Pharmaceutica Analytica Acta
ISSN: 2153-2435

Delhiraj and Anbazhagan, Pharm Anal Acta 2015, 6:7
DOI: 10.4172/2153-2435.1000400

Short Communication Open Access

A Simple, Isocratic and Ultra-Fast Liquid Chromatography / Mass Spectrometry Method for the Estimation of Barnidipine in Human Plasma

Napa Delhiraj[1]* and Sockalingam Anbazhagan[2]

[1]Department of Pharmaceutical analysis, A.S.N Pharmacy College, Tenali, Guntur (Dt), Andhra Pradesh, India
[2]Department of Pharmaceutical analysis, Surya School of pharmacy, Vikravandi, Villupuram (Dt), Tamil Nadu, India

Abstract

An Ultra Fast liquid chromatography – mass spectrometry method (UFLC-MS), for detection and quantification of the anti-hypertensive drug, Barnidipine in human plasma was developed. The Detection was performed using Shimadzu LCMS – 2010 EV mass spectrometer with Atmospheric pressure chemical ionization source in negative polarity. Indapamide was used as an internal standard. Selective ion monitoring (SIM) mode was selected with Barnidipine m/z 491 in negative ion mode. Chromatographic separation of analyte and internal standard were carried out using a reverse phase column, Phenomenex C_{18} (50 x 4.6 mm i.d., 5 µ) at a flow rate of 0.300 mL/min. Mobile Phase is composed of Acetonitrile: 0.05 % Formic acid (60:40) v/v. Extracted by Solid Phase Extraction with a sample volume of 200 µL plasma. The assay of Barnidipine is linear over the range of 50 ng/mL to 1000 ng/mL with a precision of <9.86%. Mean extraction recovery for Barnidipine was more than 61%. Samples are stable at room temperature for 6 hrs, processed samples were stable at least for 28 hrs and also stable at three freeze–thaw cycles. The limits of quantification and detection were achieved upto 10 ng/ml and 5 ng/ml respectively. This method was applied successfully to determine Barnipine in plasma samples.

Keywords: Barnidipine; LCMS method; Plasma-antihypertensive

Introduction

Barnidipine hydrochloride is ([3S]-l-benzyl-3-pyrrolidinyl methyl [4S]-2,6-dimethyl-4-[m-nitrophenyl]-l,4-dihydropyridine-3,5-dicarboxylate hydrochloride) has been synthesized and developed as an antihypertensive drug by Yamanouchi Pharmaceutical Co Ltd. (Tokyo, Japan). Physicochemically, Barnidipine hydrochloride differs from other dihydropyridines, such as nifedipine or nisoldipine, in its water solubility. The solubility of Barnidipine at room temperature is 2.89 mg/ml [1,2]. Literature review reveals that several analytical methods by HPLC and LC–MS–MS have been developed for the detection of Barnidipine in formulations and biological fluids [3,4]. Hence, authors propose a simple LCMS method that is developed and validated in achieving good extraction recoveries that willbe useful for carrying out analysis.

Material and Methods

Chemicals

All solvents and chemicals were of HPLC and LCMS grade (Acetonitrile and formic acid) and were purchased from Merck (India). Barnidipine pure powder was purchased from Sigma Aldrich (USA). Mass spectrometry grade ultrapure water was obtained from in-home MilliQ water purifier system. All solvents were filtered through 0.22 µm nylon membrane filters and degassed with ultrasonicator prior to the analysis. Pooled human plasma was obtained from the blood bank [4,5].

Chromatographic conditions

The Liquid chromatography system used for the study was Shimadzu UFLC 2020 series equipped with binary LC20AD pumps, Shimadzu SIL-HTC auto sampler with UV-visible detector. Phenomenex C18 column (diameter of 50 mm x 4.6 ID x 5 µm pore size) with particle size of 110 Å, attached with compatible guard column (Phenomenex) was used for the separations. The isocratic mobile phase used for the analysis was acetonitrile: 0.05% formic acid (60:40) with the flow rate of 0.3 ml/min. The mass spectrometer used for the detection was Shimadzu 2010EV attached with Atmospheric pressure chemical ionization (APCI Detector) and a nitrogen generator (Peaks Scientific, Japan). The detector voltage was set to 1.5 kV (positive mode) and nebulizer gas flow was set to 2.5 ml/min. Mode of detection was set to selective ion monitoring (SIM) at m/z 491.

Standards

All standards were prepared in pure LC grade milli-Q water. Initially, a stock solution of 1 mg/ml was dissolved and used to prepare desired dilutions with LC grade milli-Q water. Prepare 10 ml each of 1000.0, 1200.0, 1500.0, 2500.0, 5000.0, 9000.0, 18000.0 and 20000.0 ng/ml of Barnidipine standard solutions using the Barnidipine standard stock solution and mobile phase and store at −20 ± 2°C until analysis. Prepare 10.0 ml each of 50.0, 60.0, 75.0 ,125.0, 250.0, 450.0, 900.0 ,1000.0 ng/ml of Barnidipine Calibration curve samples using 0.5 ml of Barnidipine standard stock solution and make up the volume with blank plasma, transfer in to different 2 ml centrifuge tubes and store at −70 ± 2°C until processing.

