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Determination of Impurities in Formulated Form of Entacapone by using RP - HPLC Method

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ABSTRACT

Reverse Phase High performance Liquid Chromatography was developed for separation and quantification of entacapone from 1-[2-Cyano-3-(3,4-dihydroxy-5-nitropheyl-1-oxo-2-propenyl piperidine (entacapone Stage IV), (2Z)-2-Cyano-3-(3,4-Dihydroxy-5-Nitrophenyl)-N,N-Diethylpropenamide (entacapone Z- form) impurities. The proposed method meets all the requirements of validation and found to be specific, precise, linear, accurate, rugged and robust. Therefore the method can be used as a routine quality control method for quantitative estimation of Stage IV impurity and Z-isomer impurity in entacapone formulated form.

Key words: RP - HPLC, Entacapone, Stage IV and Z- form impurities, Validation, recoveries.

INTRODUCTION

Pharmaceutical product comprises of various ingredients other than the required target compound. The presence of the unwanted impurities even in small amounts can influence the efficacy and safety of the products. To establish a drug for biological safe the purity requirements of the pharmaceutical product in Active Pharmaceutical Ingredient's (API's) is required [1]. Therefore, the impurity profile an essential. Impurity profile includes identification, quantification and validation of the impurities [2]. High Performance Liquid Chromatography is used as an analytical tool used for developing the separation methods and for determining the impurities in drugs [3-6]. Current work is related to the validation of impurities in entacapone tablet by RP-HPLC method.

Entacapone is an orally active nitrocatechol derivative of antiparkinsonian drug [7-9]. It has a selective and reversible inhibitory effect on catechol-O-methyl transferase (COMT) enzyme [10-12]. It exists in two stereoisomeric forms: the (E) = trans-isomer and the (Z) = cis-isomer. The (E)-isomer was originally chosen because of a more favourable synthetic route. It has been used throughout the clinical and toxicological programme and the amount of (Z)-isomer has been controlled to be less than 0.5%. Both isomers are pharmacologically active as COMT-inhibitor and have an equivalent activity. Entacapone is rapidly absorbed from the gastro-intestinal tract

and undergoes extensive first pass metabolism. Entacapone is converted to its (*cis*)-isomer, (*Z*)-entacapone, the main metabolite in plasma, followed by direct glucuronidation to inactive glucuronide conjugates. Four metabolites have been observed. Elimination is mainly via faeces (80 to 90%) and the remainder in urine as glucuronide conjugates and (*Z*)-isomer.

HPLC method was used for determination of entacapone and (*Z*)-isomer from formulation [9, 13], human plasma[14] and urine [15] were proposed.

Present work emphasis on a method to quantify entacapone and the associated impurities like Stage IV and Z-isomer from the formulation simulteneously.

MATERIALS AND METHODS

Chemicals and Reagents

Entacapone standard, 1-[2-Cyano-3-(3,4-dihydroxy-5-nitropheyl-1-oxo-2-propenyl piperidine and (2Z)-2-Cyano-3-(3,4-Dihydroxy-5-Nitrophenyl)-N,N-Diethylpropenamide from M/s Sekhsaria Chemicals Limited - Watson. Ammonium acetate and O-phosphoric acid from Merck, Methanol (HPLC grade) from Burdick & Jackson.

Instrumentation

HPLC system (Shimadzu LC 2010C HT) with a quaternary gradient pump system and a fixed wavelength programmable UV/VIS detector and 'LC-solution Version 1.12' software.

A thermostated autosampler tray with cooling facility, a thermostated column oven compartment.

Chromatographic Conditions

Isocratic elution at a flow rate of 1.0 mL min^{-1} was employed on a Inertsil C8 (250 x 4.6mm, 5 micron) column maintained at 25°C $\pm$ 2°C with a Ammonium acetate buffer : Methanol (55:45 v/v, pH 3.0) as mobile phase. The UV detection wavelength was performed at 283 nm and 2 µL of sample was injected.

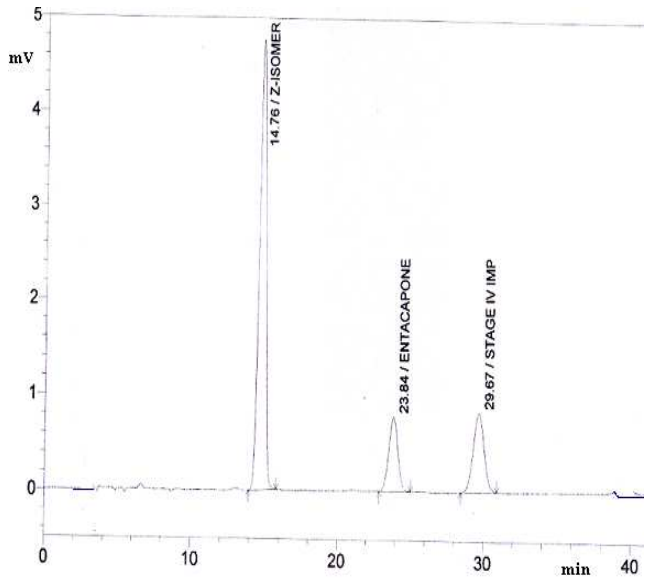

Figure 1. Typical Chromatogram of Z-isomer, Standard Entacapone and Stage IV impurity

Preparation of samples:
Entacapone Standard Solution: 0.5 ppm of Entacapone standard solution was prepared from 500 ppm stock solution of

Stage IV impurity solution: 100 ppm of Stage IV impurity was prepared from 1-[2-Cyano-3-(3,4-dihydroxy-5-nitropheyl-1-oxo-2-propenyl piperidine dissolved in methanol.

