Trade Science Inc.

June 2009 Volume 8 Issue 2

Analytical CHEMISTRY

An Indian Journal

Urgent Communication

ACAIJ, 8(2) 2009 [289-292]

Liquid chromatography-tandem mass spectrometric assay for determination of losartan in human plasma

G.Rajender*, N.G.B.Narayanan

Research and Development Division, Relisys Medical Devices Ltd., (RMDL), Hyderabad-510501 (INDIA)

E-mail : dr.grajennder@gmail.com

Received: 27th April, 2009 ; Accepted: 2nd May, 2009

ABSTRACT

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has proved to be a powerful research tool due to its sensitivity, high selectivity, and high throughput efficiency. Determination of Losartan in human plasma method was developed and validated.Losartan was extracted from plasma by two-step extraction procedure using chloroform as extracting solvent. In this study, ESI^- sense 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 (250×4.60mm 5microns).Mobile phase contains acetonitrile, water (70; 30 v/v) + 0.1% acetic acid, flow rate 0.8 mL/min.The retention time of Losartan 5.1 min, the total run time 6 min.Linearity correlation coefficients (r^2) curve was 0.999818,calibraction range 10-1000ng/mL.TheLLOQ of Losartan 10 Pico gram. The UV detection of Losartan was at 225 nm. MRM (Multiple reaction monitoring) transition of Losartan *m/z* 421.28-126.99 was selected to obtain maximum sensitivity. LC-MS/MS method has been successfully used in the pharmacokinetic analysis of Losartan in human serum.

KEYWORDS

Losartan;
Liquid chromatography-tandem mass spectrometry;
ESI^- source;
Human plasma;
Chloroform extraction.

INTRODUCTION

Losartan, the potassium salt of 2-butyl-4-chloro-1-{[2′-(1H-tetrazol-5-yl)biphenyl-4-yl] methyl}-1H-imidazol-5-yl) methanol. (**Figure** 1) is a potent orally active and highly selective AT_1 subtype, not peptide angiotensin II (AII) receptor anatagonist and antihypersensitive agent[1-3].

Having a sensitive and reliable technique is crucial for determination of losartan in biological fluids and studying its pharmacokinetics investigation in man. Several HPLC techniques, mostly based on UV detection are reported for extraction and determination of losartan in biological matrices[4-7]. These methods mostly need extracting steps that are sophisticated and time consuming with some interfering materials that could effect the overall determination of losartan. Carrying out some of these methods[4,5] we encountered difficulties during extraction of losartan from plasma, including interfering

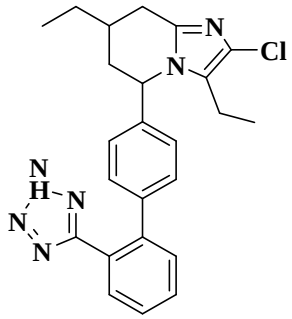

Figure 1: Losartan structure

materials that isolation of them was complex, time consuming and effected obtaining a clean extraction and reasonable peaks.

The aim of this study was to establish a simple, rapid and sensitive LC-MS/MS method to overcome the mentioned problems and allowed determination of the range of concentration of losartan in biological matrices applicable to pharmacokinetic studies.

EXPERIMENTAL

Chemical and reagents

Losartan was obtained from Dr.reddys analytical R&D (Hyderabad-INDIA).The purity of the compound was 99.9% as compared with standard.Acetonitrile, acetic acid of HPLC grade all from Merck. All other reagents were of analytical grade. Blank human plasma was obtained from healthy volunteers. Ultra pure water obtained from Milli-Q water purification system (S G waters, UK).

Preparation of standard samples

Stock solution of Losartan was prepared by dissolving 2mg of losartan in 2ml of Acetonitrile to give final concentration mg/mL. Standard solutions were obtained by diluting this solution with acetonitrile to give final concentrations over the range of 10 -1000 ng/mL for preparation of the standard curve. Calibration solutions were prepared by spiking blank human plasma with losartan standard solutions.

Sample preparation

Plasma samples were obtained from healthy volunteers. A 500 μL aliquot of plasma was placed into a test tube, 50 μL standard solution of losartan was added. The tube was vortex-mixed for 1min and kept at room temperature for 5min. After addition of 1ml of chloroform the tubes were vortex mixed for 1min and centrifuged for 5min at 1500g. The chloroform layer is completely removed and transferred to a clean test tube and evaporated to dryness under nitrogen, the residue was reconstituted with mobile phase. These samples are ready to analysis on LC/MS/MS system.

Sample extraction

Aliquots of 500 μL of drug contain plasma, 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 reconstituted with 1 ml of previously prepared mobile phase. Now the samples are ready to analysis on LC/MS/MS.

