Volume : 2 | Issue : 11 | Nov 2013 • ISSN No 2277 - 8160

Research Paper Chemistry

Analytical Method Validation of Stability-Indicating HPLC Method for Determination of Assay of Carbamazepine CR Tablets

Ajay Kumar Gupta Department of Chemistry, Shri JJTU University, Rajasthan, India.

P. K. Patel Department of Chemistry, M. G. Sciences Institute, Ahmedabad, Gujarat, India.

ABSTRACT

This paper deals with the development and validation of stability indicating high performance liquid chromatographic method for the quantitative determination of Carbamazepine CR Tablets form. Study of an analytical method for the determination of Carbamazepine drug and also validate the method as per the ICH guideline and required acceptance criteria. The method was developed by using Hypersil ODS V column (250×4.6 mm, 5 µm particle sizes) containing mobile phase Water: Methanol: Methylene chloride in ratio (30: 30: 3) total volume 630 ml. The flow rate was set at 1.5 mL/minute and the injection volume was 20µL. The run time of injection is near about 25 min. In this method use Shimadzu HPLC with UV Detector at 230 nm . The linearity of method was 100.50 to 301.50 µg/ml, the correlation coefficient was found to be 0.9998. The liquid chromatographic method was validated with respect to specificity, precision (% RSD about 0.28%), Stability an analytical solution (% deviation from initial area count found in limit ±2%), accuracy, ruggedness and robustness are uses parameter.

KEYWORDS : Carbamazepine Working Standard and sample. HPLC With UV detector.

Introduction

Carbamazepine belongs to a class of medication Anticonvulsant [1]. Which are primarily used to treat Seizures in certain type of Epilepsy. It is also used to relieve one type of nerve pain (trigeminal neuralgia). Seizures can be classified according to several different criteria and the specific symptom, reflect the affected part of the cerebral hemisphere in which the seizure originates [2]. seizure are the second most common neurological disorder after stroke that occurs in humans [3]. It is also used to treat bipolar disorder [4].Seizure any interruption of consciousness that may be accompanied by changes in sensory or behavioural activity or alternately as a recurrent paroxysmal disorder of cerebral function that is characterized by sudden, brief attacks of altered consciousness, sensory phenomena, or inappropriate behaviour caused by an abnormal excessive discharge of cerebral neurons [5]. Carbamazepine has the empirical formula $C_{15}H_{12}N_2O$ representing a molecular formula of 236.27g/mol [6 7,8,9,10]. The chemical designation is 5-H-dibenzo[b,f]azepine-5-carboxamide[1,6,8,9].It is a white to off-white powder practically insoluble in water and soluble in methanol and acetone[6,7,8,9,11]. Carbamazepine was approved only for the treatment of epilepsy and neuralgic pain (trigeminal neuralgia) by the U.S. Food and Drug Administration. This is known as labeled use [12]. Whereas reduction of glutamate release and stabilization of neuronal membranes may account mainly for the antiepileptic effects [13,14].

Experimental

Instruments and Apparatus

The chromatography was performed on a Shimadzu HPLC instrument equipped with UV detector, Hypersil ODS V; column (250×4.6 mm, 5 µm particle size) was used as stationary phase. And in mobile phase Water: Methanol: Methylene Chloride in ratio of 30: 30: 3. and flow rate 1.5 ml/minute and temperature of column 25°C. And injection volume is 20µl and run time is 25 minute. Shimadzu (Columbia, MD) HPLC instrument (LC-10AT) equipped with UV detector and LC-solution software, Mettler Toledo analytical balance and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the research work.

Preparation of Standard Solution

Transfer about 200 mg accurately weight of Carbamazepine WRS into a 100mL volumetric flask. Add about 50 ml methanol and sonicate for about 5 minutes. Dilute up to mark with methanol, mix and dilute further 5 ml of this solution to 50 ml with mobile phase. Concentration of this solution 200ppm.

Preparation of Sample Solution

20 tablets were weighed and powdered. The powder equivalent to 200mg Carbamazepine was accurately weighed and transferred in 100 mL volumetric flask, and add about 50mL of Methanol and sonicate for about 15min with intermittent shaking, Cool the mixture to room temperature and dilute to volume with Methanol, mix and filter. Further dilute 5mL of this solution to 50ml volumetric flask and dilute to volume with Mobile Phase.

