

ESTIMATION OF ENTACAPONE TABLETS BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

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ABSTRACT

A simple and reproducible method was developed for the determination of entacapone using high performance liquid chromatography. The determination was carried out on Inertsil C8, 250 x 4.6mm, column using a mobile phase consisting of ammonium acetate Buffer (pH = 3.0) : Methanol (55:45 v/v) with flow rate of 1 ml/min at 283 nm. The linearity range was 0.06 -0.75 µg/ml for entacapone. The method was applied for quantification of entacapone from tablet with percent recoveries.

Keywords: HPLC, Entacapone

INTRODUCTION

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is an important technique used for drug analysis (Chitlange 2008; Jing Yao 2007; Murakami FS 2007; Shelar 2009). Entacapone is a nitrocatechol derivative. It is used as catechol-O-methyl transferase inhibitor for the treatment of Parkinson's disease (Schrag 2005). It was introduced as an antiparkinsonian drug for the first time in late 80's (Bäckström 1988).

Parkinson's disease (PD) is a neurodegenerative, slowly progressive disorder characterized by bradykinesia, resting tremor, rigidity and postural reflex impairment with associated characteristic eosinophilic cytoplasmic inclusions. Entacapone is indicated in combination with standard preparations of L-dopa/benserazide or L-dopa/carbidopa for use in patients with Parkinson's disease with end-of-dose motor fluctuations, who cannot be stabilized on L-dopa therapy (Lyytinen 2000). Entacapone is an orally active, nitrocatechol derivative with a selective and reversible inhibitory effect on catechol-O-methyl transferase (COMT) enzyme. The film-coated tablets are made with standard core and film-coat excipients. Entacapone is given by mouth in a dosage of 200 mg at the same time as each dose of levodopa with dopa-decarboxylase inhibitor. Entacapone may also be

given as a combination preparation with carbidopa and levodopa (Website SCIENTIFIC DISCUSSION).

Entacapone exists in two stereoisomeric forms: the (E) = trans-isomer and the (Z) = cis-isomer. The (E)-isomer (Pippuri 1992) 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^[9]. 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. A HPLC method for detection of entacapone was reported by T. Wikberg *et al* (Karlsson 1992; Wikberg 1993) and Ramakrishna *et al.* (Ramakrishna2005) from rats and humans. The quantification of Entacapone was carried out by Siddiqui *et al.* (Siddiqui 2005) and Sivasubramanian *et al* (Sivasubramanian 2009)¹ at 210 and 315 nm respectively.

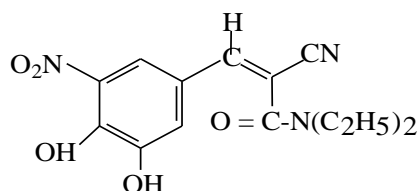
Current work presents a novel method to quantify entacapone from formulation.

GENERAL PROFILE of Entacapone

Chemical name : (2E)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethylpropanamide.

Empirical formula : C₁₄H₁₅N₃O₅

Structural Formula :



CAS Number : 130929-57-6

Solubility : Insoluble in water, soluble or sparingly soluble in acetone, and slightly soluble in anhydrous ethanol.

Mol. Weight : 305.29 Entacapone is “practically insoluble” in water.

MATERIALS AND METHODS

Entacapone standard 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.

Experimental Procedure:

Mobile phase preparation: First a buffer solution of pH =3 was prepared by dissolving 7.75 g of Ammonium Acetate in 1000 ml of distilled water and then pH of the solution was adjusted to 3.0 with O-phosphoric acid. Then mobile phase was prepared by mixing ammonium acetate buffer solution and Methanol in 55 : 45 (v /v) proportion.

Mobile phase and other working solutions were filtered through 0.45 micron membrane filter and degassed by sonication.

HPLC column was equilibrated with the mobile phase. Diluent methanol was injected as a blank. The Sample solution was injected to record the responses for the same. Standard preparation in six replicates were used to calculate the % RSD. 20 μ l of all the solutions were injected as per the method

and responses of peak areas were recorded and integrated using software.

Experimental Conditions :

Column	: Inertsil C8, 250 x 4.6mm, 5 micron.
Flow rate	: 1.0 ml/min
Detector	: UV 283 nm
Injection volume	: 20 μ l
Column Temperature	: Ambient (25°C \pm 2°C)
Mobile Phase	: Buffer pH 3.0 : Methanol (55:45)
Diluent	: Methanol
Run time	: 50 minutes

RESULTS AND DISCUSSION

a) Chromatography:

Standard stock solution of entacapone was prepared by weighing accurately 25 mg of entacapone standard in 50 ml standard volumetric flask. 30 ml of methanol was added and sonicated to dissolve the Entacapone powder. Finally the volume was made to 50 ml with Methanol. This is 500 ppm of Entacapone Stock solution. Further, 5 ppm of Entacapone solution was prepared by diluting 1 ml of the solution to 100 ml with methanol.

Working solutions were prepared by appropriate dilution of 5 ppm standard entacapone solution with methanol. Figure 1 includes the Chromatogram of Entacapone Standard.