Liquid chromatography and Mass spectrometric conditions

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 (250 × 4.60 mm-5microns). The mobile phase contains 0.1% Acetic acid (70:30-0.1%Acetic acid) was prepared and degassed. Chromatographic separations were performed at 30°C. The flow rate was set to 0.8mL/min. UV detection of Losartan at 225 nm.

Micro mass triple quadrupole mass spectrometer with an ESI^- source was used for mass analysis and detection. Mass spectrometric analysis was performed in the negative ion mode and set up in multiple reaction monitoring (MRM).Desolvation Gas flow200(L/Hr).Gas cell pirani pressure 2.40 e-3(mbar).The capillary temperature was 3.37(KiloVolt), Cone 41 (Volt) for losartan. 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 421.28 for losartan.

Method validation

The method was validated for selectivity, accuracy, precision, recovery, calibration curve range and reproducibility according to the FDA guidelines for validation of bio analytical method (FDA, 2001). The selectivity was investigated by preparing and analyzing four individual human blank plasma samples set LLOQ.

Accuracy and precision were assessed by determining QC samples at three concentration levels (five samples each concentration) on three different validation days. The precisions were determined as the RSD (%) and the accuracies were expressed as a percentage of the nominal concentration. The criteria used to assess the suitability of precision and accuracy was as

follows: the RSD should not exceed 15% and the accuracy should be within 85-115%. Furthermore, the recovery (extraction efficiency) of analytic from human plasma was determined by comparing the areas of spiked plasma samples before and after liquid extraction that represent 100% recovery.

RESULTS AND DISCUSSION

Method development

The losartan, Signal intensity was high in losartan using ESI$^-$ source. ESI$^-$, Losartan formed m/z 421.28 in full scan spectra (Figure 2). The most abundant ion in the product ion mass spectrum was at 126.99 for losartan is represented in figure 3. To determined Losartan using MRM mode, full scan and product ion spectra of the analytic work investigated. The MRM method transition of m/z 421.28-126.99 for losartan was selected to obtain maximum sensitivity. The U V detection of Losartan was at 225 nm.

Present study, a simple liquid- liquid extraction procedure was used. Extraction was carried out with different organic solvents like methanol, dichloromethane, diethyal ether, acetonitrile, chloroform. It was found that all solvents gave high extraction efficiency for losartan. Extraction efficiency was increased when liquid-liquid extraction was carried out with chloroform as extractive organic solvent. Among all, chloroform was rapid evaporation and 100 % extraction organic solvent.

Method validation

LC/MS/MS method was demonstrated high specificity only ions derived from the analytes of interest were monitored. The retention time of Losartan was 5.1 min (figure 4).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 losartan was 0.348306* x + -3.10209. The mean squared correlation coefficient (r^2) for calibration standard curve was 0.999818 **(figure** 5). Losartan gave linear response as a function of the concentrations ranges showed excellent linearity over 10-1000ng/mL.

Recovery of Losartan from human plasma extraction recoveries were 100.0 %, and were similar at all analyte concentractions, which indicated that the ex-

