



Trade Science Inc.

December 2009

Volume 8 Issue 4

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAJ, 8(4) 2009 [627-630]

LC-MS/MS method for determination of carbamazepine in human serum

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Received: 26th July, 2009 ; Accepted: 5th August, 2009

ABSTRACT

Determination of Carbamazepine in human serum method was developed and validated. Carbamazepine was extracted from serum by two-step extraction procedure using Chloroform as extracting solvent. LC-MS/MS method has proved to be a powerful research tool due to its sensitivity, high selectivity, and high throughput efficiency. In this study, ESI⁺ source was chosen as the ionization source. Signal intensity was high using ESI⁺ source provided for the quantification of samples. Chromatographic separation was performed on phenomenax C-18 column (250x4.60mm 5microns). Mobile phase contains Methanol, water (70; 30 v/v) + 0.1% acetic acid, flow rate 0.8 mL/min. The retention time of Carbamazepine 3.9 min, the total run time 6 mins. Linearity correlation coefficients (r^2) curve was 0.998070, calibration range 10-1000ng/mL. The LLOQ of Carbamazepine 10 Pico gram. MRM (Multiple reaction monitoring) transition of Carbamazepine m/z 258.87-193.33 was selected to obtain maximum sensitivity. LC-MS/MS method has been successfully used in the pharmacokinetic analysis of Carbamazepine in human serum. © 2009 Trade Science Inc. - INDIA

KEYWORDS

Carbamazepine;
LC-MS/MS;
ESI⁺ source;
Human serum;
Chloroform extraction.

INTRODUCTION

Carbamazepine is an anticonvulsant and mood stabilizing drug used primarily in the treatment of epilepsy and bipolar disorder. Carbamazepine was discovered by chemist Walter Schindler at J.R. Geigy AG, Switzerland, in 1953^[1]. Carbamazepine is known to render many hormonal contraception products ineffective, due to its action as a cytochrome P450 enzyme inducer, which is the system that metabolizes many oral contraceptives. Carbamazepine causes more cytochrome P450 enzyme to be produced, which hastens removal of the contraceptive from the blood plasma although

the clinical significance of this effect is debatable. There are also reports of an auditory side effect for carbamazepine use, whereby patients perceive sounds about a semitone lower than previously^[2]. Pregnant women taking carbamazepine put their fetuses at increased risk for teratogenic effects. As a result, they should be given folic acid supplementation and undergo prenatal ultrasonography for diagnosis.

In addition, carbamazepine has been linked to serious adverse cognitive anomalies, including EEG slowing^[3] and cell apoptosis^[4] voltage-gated sodium channels are the molecular pores that allow brain cells to generate action potentials, the electrical events that al-

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low neurons to communicate over long distances. After the sodium channels open to start the action potential, they inactivate, essentially closing the channel. Carbamazepine stabilizes the inactivated state of sodium channels, meaning that fewer of these channels are available to open, making brain cells less excitable (less likely to fire). Carbamazepine has also been shown to potentiate GABA receptors made up of $\alpha 1$, $\beta 2$, $\gamma 2$ subunits^[5]. Valproic acid and valnoctamide both interact with carbamazepine, as they inhibit microsomal epoxide hydrolase (mEH), the enzyme responsible for the breakdown of carbamazepine-10, 11 epoxide into inactive metabolites^[6]. Carbamazepine, as CPY 450 inducer, may increase clearance of many drugs, decreasing their blood levels. Increases in dose at a rate of 200mg every 1-2 weeks may be required to achieve a stable seizure threshold. Stable Carbamazepine concentrations occur usually within 2-3 weeks after initiation of therapy^[7].

EXPERIMENTAL

Chemical and reagents: Carbamazepine was obtained from Dr.reddys analytical R&D (Hyderabad-INDIA).Methanol, acetic acid of HPLC grade all from Merck. All other reagents were of analytical grade. Blank human serum was obtained from healthy volunteers. Ultra pure water obtained from Milli-Q water purification system.

Sample preparation: Serum samples were obtained from healthy volunteers. A 500 μ L aliquot of serum was placed into a test tube, 100 μ L standard solution of Carbamazepine was added. The tube was vortex-mixed for 2min and kept at room temperature for 5min. After addition of 2ml of chloroform the tubes were vortex mixed for 2min and centrifuged for 10min at 1500g. The chloroform layer is completely removed and transferred to a clean test tube and evaporated to dryness, the residue was reconstituted with mobile phase. These samples are ready to analysis on LC/MS/MS system.

Sample extraction: Aliquots of 500 μ L of drug contain serum, Added 2 mL of chloroform and vortex for 2 min. A ring was formed between aqueous and organic solvent. Chloroform was collected from test tube dried under nitrogen. This evaporated sample was reconsti-

tuted with 2 ml of previously prepared mobile phase. Samples are ready to analysis.

