvloed, wat uiteindelik weer sy biobeskikbaarheid na orale toediening affekteer (37 %).

Polimorfiese (monohidrate en dihidrate) en anhidriese vorme van asitromisien is oorsigtelik tydens hierdie studie ondersoek. Een anhidraat het ook amorfe eienskappe getoon, wat daartoe aanleiding gegee het dat die fokus van hierdie studie vanaf polimorfisme na amorfisme verskuif het. ‘n Amorfe glas van asitromisien is vervolgens berei en volledig in terme van vaste vorm eienskappe gekarakteriseer .

Die stabiliteit van die bereide amorfe asitromisien was by toestande van hoë temperatuur en relatiewe humiditeit oor ‘n tydperk van vier weke bepaal. Blootstelling aan verhoogde relatiewe humiditeit (tot en met 95 %) en verhoogde waterinhoud het voorts as stabiliteitsaanduidende toetse gedien. Die oplosbaarheid van die glas is ook in verskeie waterige mediums bepaal. Amorfe asitromisien is vervolgens in ‘n tabletvorm geformuleer, waarna dissolusie studies op die tablette in verskeie waterige mediums uitgevoer is. Die stabiliteit van amorfe asitromisien in tabletvorm is oor ‘n tydperk van drie maande bepaal. Die deurlaatbaarheid van amorfe asitromisien oor uitgesnyde intestinale varkweefsel by verskillende pH-waardes is verder ook bepaal.

Hierdie amorfe, glasagtige vorm van asitromisien het bewys gelewer dat dit baie stabiel was, ten spyte van blootstelling aan beide hoë temperatuur en relatiewe humiditeit. Verlenge blootstelling aan 95 % relatiewe humiditeit het ook geen effek op die stabiliteit van die glas getoon nie, aangesien dit die vog slegs op die oppervlak adsorbeer het. Toenemende waterinhoud (tot en met 50 %) het geen plastiserende effek op amorfe asitromisien gehad nie. Die glas het ongeveer 339 % beter wateroplosbaarheid in

teenstelling met die dihidraat van asitromisien aangetoon. Dit was voorts ook 39 % meer oplosbaar in fosfaatbuffer (pH 6.8).

Die dissolusie profiel van die bereide asitromisien (amorfe vorm) tablette in water was baie belowend, weens die verhoogde oplosbaarheid van hierdie vorm. Hoër asitromisien konsentrasies is vinniger verkry in vergelyking met die tablette wat asitromisien dihidraat-bevat het. Die deurlaatbaarheid van amorfe asitromisien oor die intestinale membraan by hoë pH-waardes (6.8 en 7.2) was beduidend beter as díe van die dihidraat. Die pH-afhanklikheid van die deurlaatbaarheid van die asitromisien glas oor die intestinale weefsel is hierdeur beklemtoon.

Die verbeterde wateroplosbaarheid van amorfe asitromisien, tesame met die vinniger dissolusie-tempo, die stabiliteit en verhoogde deurlaatbaarheid mag uiteindelik tot verhoogde biobeskikbaarheid na orale toediening lei.

Hierdie navorsingsuitkomstes het dus die behoefte laat ontstaan om die impak van die toediening van laer dosisse te ondersoek, ten einde te bepaal of dieselfde antimikrobiese effektiwiteit moontlik bereik kan word, weens die handhawing van identiese weefselkonsentrasies ten spyte van hierdie laer dosisse.

Sleutelwoorde: Makrolied; Asitromisien; Amorf; Oplosbaarheid; Stabiliteit; Dissolusie; Deurlaatbaarheid.

STUDY OBJECTIVES

Main objectives of this study

HPLC method development

- ✓ Developing a robust HPLC method for identification and quantification of azithromycin

Polymorphism of azithromycin

- ✓ Screening of different solid state forms produced through recrystallisation
- ✓ Preparation of anhydrous azithromycin
- ✓ Characterisation of anhydrous azithromycin

Amorphism of azithromycin

- ✓ Preparation of amorphous azithromycin glass
- ✓ Characterisation of azithromycin glass
- ✓ Solubility and stability of azithromycin glass

Product development

- ✓ Formulating azithromycin glass in a tablet
- ✓ Dissolution profiles of prepared tablets in various aqueous media
- ✓ Stability of azithromycin glass in tablet formulation

Permeability of azithromycin glass at various pH values