Method Validation

The methods were validated in compliance with ICH guidelines.

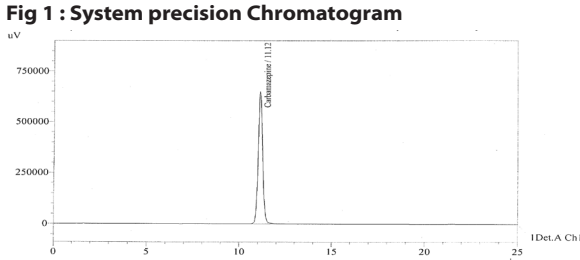
Accuracy

The accuracy of the methods was determined by calculating recoveries of Carbamzepine by the standard addition method. Recovery study was performed by spiking Carbamazepine WRS in placebo at level 80%, 100% and 120% of concentration in triplicate (total nine determinations) and then preceded with sample preparation as described under method. The result indicates that the recovery of Carbamazepine from the sample by the proposed method is satisfactory.

Mean Assay -> 99.01%

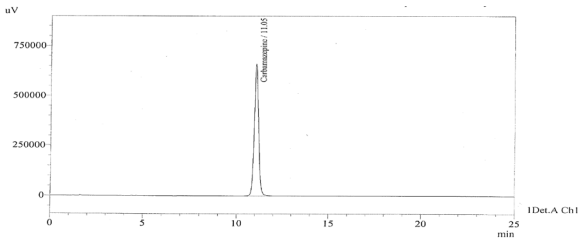
Relative standard deviation -> 0.73%

Fig 1 : System precision Chromatogram



	Name	Retention Time	Area	% Area	Height	USP Tailing	USP Plate Count
1	Blank						
2	Carbamazepine	11.12	10869918.88	100.00	649149	1.15	10363

Fig 2 : Chromatogram of Carbamazepine Sample preparation



	Name	Retention Time	Area	% Area	Height	USP Tailing	USP Plate Count
1	Blank						
2	Carbamazepine	11.050	10796533.06	100.00	659100	1.14	10727

Intermediate Precision (Ruggedness)
The procedure followed for method precision was repeated on a different day; by different analyst, using a different HPLC system and different column using same lot of sample. Calculated individual assay value, mean assay value % RSD, and overall % RSD record.

Method Precision (Repeatability)
The repeatability or reproducibility of an analytical method refer to enter-assay precision and is expressed as the degree of variation arising from consecutive and non-consecutive injection analyzed on the same day [15]. Six replicate injection of the standard preparation were made into HPLC.

Mean Assay -> 101.36%

Relative standard deviation -> 0.28%

Robustness
The robustness was studied by analyzing the same samples of Carbamazepine by deliberate variation in the method parameters. The change in the responses was noted. Robustness of the method was studied by change in flow rate ± 0.2 ml/min. composition of mobile phase by ± 5 % of organic solvent, change in pH by ± 0.2 unit and column oven temperature by ± 2°C. The parameters used in system suitability test were asymmetry of the chromatographic peak, peak resolution, theoretical plates and capacity factor, as RSD of peak area for replicate injections.

Linearity
Prepare a series of standard preparation of Carbamazepine WRS over the range of 100.50 µg/ml to 301.50 µg/ml using linearity solution. Result in Table 1.

Table 1: Linearity and Range

Concentration	Mean Area Counts	Limits
0.00		
100.50	5348333.48	
160.80	8521142.95	
201.00	10610379.6	
241.20	12800470.5	
301.50	16236218.3	
Slope	54038.50	-
Intercept	-158428.85	± 212207.59
Correlation Coefficient (r)	0.9998	0.99

Solution Stability
This was evaluated by injecting initially a freshly prepared Carbamazepine standard solution and sample solution at different time intervals. The peak response data are given below Table 2.

