Advanced Pharmaceutical Bulletin, **2013**, 3(2), 409-413
doi: http://dx.doi.org/10.5681/apb.2013.065
http://apb.tbzmed.ac.ir/

A Novel Approach using Hydrotropic Solubalization Technique for Quantitative Estimation of Entacapone in Bulk Drug and Dosage Form

Ruchi Jain[1]*, Nilesh Jain[2], Deepak Kumar Jain[2], Surendra Kumar Jain[2]

[1] *Suresh Gyan Vihar University, Jaipur, Rajasthan, India-302025.*

[2] *Sagar Institute of Research & Technology-Pharmacy, Ayodhya Bypass Road Bhopal, Madhya Pradesh, India – 462041.*

ARTICLEINFO

Article Type:
Research Article

Article History:
Received: 14 March 2013
Revised: 6 May 2013
Accepted: 8 May 2013
ePublished: 20 August 2013

Keywords:
Entacapone
Urea
Ecofriendly
Hydrotropic solubilizing agents

ABSTRACT

Purpose: Analysis of drug utilized the organic solvent which are costlier, toxic and causing environment pollution. Hydrotropic solution may be a proper choice to preclude the use of organic solvents so that a simple, accurate, novel, safe and precise method has been developed for estimation of poorly water soluble drug Entacapone (Water Solubility-7.97e-^{02} g/l). ***Methods:*** Solubility of entacapone is increased by using 8M Urea as hydrotropic agent. There was more than 67 fold solubility enhanced in hydrotropic solution as compare with distilled water. The entacapone (ENT) shows the maximum absorbance at 378 nm. At this wavelength hydrotropic agent and other tablet excipients do not shows any significant interference in the spectrophotometric assay. ***Results:*** The developed method was found to be linear in the range of 4-20 µg/ml with correlation coefficient (r^2) of 0.9998. The mean percent label claims of tablets of ENT in tablet dosage form estimated by the proposed method were found to be 99.17±0.63. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. ***Conclusion:*** As hydrotropic agent used in the proposed method so this method is Ecofriendly and it can be used in routine quantitative analysis of drug in bulk drug and dosage form in industries.

Introduction

Entacapone (ENT) is chemically (E)-2-cyano-3-(3, 4-dihydroxy-5-nitrophenyl)-N, N-diethyl-2-propenamide (Figure 1), is a drug that functions as a catechol-O-methyl transferase (COMT) inhibitor, used in the treatment of Parkinson's disease. It is a member of the class of nitrocatechols.[1,2] The drug is not official in any pharmacopoeia. Literature survey revealed few HPLC methods[3,4] has been reported for the determination of ENT in biological fluids. The reported methods for the determination of ENT in tablets includes HPLC[5-11] and spectrophotometric methods.[12,13]

As the environmental pollution it is necessary to preclude the use of organic solvents for analysis of drug. Various techniques have been employed to enhance the aqueous solubility and hydrotropy is one of them. Hydrotropic solubilization is the phenomenon by which aqueous solubility of poorly water soluble drugs and insoluble drugs increases. Maheshwari and Jain et al has used sodium salicylate, sodium benzoate, urea, nicotinamide, sodium citrate and sodium acetate as the most common examples of hydrotropic agents utilized to increase the water solubility of drug.[14-19] Various organic solvents such as methanol, chloroform, dimethyl formamide and acetonitrile have been employed for solubilization of poorly water-soluble drugs to carry out spectrophotometric analysis. Drawbacks of organic solvents include their higher cost, toxicity and pollution. Hydrotropic solution may be a proper choice to preclude the use of organic solvents. Therefore, it was thought worthwhile to employ this hydrotropic solution to extract out the drug from fine powder of tablets to carry out spectrophotometric estimation. Present work emphasizes on the quantitative estimation of ENT in their dosage form by UV Spectroscopic methods.

Materials and Methods

Instrument

UV-Visible double beam spectrophotometer, Shimadzu model-1700 having spectral bandwidth 3 nm and of wavelength accuracy ±1 nm, with 1cm quartz cells was used.