Z-isomer impurity solution: 500 ppm of Z-isomer impurity solution was prepared from 1-[2-Cyano-3-(3,4-dihydroxy-5-nitropheyl-1-oxo-2-propenyl piperidine dissolved in methanol.

Sample Preparation from Tablet: 100 mg of powdered tablet was dissolved in 200 ml methanol.
All working solutions were prepared by diluting with methanol and filtered through 0.45 micron membrane filter and degassed by sonication.

Experimental Procedure
HPLC column was equilibrated with the mobile phase. The experimental conditions were set as mentioned above. 20 µl of blank (methanol), entacapone standard solution, individual impurities and resolution solution with spiked impurities were injected. The responses of peak areas were recorded and integrated using software.

RESULTS AND DISCUSSION

Chromatogram
The stock solutions of entacapone, Stage IV impurity and Z-isomer impurity having concentrations of 500 ppm, 75 ppm and 500 ppm respectively were injected. From typical chromatogram (Figure 1) the separation of entacapone, Stage IV impurity and Z-isomer impurity was obtained. The retention times of Z-isomer, Stage IV impurity and entacapone impurity were found to be 14.76, 23.84, 29.67 minutes respectively.

A chromatogram showed a good separation of standard entacapone peak from the impurities (Z-isomer and Stage IV) peaks.

Validation of HPLC method
The proposed RP-HPLC method was validated as per ICH guidelines. Validation parameters such as specificity, Limit of detection (LOD), Limit of Quantification (LOQ), Precision, Accuracy, Ruggedness and Robustness were studied.

Limit of detection (LOD) and limit of quantitation (LOQ)
The LOD and LOQ were separately determined based on the calibration curves. The standard deviation of the y-intercepts and slope of the regression lines were used. The limit of detection (LOD) of stage IV impurity is 0.008%, Z-isomer impurity as 0.004% and entacapone is 0.004%.

Similarity, the limit of quantification (LOQ) of stage IV impurity is 0.02%, Z-isomer is 0.012% and entacapone as 0.012%.

Figure 1 indicated that the impurities can be detected.
Precision: In method precision, repeatability of sample analysis was verified in six replicates on a batch of entacapone tablets. The data (Table 1) indicated that the method is precise under the specified experimental conditions.

Table 1 Method Precision

	Z isomer	Stage IV
	0.0125	0.0184
	0.0126	0.0191
	0.0125	0.0182
	0.0125	0.0189
	0.0118	0.019
	0.0123	0.019
Mean	0.0123667	0.0187667
% RSD	2.17	1.81

The Intermediate Precision (ruggedness) study was done by the two analysts (Table 2).

Table 2 Intermediate Precision

	Z isomer	Stage IV
	0.0123	0.0184
	0.0125	0.0182
	0.0128	0.0182
	0.0125	0.0187
	0.0122	0.0182
	0.0124	0.0184
Mean	0.01245	0.01835
% RSD	1.5204574	0.982439

In the sample solution there are no known and unknown impurity peaks eluted. The results of repeatability and intermediate precision are within acceptance criteria. Hence method is rugged with respect to analyst to analyst and also system to system.

Accuracy : Recovery studies were performed by standard addition method at three levels i.e., 50%, 100% and 150%. Known amounts of standard z-isomer and Stage IV impurity were added to pre-analyzed samples and they were subjected to proposed HPLC method. Results of recovery studies are shown in (Table 3). The method is capable of quantifying impurities accurately from entacapone drug product over the range of LOQ to 50% of specification level.

Table 3. Accuracy

	% Recovery of impurities							
	LOQ		150%		100%		50%	
	Z isomer	Imp IV	Z isomer	Imp IV	Z isomer	Imp IV	Z isomer	Imp IV
%RSD	2.35	1.86	0.36	3.58	0.39	2.67	0.27	0.60

Robustness

To evaluate robustness of the method, the three test samples were analyzed for related substances of entacapone using chromatographic conditions recommended in test procedure except following method variables. The system suitability was evaluated for the each variable in chromatographic conditions.

The following parameters were altered (one change at a time was made). Different pH of the buffer used in mobile phase (pH 2.8 and 3.2), Column temperature (30°C), different flow rate of mobile phase (0.8 ml/min and 1.2 ml/min), different detection wavelength (281 nm and 285 nm), different mobile phase composition (buffer: methanol 55:43 and buffer: methanol 55:47). The summary of results is given in Table 4.

Table 4. Variation of Experimental Parameters

Validation Characteristics	Cumulative %RSD of results	
	Stage IV	Z isomer
Buffer pH 2.8	2.07	3.21
Buffer pH 3.2	1.28	4.71
Column Temperature 30°C	0.39	1.09
Wavelength 281 nm	1.52	0.96
Wavelength 285 nm	0.67	1.98
Flow rate 0.8 ml/min	0.17	0.81
Flow rate – 1.2 ml/min	0.58	2.64
Mobile Phase 55:43	0.6	1.46
Mobile Phase 55:47	0.63	0.42

The above data indicates that the impurities can be detected and quantified at the respective concentrations.

CONCLUSION

The results of analysis have been validated statistically and by recovery studies. It was used successfully for identification and quantification of the entacapone and formulated form of entacapone. The validated method confirms the accuracy of the method. Thus the method can be used for quantification of the stage IV and Z-isomer impurity.

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