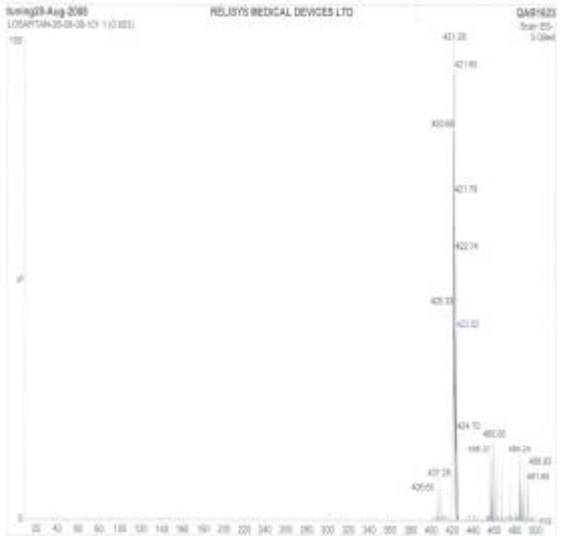

Figure 2: Losartan parent molecule

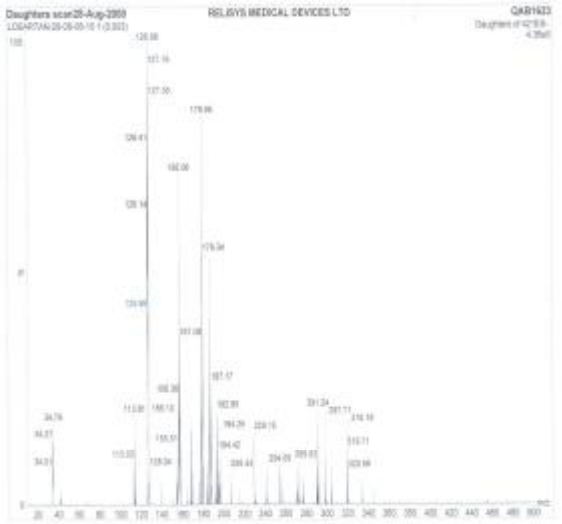

Figure 3: Product ion of losartan

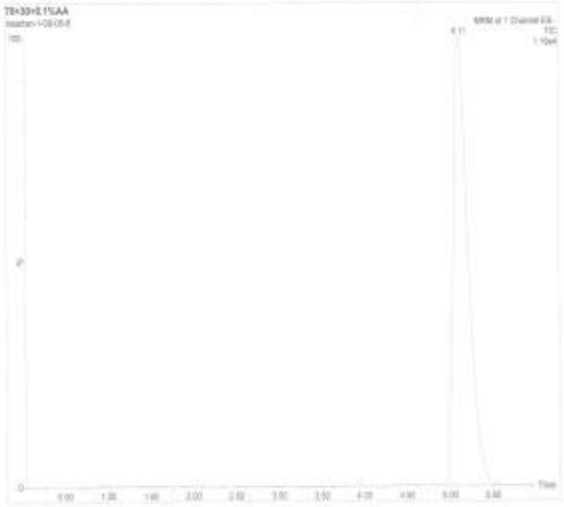

Figure 4: Retention time of losartan

Urgent Communication

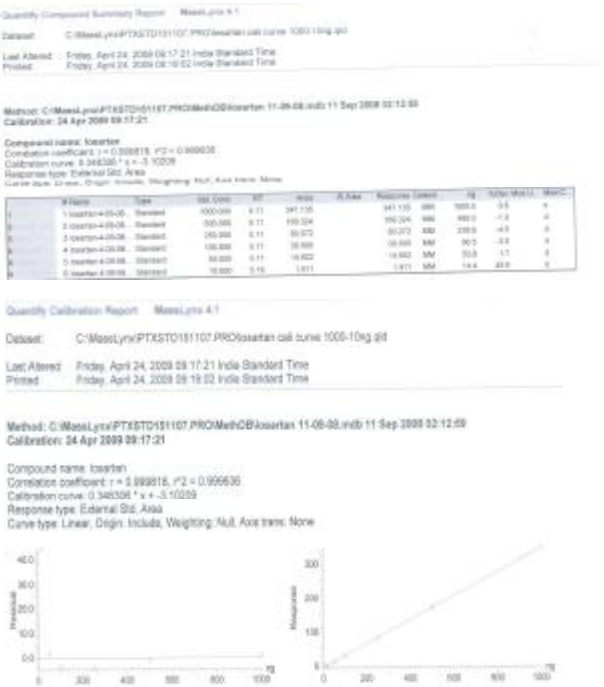

Figure 5: (a) Calibration curve of Losartan; (b): Calibration curve of losartan

traction efficiency for Losartan using chloroform was satisfactory.

In this study, ESI^- was chosen as the ionization source. Signal intensity was high using ESI^- source provided for the quantification of samples . Losartan formed molecules$[M-H]^-$ of m/z 421.28 in full scan spectra. The most abundant ion in the product ion mass spectrum was at 126.99.The MRM transition of m/z 421.28-126.99 for losartan was selected to obtain maximum sensitivity. For quantification of Losartan coated drug eluting stents a new MRM method was created. Losartan the parent molecule was fragmented into the daughter ions through the collision energy. Argon was used as collision energy. A standard curve of losartan in different range of concentrations 10, 50,100,250,500, 1000ng/ml was prepared. The calibration curve displayed excellent linearity (r^2>0.999818) 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 Losartan approximately 5.1 min. 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 Losartan 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 percentages. 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] A.H.Grandman, K.E.Arcuri, A.E.Goldburg, L.S. Ikeda, E.V.Nelson, D.B.Snavely; Hypertension., **25**, 1345 **(1995)**.

[2] M.A.Weber, R.L.Byyny, J.H.Pratt, E.P.Fasion, D.B.Snavely, A.E.Goldburg; Arch.Intern.Med., **155**, 405 **(1995)**.

[3] A.I.Goldburg, M.C.Dunlay, C.S. Sweet; Am J Cardiol., **75**, 793 **(1995)**.

[4] C.I.Furtek, M.W.Lo; J.Chromatogr., **573**, 295 **(1992)**.

[5] M.A.Ritter, C.I.Furtek, M.W.Lo; J.Pharm.Biomed. Anal., **15**, 1021 **(1997)**.

[6] D.Farthing, D.Sica, I.Fakhry, A.Pedro, T.W.B.Gehr; J.Chromatogr., **704**, 374 **(1997)**.

[7] A.Soldner, H.Spahn-Langguth, E.Mutschlur; J. Pharm.Biomed Anal., **16**, 863 **(1998)**.