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Original Article

SOLID STATE CHARACTERIZATION AND QUANTIFICATION OF ABACAVIR SULPHATE, LAMIVUDINE AND ZIDOVUDINE AND ITS TABLET FORMULATION BY X-RAY POWDER DIFFRACTION METHOD

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ABSTRACT

Objective: To determine simultaneously the content of crystallinity of Abacavir sulphate (ABC), Lamivudine (LMD) and Zidovudine (ZVD) using X-ray Powder Diffraction (XRPD) technique and to validate the developed analytical methods and to statistically perform correlations by ANOVA technique.

Methods: Characteristic non-interfering peaks of ABC, LMD and ZVD were identified by using X-Ray Powder Diffraction method for assessment of the content of crystallinity.

Results: A working range 70 % to 130 % was taken for the establishment of linearity of the ABC, LMD and ZVD in the formulation and the coefficient of regression of ABC was 0.999, LMD was 0.998 and ZVD was 0.998. The F value by ANOVA was found to be within limits and satisfactory.

Conclusion: The developed method was adapted to the samples exposed to 40 °C/75 % RH, accelerated stability conditions and hence proved that it can be used for monitoring real-time samples.

Keywords: Abacavir sulphate, Lamivudine, Zidovudine, Crystallinity, XRPD, Validation

INTRODUCTION

Solid state studies of active pharmaceutical ingredients are vital during the research and development of a suitable large scale preparative technique and dosage form or a drug since polymorphism impacts three key areas during drug development and dosage form selection. These three areas include (1) the stability of the drug (both the physical and the chemical stability of the drug) in the dosage form, (2) the solubility of the drug (which includes the bioavailability of the drug from the site of absorption), and (3) the application and selection of a specific manufacturing process for a drug and its corresponding dosage form.

Polymorphs are chemically identical, but have different crystal lattice energies, melting points, intrinsic solubility, the rate of dissolution, densities, mechanical properties, physicochemical stability, hygroscopicity and different crystal habits. Solid drug forms have implications not only on biopharmaceutical performance and on the method of manufacture but also on intellectual property.

There are several practical examples in the pharmaceutical industry, where unwanted polymorphic changes caused bio-availability differences or intellectual litigations.

A three-drug combination of Abacavir sulphate, Lamivudine and Zidovudine includes two nucleoside reverse transcriptase inhibitor and one non-nucleoside reverse transcriptase inhibitor as a fixed dosage form used in the effective management of Human Immunodeficiency Virus (HIV) infection in adults [1]. Abacavir sulphate, chemically known as [(1R)-4-[2-amino-6-(cyclopropyl amino)-9H-purin-9-yl)-2- cyclopenetene]-1-methanol, is a carbocyclic synthetic analogue [2]. The active triphosphate metabolites of Abacavir sulphate, namely Lamivudine (4-amino-1-((2R, 5S-2-hydroxymethyl)-1,3-oxathiolan-5-yl)pyrimidin-2-(1H)-one) [3] and Zidovudine (3-azido-3-deoxythymidine) [4] and acts against HIV by inhibiting the reverse transcriptase. LMD reported existing in three crystalline forms [5-8] namely from I, II and III, where Form I is obtained from Form II by dissolving in hot water. Synthetic process yield form II. Form III obtained by controlled cooling of form I in a hot saturated solution of water.

Only one simultaneous RP-HPLC method [9] for combined dosage forms of ABC, LMD and ZVD in tablet dosage forms was reported in the literature.

No detailed solid state characterization of ABC, LMD and ZVD by X-Ray diffraction was reported in the literature and hence it was thought worthwhile to perform the physical characterization of ABC, LMD and ZVD and its tablet formulation to ascertain the most stable form of API during pre-formulation and in tablet formulation.

MATERIALS AND METHODS

The three drugs ABC, LMD and ZVD were confirmed using X-Ray Powder Diffraction (XRPD). The working ranges 70 %-130 % were prepared accurately using Sensitive high Microbalance (Mettler Toledo XP2U, Max 2.1g-Min-1 mg).

XRPD analysis

Diffraction patterns were collected using Bruker D8 advance X-ray diffractometer with Cu anode and Lynx eye detector. ABC, LMD and ZVD were scanned from 3 ° 2θ to 45 ° 2θ, with step size 0.01 2θ and time per step of 1.4 sec for the identification purpose. After selecting the non-interfering peak, the patterns of spiked standards were collected from 3 ° 2θ to 25 ° 2θ, with step size 0.01 2θ and time per step of 1.4 sec. The instrument was operated at 40 kV generator voltages and 40 mA generator current. Variable divergent slit and Anti-scattering slit were used of V20 mm, Nickel filter was used in the secondary beam path. Eva (version 10.0 revision I) which is part of the Diffrac software package was used for data processing and evaluation.

