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RP-HPLC Estimation of Entacapone in Bulk and Dosage Form

***Lakshmi Sivasubramanian,K.S.Lakshmi and Ranga Rao Pathuri**

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM University, Kattankulathur – 603 203.

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ABSTRACT

A simple, efficient and reproducible method for the determination of entacapone in tablets has been developed using reverse phase high performance liquid chromatographic method. The elution was done using a mobile phase consisting of acetonitrile and methanol (50:50 v/v) pH adjusted to 2.75 with ortho-phosphoric acid on inertsil C18, 4.6 × 250 mm analytical column with flow rate of 1 ml/min with detection at 315 nm. An external standard calibration method was employed for quantitation. The elution time was 3.15 ± 0.02 min. The linearity was 2-12 µg/ml for entacapone.

Keywords: -HPLC, Entacapone, quantitative analysis

INTRODUCTION

Entacapone, 2-cyano-3 [3,4-dihydroxy-5-nitrophenyl]-N,N-diethyl-2-propenamide is used in the treatment of Parkinson's disease [1]. It blocks the COMT enzyme that helps more levodopa become available to the brain. When levodopa reaches the brain, it is converted in to dopamine. A survey of literature revealed a LC-MS [2] and HPLC methods [3,4] for its determination in human plasma and urine and a spectrophotometric method [5] for its estimation from dosage form. The present paper aims at reporting an isocratic RP-HPLC method for the determination of entacapone in tablets.

The apparatus used was Shimadzu HPLC – LC 10 VP series chromatograph equipped with dual pump and rheodyne injector with 20 µl external loop. The column used was inertsil C18, 4.6 × 250 mm analytical column, the elution was carried out isocratically at the flow rate of 1 ml/min using acetonitrile:methanol in the ratio 50:50 v/v, pH adjusted to 2.75 with ortho phosphoric acid as mobile phase. The detector was set at 315 nm. Responses of peak areas were recorded and integrated using software. Entacapone was obtained from M/s Intas Labs, Hyderabad. Acetonitrile and methanol used were of HPLC grade and obtained from S.D Fine Chemicals, Mumbai.

Standard stock solution of the drug was prepared by dissolving 25 mg of entacapone in mobile phase and made up to 25 ml with the same (1000 µg/ml). Working standard solution was prepared by diluting 0.5 ml of the stock solution to 25 ml with mobile phase (20µg/ml). The gradient dilutions were prepared by taking 1, 2, 3, 4, 5 and 6 ml of solutions and made up to 10 ml with the mobile phase. Twenty microlitres of the solution from each flask was injected two times. Calibration curve was constructed by plotting mean peak areas against the corresponding drug concentrations (Fig 1). The detector response was found to be linear in the concentration range of 2-12 µg/ml.

For the estimation of drugs from commercial formulations, 20 tablets of entacap (Sun Pharma, Hyderabad) each containing 200 mg of entacapone were powdered finely. A quantity equivalent to 25 mg was transferred in to a 25 ml volumetric flask and dissolved in 10 ml of methanol. The volume was then diluted with the mobile phase and filtered through Whatman No.1 filter paper. One milliliter of the resulting solution was then diluted to 50 ml with mobile phase.

From this 1, 2 and 3 ml samples were taken and their volume was made up to 10 ml each. Chromatogram of these solutions was obtained by injecting 20 µl of each sample in to the chromatographic system. There was no interference from diluents and lubricants. The retention time of the drug was 3.15 ± 0.02 min (Fig 2). Chromatographic parameters such as peak asymmetry (A_s) and capacity factor (k) were found to be 1.23 and 2.15 respectively. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.143 and 1.478 µg/ml respectively. Analytical recovery studies were carried out from a series of spiked concentrations added to the preanalysed dosage form (Table 1).

***Corresponding author.**
Lakshmi Sivasubramanian
Department of Pharmaceutical Analysis,
SRM College of Pharmacy, SRM University,
Kattankulathur – 603 203.

Table 1: Determination of Entacapone in Tablets

Drug Entacapone	Label Claim /tablet (mg)	Amount Found*(mg)	Recovery Studies Amount added (µg/ml)	Amount recovered (µg/ml)	Percentage recovery (%)
A	200	200.56	6	6.11	101.83
			8	8.21	102.62
			10	10.08	100.80

* *Mean of three determinations*

A – Entacap, Sun Pharma, Hyderabad

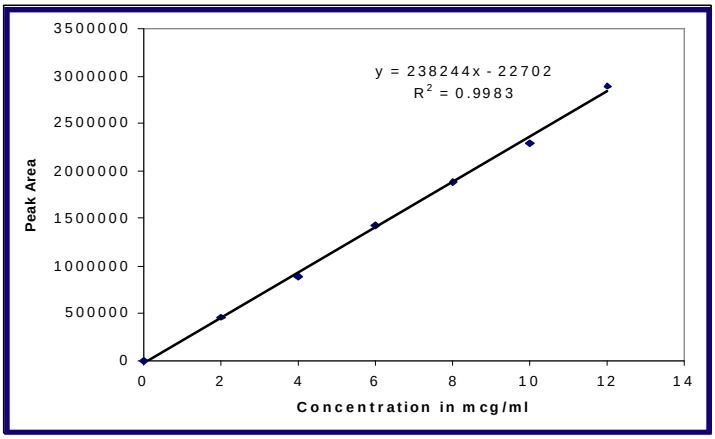

Fig 1: Linearity Curve of Entacapone

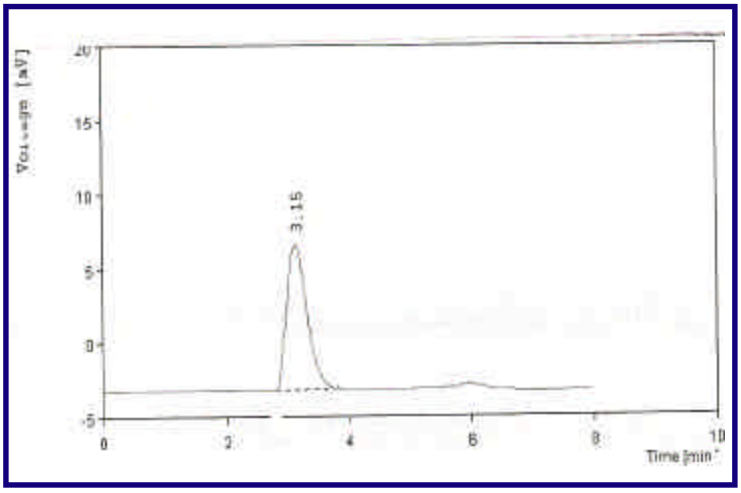

Fig 2: Chromatogram of Entacapone showing retention time at 3.15 min.

Analysing five replicates of fixed amount of entacapone enabled checking the precision and accuracy of the proposed method. The precision of the method was calculated in terms of the relative standard deviation (0.528%) and percentage errors at 95% confidence limits (0.78) indicated high precision and accuracy of the proposed method. Hence the present method can be used for the routine analysis of entacapone in formulation.

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