LCMS/MS: Quattro micro API, triple quadrupole. Mass Lynx software, version 4.1. Consisted series of 2695 separation module and PDA (2996) detector all from Waters (Milford, MA, USA). Separation was achieved using phenomenax C-18 column (250x4.60 mm-5microns). The mobile phase contains 0.1% Acetic acid was prepared and degassed. Chromatographic separations were performed at $30^{\circ} \pm 2C$. The flow rate was set to 0.8mL/min.

Micro mass triple quadrupole mass spectrometer with an ESI⁺ source was used for mass analysis and detection. Mass spectrometric analysis was performed in the positive ionization mode and set up in multiple reaction monitoring (MRM).Desolvation Gas flow150(L/Hr).Gas cell pirani pressure 2.80 e-3(mbar).The capillary temperature was 2.89 (KiloVolt), Cone 35 (Volt) for Carbamazepine. Based on the full scan mass spectra of the analyte the most abundant ions were selected and mass spectrometer was set to the monitor the transitions of the precursors to the product ions as m/z 258.87 for Carbamazepine. (Na⁺ was adding as adduct for carbamazepine)

Method validation: The method was validated for Sensitivity, Accuracy, Precision, Recovery, calibration curve range and lower limit of detection (LLOD) and reproducibility according to the FDA guidelines for validation of bio analytical method. The selectivity was investigated by preparing and analyzing four individual human blank serum samples set LLOQ.

RESULTS & DISCUSSION

Method development: The Carbamazepine, Signal intensity was high in Carbamazepine using ESI⁺ source. ESI⁺, Carbamazepine formed m/z 258.87 in full scan spectra (Figure 1). The most abundant ion in the product ion mass spectrum was at 193.33 for Carbamazepine is represented in Figure 2. To determined Carbamazepine using MRM mode, full scan and product ion spectra of the analytic work investigated. Present study, a simple liquid- liquid extraction procedure was used. Extraction efficiency was increased when liquid-liquid extraction was carried out with chloroform as extractive organic solvent.

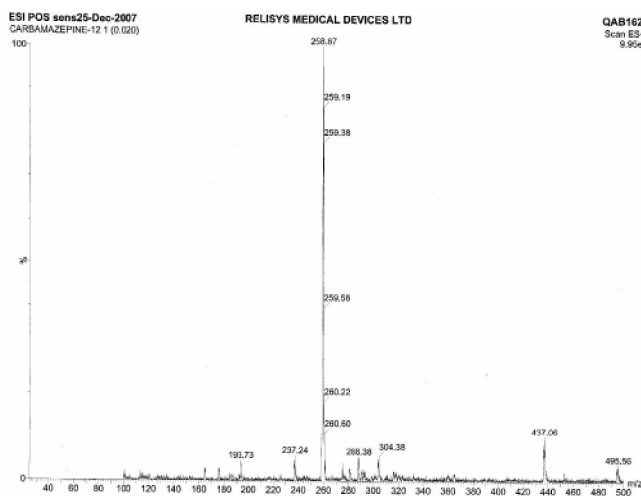


Figure 1 : Carbamazepine parent molecule.

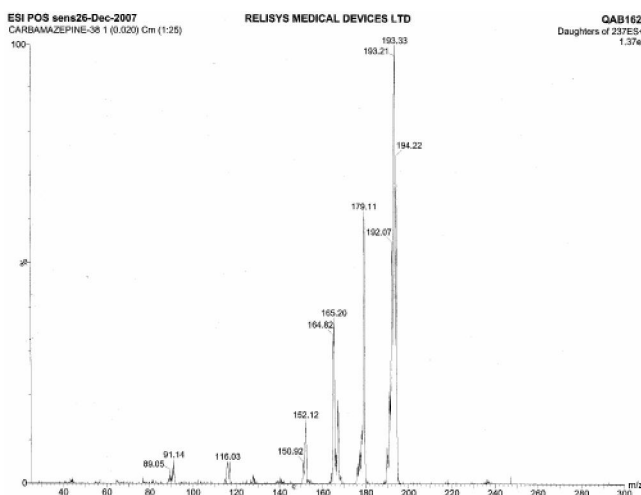


Figure 2 : Product ions of carbamazepine.

Method validation: LC/MS/MS method was demonstrated high specificity only ions derived from the analytes of interest were monitored. The retention time of Carbamazepine was 3.9 min (Figure 3). The slope, the intercept and correlation coefficient (r) for each standard curve from analytical run was determined automatically by mass lynx software. The representative standard curve for Carbamazepine was $0.19.9564 * X 348306 * x + -67.2692$. The mean squared correlation coefficient (r^2) for calibration standard curve was 0.993621 (Figure 4). Carbamazepine gave linear response as a function of the concentrations ranges showed excellent linearity over 10-1000ng/mL.