Reagents and chemicals

Analytical pure sample of ENT was supplied as gift sample from Sun Pharmaceuticals Ind. Ltd. Urea obtained from Merck Chemical Division, Mumbai.

***Corresponding author:** Ruchi Jain, Research Scholar, Suresh Gyan Vihar University, Jagatpura Mahal, Jaipur, Rajasthan, India-302025.
Email: jainruchi02@gmail.com

Reverse Osmosis (R.O.) Water was used throughout the study.

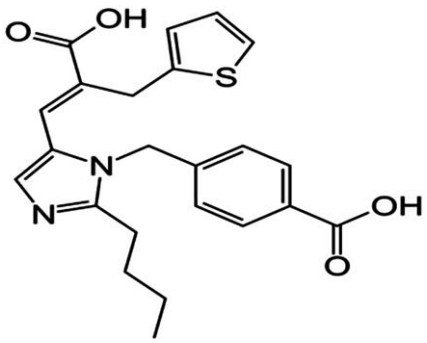

Figure 1. Chemical structure of ENT

Preliminary solubility studies of drugs

An excess amount of drug was added to a screw capped 25 ml of volumetric flask containing different aqueous systems viz. distilled water, different combination of hydrotropic agent. The volumetric flasks were shaken mechanically for 12 hrs at 25±1°C in a mechanical shaker. These solutions were allowed to equilibrate for next 24 hrs and then centrifuged for 5 min at 2000 rpm. The supernatant liquid was taken for appropriate dilution after filtered through whatman filter paper no.41 and analyzed spectrophotometrically against corresponding solvent blank. After analysis, it was found that the enhancement in the solubility of ENT was to be more than and 67 folds in 8 M Urea as compared to solubility studies in other solvents.

Selection of hydrotropic agent

ENT was scanned in hydrotropic agent in the spectrum mode over the UV range (200-400) and 8 M Urea as hydrotropic agent were found to be most appropriate because:

- ENT is soluble in it (67 fold enhancement of solubility)
- ENT is stable in hydrotropic agent (as shown in Figure 2)
- ENT exhibit good spectral characteristics in it.
- Urea solution has no interference with the λ_{max} of ENT i.e 378nm.

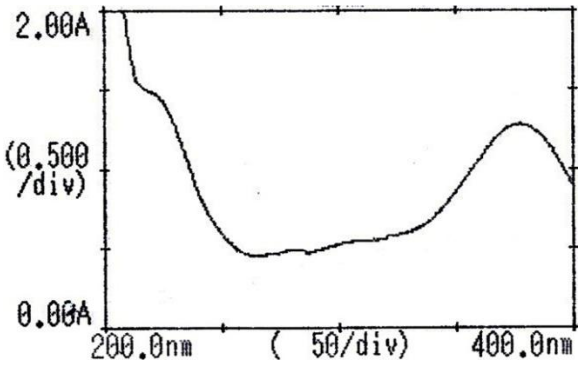

Figure 2. Spectra of ENT in 8 M Urea as Hydrotropic Agent

Establishment of stability profile

Stability of ENT was observed by dissolving in 8 M Urea as hydrotropic agent. Solution of ENT was prepared in the conc. of 12 µg/ml and scanned under time scan for 30 min. Spectra of drug under time scan shows that drug are stable in hydrotropic solution.

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A)

Accurately weighed 100 mg of the ENT was transferred in to 100 ml volumetric flask containing 80 ml of hydrotropic agent and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with mixed hydrotropic agent to get a concentration of 1000 µg/ml (Stock-A).

Preparation of Working Standard Solution

The standard solution (1000 µg/ml) was further diluted with distilled water to obtain 4, 8, 12, 16 and 20µg /ml solution and absorbance were noted at 378 nm against distilled water as blank.

Analysis of Marketed Formulation

Marketed formulation Entacom (Intas Pharmaceuticals) was selected for tablet analysis, i. e containing 200 mg ENT. Twenty tablets were accurately weighed, average weight determined and ground to fine powder. An accurately weighed quantity of powder equivalent to 100 mg of ENT was transferred into 100 ml volumetric flask containing 80 ml of hydrotropic solution. The flask was sonicated for about 20 min to solublize the drug; volume was adjusted to mark with hydrotropic agent and filtered through whatman filter paper no. 41. The Absorbance of sample solutions was analyzed on UV spectrophotometer at 378 nm against R.O. water as blank.