The chemical structures of ABC, LMD and ZVD are represented in fig. 1. Spiked standard working range concentrations 70 % to 130 % were prepared by geometrical mixing of ABC, LMD, ZVD and placebo.

Validation

Validation Studies were done with 70 %, 95 %, 105 % and 130 % concentrations of ABC, LMD and ZVD in formulation and linearity, specificity, recovery and precision were established.

Statistical calculation

The validation results were used to calculate the Regression Analysis and ANOVA values by using Microsoft Excel Analysis data pack, version 2010, to check the statistical reliabilities of the obtained data.

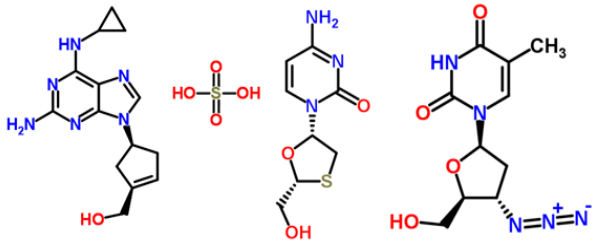

Fig. 1: Chemical structure of ABC, LMD and ZVD

RESULTS AND DISCUSSION

Knowledge of the solid state is of great importance in the development of a new active pharmaceutical ingredient since the solid form often dictates the properties and performance of the drug. Pharmaceutical substances are known to exist in different solid-state forms, and there are many analytical techniques available to characterize the solid-state pharmaceuticals and their solid-state transformations. Solid-state characterization technique is used to detect and quantitate physical forms of drugs as such and in solid dosage forms.

In the present study, we described, X-Ray Powder Diffraction (XRPD) technique to identify physical forms of ABC, LMD and ZVD and to detect the physical forms within the formulations, development of methods for physical quantification forms of drugs within formulations. Development of a method to detect and quantitate the undesired form, Form-I in Form-II of LMD in API, development of a method to estimate the content of crystallinity of the three API's in the formulation was also described in the study. The developed methods were validated to determine the statistical reliabilities of them.

The appearance of sharp peaks in XRPD for the three drugs ABD, LMD and ZVD confirmed their crystallinity, and they were identified and characterized with XRPD and overlayed with a formulation to identify characteristic non-interfering peaks (fig. 2).

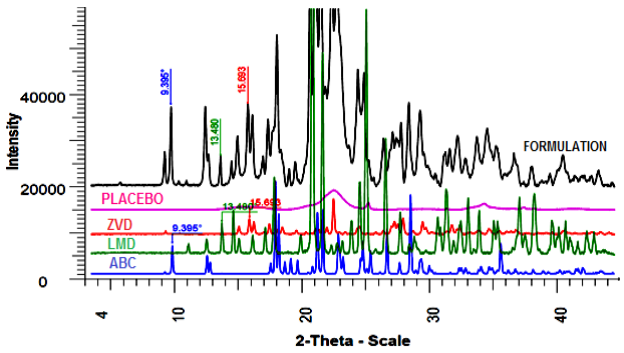

Fig. 2: Overlay diffractogram patterns of ABC, LMD, ZVD, placebo and formulation

The calibration curves (fig. 3, 4 and 5) were drawn by plotting working range standard preparations against net area (counts per seconds (Cps) x 2θ °). Diffractogram overlay of 70 % to 130 % working range standard preparations is represented in fig. 6. The standard calibration curve for ABC, LMD and ZVD was found to be linear over a working range of concentrations 70 %-130 %. The correlation coefficient (R^2) was 0.999 for ABC, 0.998 for LMD and 0.998 for ZVD which are well within the acceptable limit.

Four standard preparations at 70 %, 95 %, 105 % and 130 % concentrations, Prep 1, Prep 8, Prep 9 and Prep 7 respectively were prepared and measurements are recorded for three replicates and corresponding peaks of ABC, LMD and ZVD were integrated for the evaluation of precision, recovery, accuracy and reproducibility (table 1). The mean recovery values of 96.5 % for ABC, 100.3 % for LMD and 101.97 % for ZVD were obtained. The average recovery for each drug was above 99 %.