Recovery of Carbamazepine from human plasma extraction recoveries were 100.0 %, and were similar at all analyte concentrations, which indicated that the

extraction efficiency for Carbamazepine using chloroform was satisfactory.

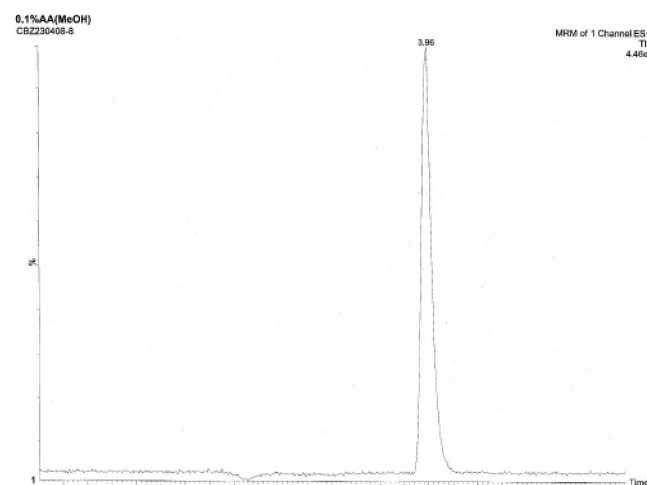


Figure 3 : Retention time of carbamazepine.

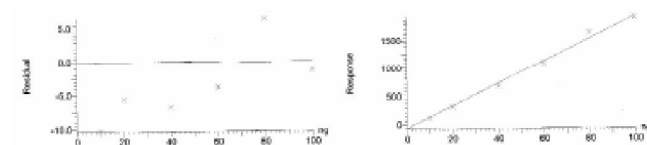


Figure 4 : Calibration curve of carbamazepine.

In this study, ESI+ was chosen as the ionization source. Signal intensity was high using ESI+ source provided for the quantification of samples. Carbamazepine formed molecules $[M-H]^+$ of m/z 258.87 in full scan spectra. The most abundant ion in the product ion mass spectrum was at 126.99. The MRM transition of m/z 258.87-193.33 for Carbamazepine was selected to obtain maximum sensitivity. Carbamazepine the parent molecule was fragmented into the daughter ions through the collision energy. Argon was used as collision energy. A standard curve of Carbamazepine in different range of concentrations 10, 50, 100, 250, 500, 1000ng/ml was prepared. The calibration curve displayed excellent linearity over the concentration range investigated.

In the present study, a simple Liquid-Liquid extraction procedure was used. The extraction efficiency was increased when liquid-liquid extraction solvent as chloroform. The proposed chromatographic conditions of LCMS/MS analysis was carried out. Retention time of Carbamazepine approximately 3.9 mins (Figure 3). Optimization was achieved by monitoring varying reversed phase column, mobile systems, flow rate and wavelength. In the present study the most important LCMS/MS technique for determination of

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Carbamazepine in biological fluids were studied. This technique is rapid and reliable and simple extraction method. LCMS/MS method was developed and validated.

In this study, Liquid-Liquid extraction procedure is used chloroform as organic extraction solvent. Mobile phase contains acetic acid as 0.1%, to enhance the ions in drug sample. While extraction with organic solvent (Chloroform) a ring was formed between the organic and aqueous medium. The drug was extracted into organic solvent (CHCl_3) and then evaporated under nitrogen. These evaporated samples were reconstituted with previously prepared mobile phase. The recovery of the drug from the sample was 100 percentage. LCMS/MS has proved to be a powerful research tool due to its sensitivity, high selectivity, and high throughput efficiency. Derivatization techniques to improve the detect ability for LCMS/MS have been successfully used in the pharmacokinetic analysis.

REFERENCES

- [1] W.Schindler, F.Häfliger; *Hel Chem Acta.*, **37**, 472 (1954).
- [2] H.Yoshikawa, T.Abe; *T.Brain Dev.*, **25**, 127 (2003).
- [3] M.C.Salinsky, L.M.Binder, B.S.Oken, D.Storzbach, C.R.Aron, C.B.Dodrill; *Epilepsia*, **43**, 482 (2002).
- [4] X.M.Gao, R.L.Margolis, P.Leeds, C.Hough, R.M.Post, D.M.Chuang; *Brain Res.*, **703**, 63 (1995).
- [5] P.Granger; *Mol.Pharmacol.*, **47**, 1189 (1995).
- [6] Gonzalez J.Frank, H.Robert; 'Biasis of therapeutics'. New York, McGraw-Hill (2nd ed. Ed) pp. 79 (2006).
- [7] Larry A.Bauer; McGraw-Hill. (2nd ed. Ed), (2008).