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Extractive Spectrophotometric Methods for the Determination of Emtricitabine in Dosage Form Using *Safranin O*

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Abstract: Two simple, rapid, accurate, precise, cost effectiveandreproducible UV spectroscopic methods have been developed for the simultaneous estimation of Emitricitabine (EM) in bulk and tablet dosage form. These methods are based on the formation of Safranin O ion-association complexes maximum at 265 nm. Reaction conditions were optimized to obtain the maximum colour intensity. The first method is based upon the simultaneous equation and second upon the determination of Q value. Emitricitabine (EM) has absorption maxima at 263 nm. Beer's law obeyed in concentration range of 5-30 µg/ml for Emitricitabine (EM). The method of Q analysis is based on measurement of absorptivity at 229.1 nm and at isobestic point 244.2 nm. The recovery studies from tablet are indicative of accuracy of method and are found in between 99.97-101.18 % at three different levels of standard additions. Precision studies showed satisfactory results.

Key words: Emitricitabine · UV Spectroscopy · Simultaneous equation · Q analysis

INTRODUCTION

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTIs). Chemically it is 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. Emtricitabine is the (-) enantiomer of thio analog of cytidine which differs from other cytidine analogs, in that it has a fluorine in 5th position. FTC is an antiviral agent used for the prevention of perinatal HIV-1 reverse transcriptase [1]. Extensive literature survey revealed that only LC/MS/MS and RP-HPLC can be used for estimation of Emtricitabine [2, 3]. Emtricitabine in plasma by HPLC with fluorometric detection, stability indicating LC has been so far reported [4-9]. Author of the article and his research team has developed a UV Method development different pharmaceutical dosage form [10-24] using *Safranin O* [25]. To the best of our knowledge, there is no reported Spectrophotometric or pharmacopoeia method for simultaneous determination of Emitricitabine using Ferric Chloride in pharmaceutical formulations. The aim of the present work is to find out a simple, specific, sensitive, spectrophotometer method developed for the detection of Emitricitabine (EM) in pure form and in pharmaceutical formulation. The methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of Emitricitabine in bulk and dosage form.

MATERIALS AND METHODS

Instrumentation and Stock Solution: Spectrophotoscopic studies were carried out using Shimadzu UV Visible spectrophotometer, model-1700 (Japan) with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used. The commercially available tablets, Emitricitabine (EM) was procured from local market. Chloroform and Methanol (HPLC) grade, S.D. Fine Tablet formulation containing 200 mg of Emitricitabine (EM). The solubility of the EM was studied as per I.P. using many polar and non-polar solvents. It was found that both the drugs were freely soluble in ortho phosphate buffer pH 3.5. Thus ortho phosphate buffer was selected for as the solvent of choice further studies. Fifty (50) mg of Safranin O in 100 ml of distilled water and Emtricitabine were weighed separately (200 mg) and dissolved in buffer and made up to 1000ml in volumetric flasks to get a concentration of 2000µg/ml. The standard stock solutions of EM were further diluted separately to get a concentration of 20µg/ml. The absorbance of the solutions were screened in the UV region and found that EM showed maximum absorbance at 288 nm. The stability of Emitricitabine in ether solution was studied by the UV method. Sample solutions were prepared in triplicate and stored at 10 to 32°C for 2, 6, 9and24 hours. The stability of these

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solutions was studied by performing the experiment. The standard stock solution of EM was diluted to get a concentration ranging 5-40 µg/ml. The absorbencies of the resulting solutions were measured at 263 nm. It was found that the EM showed good linearity at concentrations ranging 5-40 µg/ml.

Method-I Simultaneous Equation Method [26]: Simultaneous equation method of analysis is based on the absorption of drugs EM at the wavelength maximum of the each other. Two wavelengths were selected for the developments of the simultaneous equations were 255 nm and 270 nm, λ max of EM, respectively. The absorptivity values E (1%, 1cm) determined for Esomeprazole at 255 nm and 270 nm were. These values were means of six estimations. The absorbencies and absorptivity at these wavelengths were substituted in following equations to obtain the concentration of both drugs. The two equations were constructed based upon the fact that at $ë_1$ and $ë_2$ the absorbance of the mixture is the sum of individual absorbencies of EM.

At λ1, A1=ax1bcx + ay1bcy…… (1)
At λ2, A2=ax2bcx + ay2bcy…… (2)

Where, A1 and A2 are absorbances of mixture at 255 nm and 270 nm, respectively.

Method II Absorbance Ratio Method [26]: The overlain spectrum of the two candidate drugs was obtained. The overlain spectrum showed Isobestic point at 244.2 nm. The two wavelengths were selected one as 244.2 nm (Isobestic point). The serial dilutions were prepared and absorbencies were measured and absorptivity for the both the drugs at selected wavelengths were also calculated. The Q value is used for the estimation of concentrations of drugs in sample solutions.

Assay Procedure for Tablet Formulation: The absorbance of prepared sample solution was determined at 301 nm for the estimation of EM. The 5 ml of remaining sample stock solution was diluted to 10 ml to get 1500µg/ml of EM. From the above solution 1ml is transferred to 10ml volumetric flask and treated in same manner as given for working standard of EM and absorbance was noted at 231 nm. The concentrations of the drugs were calculated by equation of standard curve method and double point standardization.