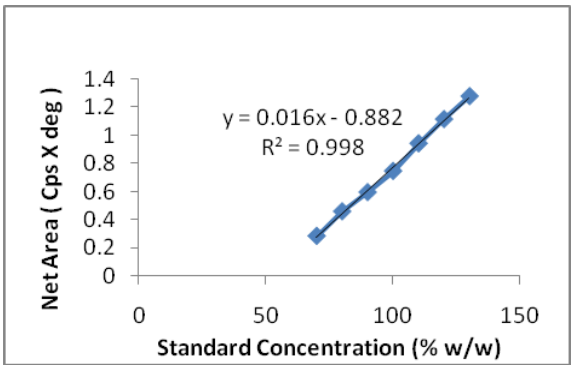

Fig. 3: Calibration plot of ABC

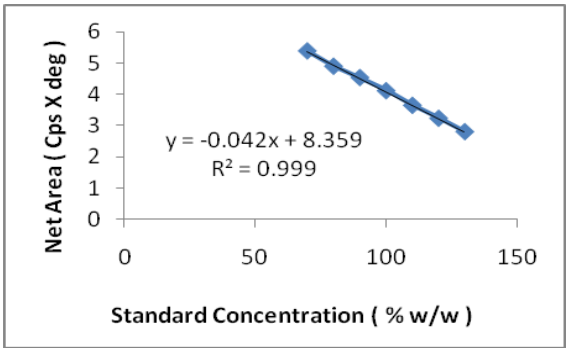

Fig. 4: Calibration plot of LMD

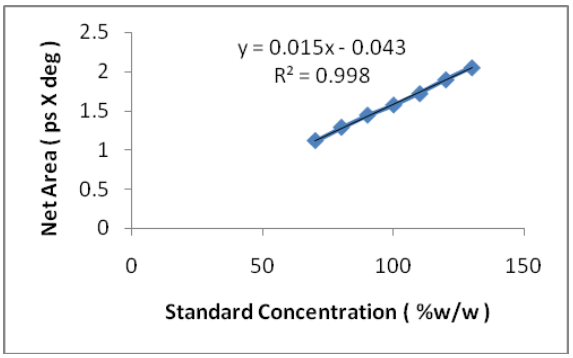

Fig. 5: Calibration plot of ZVD

Regression statistics were evaluated for working range concentrations prepared for validation versus Net area of each peak obtained. Regression parameters Multiple R, R^2, Adjusted R^2 and Standard error, were calculated. The correlation coefficient R (Multiple R), R^2 and Adjusted R^2 values for ABC, LMD and ZVD were given in table 2. Analysis of variance (ANOVA) table for the regression is constructed and is represented in table 2.

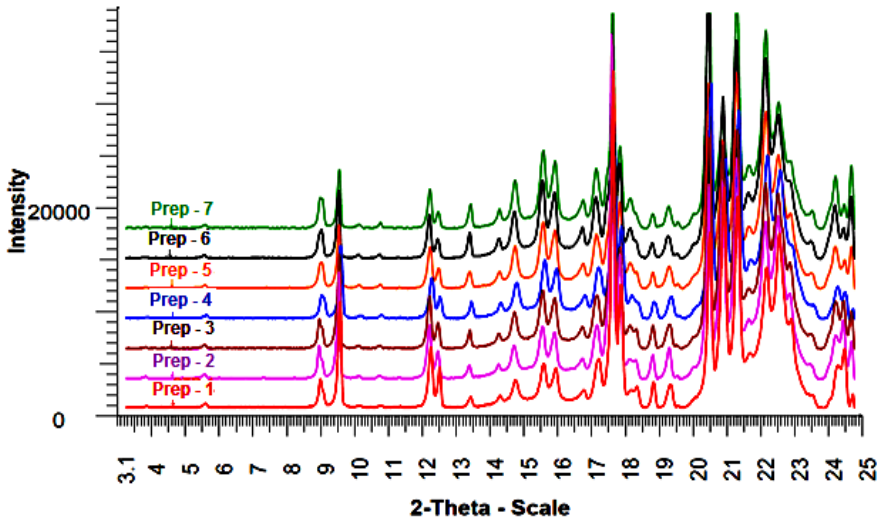

Fig. 6: Overlay diffractogram pattern of preparations, working range 70 % to 130 % for assessing the crystallinity in tablet formulation

Table 1: Precision, accuracy and reproducibility data of ABC, LMD and ZVD

Drug	Preparations	Concentration	Average Area	Average Recovery	% RSD
Abacavir sulphate	Prep-1	130.38	5.363	97.1148	0.5698
	Prep-8	105.14	4.425	99.5350	0.3883
	Prep-9	95.04	3.378	93.5821	0.2007
	Prep-7	70.02	2.574	97.5606	0.5186
Lamivudine	Prep-1	70.21	0.2813	100.47	0.2762
	Prep-8	95.17	0.6547	97.8961	0.3274
	Prep-9	105.04	0.8873	102.1218	0.5688
	Prep-7	129.69	1.2783	100.9836	0.8662
Zidovudine	Prep-1	69.5	1.2160	109.5	1.4
	Prep-8	94.9	1.5107	100.4	0.6
	Prep-9	104.77	1.6490	99.5	0.8
	Prep-7	129.99	2.0147	98.5	1.2

The low %RSD value obtained by the three measurements of each of the concentration of ABC, LMD and ZVD indicates that the method is precise and reproducible.