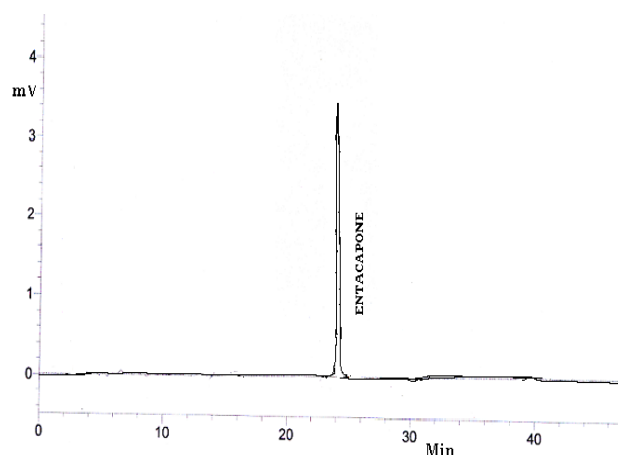


Fig. 1 Chromatogram of Entacapone Standard

From the chromatogram it was concluded that the Retention time for Entacapone was 24.2 ± 0.05 minutes (Fig. 1). There was no interference of diluents.

b) Quantification of entacapone:

Quantification of entacapone was carried out by

external standard calibration method. Calibration curve was constructed by plotting mean peak areas against the corresponding entacapone concentrations (Fig. 2).

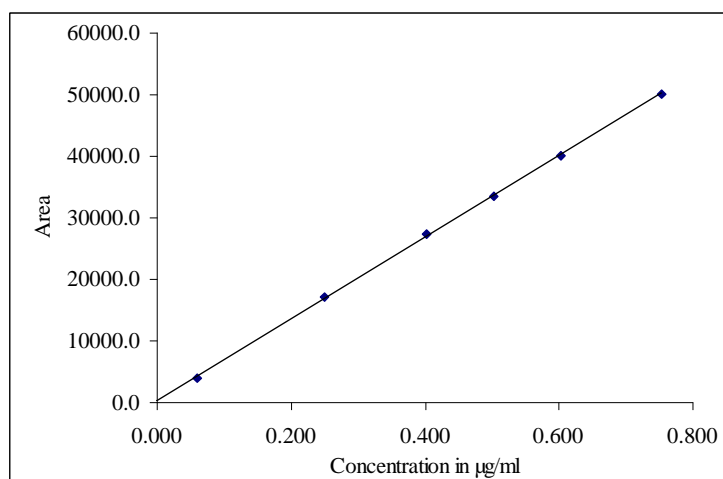


Fig. 2. Calibration plot for Linearity

Form linearity of curves, it was indicated that, entacapone can be detected and quantified at the respective concentrations. The linearity range was 0.06 -0.75 µg/ml for entacapone.

Table 1. Repeatability of entacapone standard

Injection No	RT	Area
1	24.60	30269
2	24.59	30174
3	24.59	30219
4	24.61	30167
5	24.64	30217
6	24.66	30046
Mean	24.62	30182.00
%RSD	0.12	0.25

Table 2. Comparison of the method with acceptance Criteria

	Acceptance Criteria RSD (%)	Experimental Values RSD (%)
Area of peak due to entacapone in standard solution	Not more than 5	0.25
Retention time of peak of entacapone standard	Not more than 2	0.12

From the results (Table 2) indicated that the method was in acceptance criteria

c) Limit of Detection (LOD) and Limit of Quantification (LOQ):

From 5 ppm solutions of entacapone lower concentrations of entacapone were prepared by appropriate dilutions. Each of these solutions and blank as diluent were injected. Signal to noise ratios was calculated. Based on the calculations for signal to noise ratio the concentration of entacapone as 0.004% (with S/N ratio as 4.3) were qualified as limit of detection.

The concentration of entacapone as 0.012% (with S/N ratio as 12.3) were qualified as limit of quantification. 0.012%.

d) Determination of Entacapone in Tablets Sample Preparation of entacapone Tablets:

20 tablets of entacapon each containing 200 mg of entacapone were powdered finely. The powdered tablet equivalent to 100 mg of entacapone was transferred into a 200 ml volumetric flask. Add about 120 ml of metanol and sonicate for about 15 min. Cool & dilute upto the mark with diluent and mix. Filter through 0.45µ nylon filter paper by using syringe. Analytical recovery studies were carried out from a series of sample dosage form (Table 3).

System Precision: Analyzing four replicates of fixed amount of entacapone enabled checking the precision and accuracy of the proposed method.

Table 3: Determination of entacapone from Tablet

Sample ID	Recovery from Sample (%)
EP1	99.8
EP2	100.8
EP3	101
EP4	100.5
Mean	100.53
% RSD	0.99

The method has shown good and consistent recoveries for entacapone in bulk drugs (99.8 –101 %). The percent RSD was observed as 0.99 % indicating the results are within the acceptance criteria.

CONCLUSIONS

The elution of entacapone standard was carried out on inertsil C18, 4.6 × 250 mm analytical column, at the flow rate of 1 ml/min isocratically using the mobile phase consisting of ammonium acetate Buffer (pH = 3.0) : Methanol (55:45 v/v) with flow rate of 1 ml/min at 283 nm.

The limit of detection (LOD) and limit of quantification (LOQ) of entacapone were found to be 0.004% and 0.012% respectively. The precision of the method was calculated in terms of the relative standard deviation (0.12 %) and percentage errors at 98% confidence limits indicated high precision and accuracy of the proposed method.

The method can be used successfully for identification and quantification of the active pharmaceutical ingredient, entacapone from pharmaceutical ingredient. Hence this method can be used for the routine analysis of entacapone in formulation.

Figures and Tables:

Figure 1. Chromatogram of entacapone Standard

Figure 2. Calibration plot for Linearity

Table 1. Repeatability of entacapone standard

Table 2. Comparison of the method with acceptance Criteria

Table 3. Determination of entacapone from Tablet

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