Table 2: Solution stability for standard

Time (Hrs)	Area Counts		% Deviation From Mean Initial Area Counts	% Deviation From Mean Initial Area Counts
	Standard	Sample	Std. (NMT ±3.0%)	Spl. (NMT ±3.0%)
0	10800483.61	10835801.04	0.0	0.0
2	10776220.12	10919430.48	-0.2	0.8
4	10807571.22	10849684.73	0.1	0.1
6	10683350.25	10835214.69	-1.1	0.0
8	10740504.19	10861698.26	-0.6	0.2
10	10722829.4	10895026.9	-0.7	0.5
12	10789244.51	10903163.69	-0.1	0.6

Results and Discussion:
The responses of sample solutions were measured by UV detector for quantitative of Carbamazepine by the proposed methods. The amount of Carbamazepine present in the sample solutions was determined. The mobile phase consisting of Water: Methanol: Methylene chloride in ratio (30: 30: 3), at a flow rate of 1.5 ml/min was found to be satisfactory to obtain good peak symmetry, better reproducibility and repeatability for Carbamazepine.

Linear correlation was obtained between peak area and concentration for Carbamazepine in the range of 100.5-301.50 µg/ml (Table 1).

The method was found to be specific as no significant change in the responses of Carbamazepine was observed after 12 hrs. (Table 2)

Acknowledgements
The authors are thankful to RPG Life Science Ltd, Ankleswar for providing laboratory facilities and also thankful to Principal, M.G.Science Institute, Ahmadabad for providing library and computer facility to carryout research work.

REFERENCES

1. Isaacson, E.I. Central Nervous system Depressants. In Textbook of Organic Medicinal and Pharmaceutical Chemistry. Block, J.H., Beale Jr., J.M.(eds).Lippincott, Williams, Wilkins, 2004, pp. 506. | 2. The Merck Manual, de Korte D.F.(ed), Mearck and co., 1992, pp 1436-1444. | 3. Basic and Clinical Pharmacology, Katzung B.G. (ed),Appleton and Lange,7th Edition, 1998. Pp 386-393. | 4. "Carbamazepine" The American Society of Health-System Pharmacists. Retrieved April 2011. | 5. Winter, M.E. Carbamazepine.In Basic Clinical Pharmacokinetics.Koda-kimable, M.A, Young, L.Y (eds.),2nd Ed. Applied Therapeutics,Inc.1990,pp. 7-78,139-145. | 6. Antiepileptic Drugs. Woodbury, D.M., Penry, J.K., Pippenger, C.E. (eds.). Second Ed, 1982, Raven Press, New York,pp. 453-547. | 7. Therapeutic Drugs. Boobis,A.R., Burley, D., Davies,D.S., Harrison,P.I., Orme, M.L'E., Park, B.K., Goldberg, L.I. Vol. 1, Churchill Livingstone, New York,1991, pp. 49-53. | 8. United States Pharmacopoeia incorporating 'The National Formulary', United State Pharmacopoeial Convention, Washington, 28 th Edition,2005, pp. 341. | 9. The British Pharmacopoeia Incorporating the requirements of the 5th Edition of the European Pharmacopoeia 2004 as amended by supplements 5.1 and 5.2, The Stationery Office, London, 2005, pp.348-350. | 10. Mackichan, J.J., Kutt, H. Carbamazepine Therapeutic Use and Serum Concentration Monitoring. In Individualizing Drug Therapy, Practical Applications Of Drug Monitoring, Vol.2. Taylor, W.J., Finn,A.L.(eds.), Gross, Townsend, Frank, Inc., New York, 1981, pp.2-22. | 11. Martindale: The Extra Pharmacopoeia. Pharmaceutical Press, London, 33rd Ed., 2002, pp.342-347. | 12. "Carbamazepine"(http://www.merck.com/mmpe/lexicomp/carbamazepine.html). The Merck Manual Professional. Archived (http://www.webcitation.org/5uKvOP99i) From the original on 2010-11-18.Retrieved on May 2009. | 13. Goodman and Gilman's The Pharmacological Basis of therapeutics. McNamara,J.O., Hardman, J.G., Limbird,L.E.(eds.).Tenth ed.McGraw Hill,2001,pp. 521-535. | 14. Granger, P.et al. Modulation of the gamma-amino butyric acid type A receptor by the antiepileptic drugs carbamazepine and phenytoin. Mol. Pharmacol.47,1995,pp.1189-1196. | 15. Analytical Method Development and Validation. Swartz, M.E, Krull, I.S. Marcell Dekker Inc., 1997, pp. 17-83.