Table 2: Regression statistics ABC, LMD and ZVD

Parameters	ABC	LMD	ZVD
Multiple R	0.999	0.999	0.999
R Square	0.999	0.998	0.998
Adjusted R Square	0.999	0.997	0.998
Standard Error	0.026	0.016	0.013
Observations	7	7	7

Table 3: XRPD Crystallinity study content instability batches of ABC, LMD and ZVD

	Abacavir sulphate			Lamivudine			Zidovudine		
	Net area	% recovery	% RSD	Net Area	% recovery	% RSD	Net area	% recovery	% RSD
Initial	4.199		0.36	0.744		0.80	1.573		0.15
15 D	4.175	99.42		0.74	99.46		1.571	99.87	
30 D	4.17	99.30		0.733	98.52		1.569	99.74	
45 D	4.166	99.26		0.73	98.11		1.566	99.55	
60 D	4.159	99.22		0.726	97.58		1.566	99.55	

In order to assess the real-time application of the developed XRPD method it has been adopted in the determination of the crystalline content of drug formulation of unknown compositions initially and kept at 40 °C, 75 % RH stability conditions. The results are evaluated from their respective calibration models. The results obtained are tabulated in table 3. From the results obtained we conclude that the proposed method can be applied easily to analyze a large number of samples to determine crystallinity content of drug product.

CONCLUSION

X-ray powder diffraction technique is a very promising technique to identify, characterize and assess percentage crystallinity of the active pharmaceutical ingredient in tablet formulations. The developed XRPD method was able to measure the crystallinity content accurately. Regression statistics and ANOVA calculations proved the statistical reliability of the method.

CONFLICT OF INTERESTS

Authors declare no conflict of interest.

REFERENCES

1. Spaulding A, Rutherford GW, Siegfried N. Tenofoviror, Zidovudine in three-drug combination therapy with one nucleoside reverse transcriptase inhibitor and one non-nucleoside reverse transcriptase inhibitor for the initial treatment of HIV infection in antiretroviral-naïve individuals. Cochrane Database Systematic Rev 2010. Doi:10.1002/ 14651858. CD008740. [Article in Press]
2. Yuen GJ, Weller S, Pakes GE. A review of the pharmacokinetics of abacavir. Clin Pharmacokinet 2008;47:351-71.
3. Fox Z, Dragsted UB, Gerstoft J. A randomized trial to evaluate continuation versus discontinuation of lamivudine in individuals failing a lamivudine-containing regimen: the COLATE trial. Antiviral Ther 2006;11:761-70.
4. Schrive I, Plasse JC. Quantification of zidovudine and one of its metabolites in plasma and urine by solid-phase extraction and high-performance liquid chromatography. J Chromatogr B: Biomed Sci Appl 1994;657:233-7.
5. Jozwiakowski MJ, Nguye NAT, Sisco JM, Spancake CW. Solubility behavior of lamivudine crystal forms in recrystallization solvents. J Pharm Sci 1996;85:193-9.
6. Harris RK, Yeung RR, Lamont RB, Lancaster RW, Lynn SM, Staniforth SE. 'Polymorphism' in a novel anti-viral agent: lamivudine. J Chem Soc Perkin Trans 1997;2:2653-4.
7. Singh GP, Srivastava D, Saini MB, Upadhyay PR. A novel crystalline form of lamivudine. WO; 2007.
8. Renu Chadha, Poonam Arora, Swati Bhandari. Polymorphic forms of lamivudine: characterization, estimation of transition temperature, and stability studies by thermodynamic and spectroscopic studies. ISRN Thermodyn 2012;2:62-70.
9. Savaser A, Goraler S, Tasoz A, Uslu B, Lingeman H, Ozkan SA. Simultaneous RP-LC determination of abacavir sulphate, lamivudine and zidovudine in pharmaceutical tablets, human serum and in drug dissolution studies. Chromatographia 2007;65:259-66.