Validation Parameters

The developed method was validated as per ICH guidelines (Linearity, Accuracy, Precision and Robustness).[20]

Linearity

Linearity of ENT was established by response ratios of drug. Response ratio of drug was calculated by dividing the absorbance with respective concentration.

Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. In preanalyzed tablet solution, a definite amount of drug was added and then its recovery was studied. These studies were performed in by adding fixed amount of pure drug solution to the final dilution while varying the concentration of tablet sample solution in the final dilution.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility.

Repeatability was performed by analyzing same 5 concentrations of drug for 5 times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week.

Reproducibility was performed by analyzing same concentration of drugs for five times in different lab.

Results and Discussions

Based on the solubility, stability and spectral characteristics of the drug, 8M Urea was selected as hydrotropic agent. There was more than 67 fold solubility enhanced in hydrotropic solution as compare with distilled water. After solubilizing the Entacapone in selected hydrotropic agent, it was scanned in spectrum mode and the working wavelength for the estimation, considering the reproducibility and variability was found to be 378 nm. Spectra of ENT is shown in Figure 2, Calibration curve was plotted between concentrations versus absorbance Figure 3. Observation of linearity data has reported in the Table 1. The Result of their optical characteristics has been reported in Table 2.The developed method was found to be linear in the range of 4-20 µg/ml with linear equation was Y=0.098X + 0.011 and correlation coefficient (r^2) of 0.9998. Drug content of tablet formulation was calculated using calibration curve and values are reported in Table 3. The mean percent label claims of tablets of ENT in formulation-I estimated by the proposed method were found to be 99.17±0.63. These values are close to 100, indicating the accuracy of the proposed analytical method.

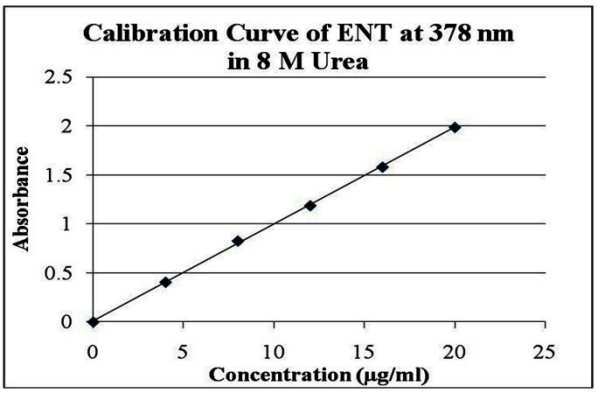

Figure 3. Calibration Curve of ENT at 378 nm in 8 M Urea

Table 1. Linearity ENT at λ_{max} =378 nm in 8 M Urea

Standard Conc. (µg/ml)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean
0	0	0	0	0	0	0
4	0.403	0.408	0.41	0.411	0.401	0.4066
8	0.821	0.823	0.812	0.841	0.842	0.8278
12	1.182	1.193	1.195	1.184	1.185	1.1878
16	1.586	1.593	1.554	1.579	1.599	1.5822
20	1.976	1.996	1.99	1.986	1.988	1.9872
Correlation Coefficient (r^2)	-	-	-	-	-	0.9998
Slope (m)	-	-	-	-	-	0.0987
Intercept (c)	-	-	-	-	-	0.0113

Table 2. Optical Characteristic and Linearity Data of ENT in 8 M Urea

S. No.	Parameter	8 M Urea as Hydrotropic Agent
1	Working λ	378 nm
2	Beer's law limit (µg/ml)	4-20
3	Correlation Coefficient (r^2)*	0.9998
4	Slope (m)*	0.098
5	Intercept (c)*	0.011
6	Number of samples (n)	25

*Average of 5 determination of 5 concentrations

Result of Validation Parameters

Linearity

Linearity was established in the range of 4-20µg/ml and it was reported as response ratio; Table 4. Then a graph was plotted between concentration and response ratio (Figure 4) which assure the linearity of the method.