Method Validation: Validation of the analytical method for the determination of Emitricitabine (EM) in pure form and in pharmaceutical formulation was carried out as per ICH guidelines [27].Linearity of the method was validated according to ICH Q2B guidelines (ICH, 1996) for validation of analytical procedures in order to determine the linearity, sensitivity, precision and of the analyte. For Emitricitabine, five point calibration curves were generated with the appropriate volumes of the working standard solutions for UV methods. The linearity was evaluated by the least-square regression method using unweighted data. Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision were determined with standard quality control samples (in addition to calibration standards) prepared in triplicate at different concentration levels covering the entire linearity range. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on three different days and the results are documented as the standard deviation and RSD %. Accuracy is the percent of analyte re-covered by assay from a known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained. The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were determined based on the standard deviation of the response and the slope of the corresponding curve using the following equations: LOD = 3.3 s/m; LOQ = 10 s/m Where s, the noise of estimate, is the standard deviation of the absorbance of the sample and m is the slope of the related calibrations graphs. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability.

RESULTS AND DISCUSSION

The proposed methods can be successfully applied for Emitricitabine assay in tablet dosage forms without any interference. This method utilizes the active analogue principle that lies at the spectroscopic method [10-24]. The assay showed the drug content of this product to be in accordance with the labelled claim 200 mg. The values of LOD and LOQ are given in Table 3. The stability of Emitricitabine in methanol solution was studied by the UV method. Sample solutions were pre-pared in triplicate and stored at 10 and 32°C for 24 hrs. The stability of these solutions was studied by performing the experiment.

Table 1: Optical Characteristics Data for Method

	Method I		Method II	
Parameters / Working λ	255 nm	270 nm	244.2 nm	311 nm
Beer's law limit (µg/ml)	5-40	5-40	5-40	5-40
Absorptive E (1%,1cm)*	256	288	277	293
Molar absorptivity (l/mol.cm)*	2155	3654	4763	5378
Correlation coefficient*	0.9991	0.9993	0.9987	0.9990
Intercept*	0.324	0.437	0.164	0.472
Slope*	0.462	0.315	0.0531	0.0943

*Average of six estimation Method-I (Simultaneous equation method), Method II (Absorbance ratio method)

Table 2: Analysis Data of Tablet Formulation, Statistical Validation and Recovery Studies

			Amount found*					Amt. Added		
Method	Drug	Lab. Claim (mg/tab)	mg/tab.	%	S.D.*	% COV	S.E*.	At (%)	mg/ml	% Rec.#
I	EM	200	200.14	100.08	0.652	0.0564	0.162	80	200.03	100.02
								100	200.34	100.08
								120	200.17	101.03
II	EM	200	199.97	99.96	0.211	0.639	0.310	80	199.60	99.99
								100	200.50	100.05
								120	200.53	101.16

EM- Emitricitabine, Method-I (Simultaneous equation method), Method II (Absorbance ratio method) S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation, # Average of three estimation at each level of recovery

Table 3: Validation Parameters

				Precision (% COV)			
					Inter day*		
Method	Drug	LOD*µg/ml	LOQ*µg/ml	Intraday n=6	First day	Second day	Third day
I	EM	2.436	0.5723	1.7432	0.7653	0.64270	0.8765
II	EM	3.544	0.4761	2.5543	0.9865	0.75432	0.6332

EM- Emitricitabine, Method-I (Simultaneous equation method), Method II (Absorbance ratio method), S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation

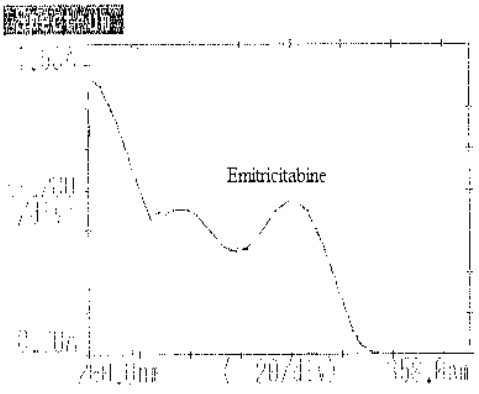

Fig. 1: Overlain spectra Emitricitabine

The stability of Emitricitabine in methanol solution was evaluated to verify whether any spontaneous degradation occurs, when the samples were prepared. The stability profile for 24 hrs was studied. The results were expressed as a percentage of the drug remaining. The obtained data showed that the sample solutions were stable up to 24hrs. The recoveries of EM from the standard mixture solution were found to be 101.24% and 99.92% respectively. The recovery results indicated that EM could be quantified by this procedure simultaneously. The absorptivity were found approximately same for all the concentrations hence both drugs obeyed Beer's law in indicated concentration range. The high values of correlation coefficients (r^2) also indicate good linearity of calibration curve for both the drugs. The spectra of EM exhibit λ max of 263 nm respectively. Additionally one Isobestic point was observed at 244.2 nm. These wavelengths were selected for simultaneous estimation and Q analysis of EM and are assumed to be sensitive wavelengths. Standard calibration curves for EM were linear with correlation coefficient of 0.9987- 0.9996 at all selected wavelengths. The accuracy of the method was confirmed by recovery studies from tablet at three different levels of standard additions, recovery in the range of 99.97-101.18 % justifies

the accuracy of method. To ascertain accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120 %) in Table 2.

CONCLUSION

The literature review encompasses the literature reports on various analytical methods of Emitricitabine estimation useful in the study. The corroborating experimental values suggest the bulk sample drug obtained is pure. The solubility study of Emitricitabine suggests that the drug is highly soluble in polar solvents, moderately soluble in alkaline borate buffer pH 8.3andleast soluble in water. The analytical method developed using UV spectrophotometer is linear and LOQ is 5 µg /ml and the inter-day and intra-day variation is minimum. Therefore it could conclude that the proposed investigation is a novel work and the investigation would help in estimation of drug candidate spectrophotometrically in the bulk.

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