Accuracy

The values of mean percentage recoveries were also found to show variability in ranging from 97.83±1.03 to 98.92±0.82%. Low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method (Table 5)**.** All these values were very close to 100.

Table 3. Results and Statistical Parameters for Entacom 200 mg Tablet Analysis using 8M Urea

Drug	Label Claim (mg)	Amount Found (mg)	% MEAN*	S.D.*	%COV*	Std. Error*
Entacom200	200	198.67	99.33	0.26	0.262	0.048
Entacom200	200	198.43	99.21	0.73	0.736	0.133
Entacom200	200	197.93	98.96	0.89	0.899	0.163
Mean	-	**198.34**	**99.17**	**0.63**	**0.632**	**0.115**

*Average of five in 3 replicates determination

Table 4. Response Ratio of ENT in Hydrotropic Solution

S. No.	8 M Urea as Hydrotropic Agent		
	Conc. (µg/ml)	ABS	Response Ratio
1.	4	0.402	0.10
2.	8	0.81	0.10
3.	12	1.194	0.10
4.	16	1.584	0.10
5.	20	1.99	0.10

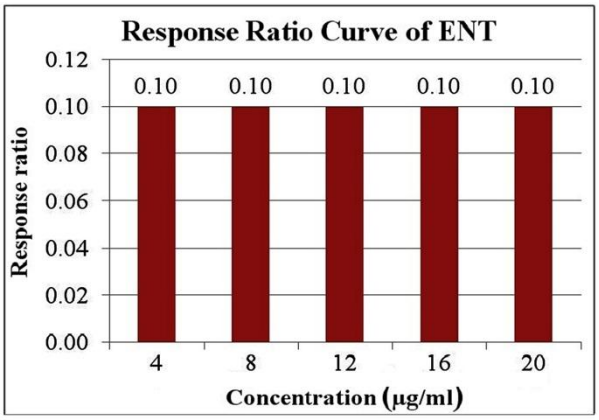

Figure 4. Response Ratio Curve of ENT in 8 M Urea

Table 5. Result of Recovery Studies of Tablet Formulation with Statically Evaluation

Drug	QC Conc. (µg/ml)	Recovery Level % (Amount Drug Added)	Amount of Drug Found (Mean±SD)*	% RSD
ENT	**10**	**80**	98.47±1.23	0.332
-	-	**100**	98.39±1.08	0.447
-	-	**120**	97.83±1.03	0.40
ENT	**12**	**80**	98.86±0.63	0.90
-	-	**100**	98.92±0.82	0.30
-	-	**120**	98.29±0.74	0.63

*Average of five determination

Precision

Result of precision at different level were found be within acceptable limits (RSD<2). The results have been reported in Table 6. Presence of hydrotropic agent do not shows any significant interference in the spectrophotometric assay thus further confirming the applicability and reproducibility of the developed method.

Table 6. Result of Precision of ENT

-	Validation Parameter	Percentage Mean ± S.D*. (n=6)	Percentage RSD
With 8 M Urea as Hydrotropic Agent	Repeatability	98.64±1.31	1.33
	Intermediate Precision	-	-
-	Day to Day	98.92±1.42	1.435
-	Analyst to Analyst	98.74±0.86	0.870
-	Reproducibility	98.39±0.70	0.711
-	-	-	-

* Mean of fifteen determinations (3 replicates at 5 concentrations level)

Conclusion

Hence, it is concluded that the proposed methods are new, simple, cost effective, accurate, safe and precise and can be successfully employed in the routine analysis of Entacapone in bulk drug sample and tablet dosage form. Advantage of these methods is that the organic solvent is not essential for the analysis and there was no interference of 8 M urea during the estimation. There is a good scope for other poorly water-soluble drugs which may be tried to get solubilized in 2 M urea solution (as hydrotropic agent) to carry out their spectrophotometric analysis excluding the use of costlier and unsafe organic solvents.

Conflict of Interest

The authors report no conflicts of interest.

References

1. Sweetman SC. Martindale-the complete drug reference. 32nd ed. London: The Pharmaceutical Press; 1999.
2. O'Neil MJ, Smith A, Heckelman PE, Obenchain JR, Gallipeau JR, D'Arecca MA. The Merck Index : An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th ed. Whitehouse Station, NJ: Merck Research Laboratories; 2001.
3. Karlsson M, Wikberg T. Liquid chromatographic determination of a new catechol-O-methyltransferase inhibitor, entacapone, and its Z-isomer in human plasma and urine. *J Pharm Biomed Anal* 1992;10(8):593-600.
4. Bugamelli F, Marcheselli C, Barba E, Raggi MA. Determination of L-dopa, carbidopa, 3-O-methyldopa and entacapone in human plasma by HPLC-ED. *J Pharm Biomed Anal* 2011;54(3):562-7.
5. Sivasubramanian L, Lakshmi KS, Pathuri RR. RP-HPLC estimation of entacapone in bulk and dosage form. *J Pharm Res* 2009;2:1850-1.
6. Zaveri M, Dhru B, Khandhar A. Simultaneous estimation of levodopa, carbidopa and entacapone in pharmaceutical dosage by validated reverse phase high performance liquid chromatography. *Int J Inst Pharm Life Sci* 2012;2:10-9.
7. Ramakrishna NV, Vishwottam KN, Wishu S, Koteshwara M, Chidambara J. High-performance liquid chromatography method for the quantification of entacapone in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005;823(2):189-94.
8. Sarsambi PS, Gowrisankar D. Reverse phase HPLC method for the analysis of entacapone in pharmaceutical dosage forms. *Pharm Rev* 2009;7(41):111-2.
9. Issa YM, Hassoun MM, Zayed AG. Application of high performance liquid chromatography method for the determination of levodopa, carbidopa and entacapone in tablet dosage forms. *J Liq Chromatogr Related Technol* 2011;34:2433-47.
10. Tekale P, Mhatre VS, Pai NR, Maurya C, Tekale S. Estimation of entacapone tablets by reverse phase high performance liquid chromatographic method. *J Biosci Discov* 2011;2(3):294-8.
11. Mohamed NG, Mohamed MS. Determination of antiparkinsonism drug entacapone. *J Chil Chem Soc* 2010;55:85-9.
12. Koradia SK, Agola AS, Jivani NP, Manek RA, Pandey S. Development and validation of derivative spectrophotometric method for determination of entacapone in pharmaceutical formulation. *Int J Pharm Res Dev* 2009;1:1-8.
13. Shah J, Banerjee SK, Chhabra GS. UV Spectrophotometric method development and validation for entacapone in bulk and formulation. *J Bull Pharm Res* 2011;1:7-9.
14. Maheshwari RK. A novel application of hydrotropic solubilization in the analysis of bulk samples of ketoprofen and salicylic acid. *Asian J Chem* 2006;18:393-6.
15. Maheshwari RK. Analysis of frusemide by application of hydrotropic solubilization phenomenon. *Indian Pharmacist* 2005;4:55-8.
16. Jain N, Jain R, Thakur N, Gupta BP, Banweer J, Jain S. Novel spectrophotometric quantitative estimation of torsemide in tablets using mixed hydrotropic agent. *Der Pharmacia Lettre* 2010;2(3):249-54.
17. Jain N, Jain R, Thakur N, Gupta BP, Banweer J, Jain S. Novel spectrophotometric quantitative estimation of Hydrochlorothiazide in bulk drug and their dosage forms by using hydrotropic agent. *Int J Appl Pharm* 2010;2:11-4.
18. Jain N, Jain R, Kulkarni S, Jain DK, Jain S. Ecofriendly spectrophotometric method development and their validation for quantitative estimation of Pramipexole Dihyrochloride using mixed hydrotropic agent. *J Chem Pharm Res* 2010;3:548-52.
19. Jain N, Jain R, Jain D, Jain A. Spectrophotometric quantitative estimation of amlodipine besylate in bulk drug and their dosage forms by using hydrotropic agent. *Eur J Chem* 2010;5:212-7.
20. International Conference on Harmonization. ICH, Validation of Analytical Procedures: Text and Methodology (Q2(R1)), IFPMA, Geneva. 2005.