Sample preparation

At the time of analysis, the samples were removed from the deep freezer and kept in the room temperature and allowed to thaw. Sam prep SPE Columns C18- (33 µm, 70 Å) 60 mg / 3 ml solid phase extraction cartridge was conditioned with Acetonitrile, water sequentially. To this 0.5ml of plasma samples and 0.5 ml of 100.0 µg/ml of internal standard

***Corresponding author:** Delhiraj N, Associate Professor, Department of Pharmaceutical Analysis, A.S.N Pharmacy College, Tenali-522201, Andhra Pradesh, India, Tel: 91-9941121000,9010433905; E-mail: pharmaraj1981@gmail.com

Received July 09, 2015; **Accepted** July 28, 2015; **Published** July 31, 2015

Citation: Delhiraj N, Anbazhagan S (2015) A Simple, Isocratic and Ultra-Fast Liquid Chromatography / Mass Spectrometry Method for the Estimation of Barnidipine in Human Plasma. Pharm Anal Acta 6: 400. doi:10.4172/21532435.1000400

was added. The cartridge was washed with 1.0 ml of methanol:water (35:65). The drug and internal standard was eluted from the cartridge using Acetonitrile. The Resulting Solution was used for the analysis.

Method validation [5]

Linearity: The different concentrations of standard solutions were prepared to contain 50.0 – 1000.0 ng/ml of Barnidipine containing 100.00 µg/ml of internal standard. These solutions were analysed and the peak areas and response factors were calculated. The calibration curve was plotted using response factor vs. concentration of the standard solutions. Linear regression analysis (linear equation y = mX + C) of calibration data was applied to calculate the slope (m), intercept (c) and correlation coefficient (r > 0.999).

Precision: The precision of the method was determined by intraday precision and interday precision studies. The intraday precision was evaluated by analysis of blank plasma sample containing Barnidipine at three different concentrations of LQC, MQC and HQC (50, 250 and 1000 ng/ml) using nine replicate determinations for three occasions. The interday precision was similarly evaluated over two-week period. The mean concentration, standard deviation and % CV were calculated.

Specificity: This method involves the peak purity test method using MS spectrum and diode array detector. The standard and sample peaks were recorded and compared. The sample peaks were seen to match with corresponding standard drug peaks. Some additional peaks observed in the sample chromatograms. These peaks, however, did not interfere with the Barnidipine and internal standard peak (Figure 1 and 2).

Accuracy: The absolute recovery of Barnidipine was determined by comparing the response factor of the drug obtained from the plasma with response factor obtained by the direct injection of Barnidipine in mobile phase at three different levels. Recovery studies were carried out for three levels at six times and the % recovery, mean, standard deviation and % CV was calculated.

Stability: The stability studies of plasma samples spiked with Barnidipine were subjected to three Freeze - thaw cycles, Short term stability at room temperature for 3 hrs and Long term stability at –70°C over four weeks. In addition, stability of standard solutions was performed at room temperature for 6 hr and freeze condition for four weeks. The stability of triplicate spiked human plasma samples following three freeze thaw cycles was analyzed. The mean concentrations of the stability samples were compared to the theoretical concentrations.

Results and Discussion

Optimization of chromatographic conditions

Different run conditions, columns and mobile phases were tested for optimizing chromatographic conditions, Phenomenex C_{18} (50 x 4.6) showed good peak shape and short retention time with good resolution without any peak tailing. Further, we tried different mobile phases such as Formic acid (in varying percentages from 0.05 – 0.5%), Ammonium acetate and ammonium formate. Formic acid at 0.05% showed good peak shape, good protonation in APCI detector, and retention time within five minute. 100% Acetonitrile act as organic modifier showed quick retention without losing peak resolution. Among different flow rates (0.1 ml/min to 0.5 ml/min), 0.3 ml/min showed no peak tailing with short retention time to allow high sample throughput analysis. Although, Barnidipine along with indapamide which has selected as Internal standard, was eluted at an average retention time of 3.9 minutes, total run time was set to five minutes to allow complete column re-equilibrium between successive injections. The analysis was run in selective ion monitoring mode (SIM), the Drug and Internal standard peak was found well resolved with baseline. Although, we detected matrix effect of plasma in the chromatogram, significant interference from endogenous compounds at specific retention time of drug and internal standard was found negligible. Good linearity was achieved at concentrations of Barnidipine (50- 1000 ng/ml in plasma). The results indicated little interday variability of slopes and intercepts, as well as good linearity ($r^2 > 0.99$) over the concentration range studied, which indicates good precision and linearity of the method.

The Barnidipine spiked plasma samples were extracted and quantified to check intra- and inter-day accuracy and precision. Three representative concentrations (low, medium and high) of Barnidipine were analyzed on a single day and between batches analyzed at different days of interval showed good accuracy and precision. % CV values of intra-day analysis of Barnidipine at three concentrations (50, 250 and 1000 ng/ml) are 11.92, 4.39 and 1.09 respectively. The % CV of precision between days (inter-day) was 0.12, 0.63 and 0.52 for the corresponding three concentrations .The results are given in Table 1.The mean extraction recovery percentages of Barnidipine spiked in plasma at three levels of concentrations, low (50 ng/ml), medium (250 ng/ml) and high (1000 ng/ml), were 96.0, 98.4 and 98.7, respectively. The recovery data is given in Table 2. An analysis of the results shows that the % CV of absolute and the relative recovery values are less than 15.00 % thus establishing that the developed method is accurate and reliable. The LOD and LOQ values were 5ng/ml and 10ng/ml. The data show that the developed methods have adequate sensitivity. Stability of spiked in plasma was evaluated in three different conditions viz., short term stability at 25°C for 3 hours, long term stability (at -70°C for 4 weeks) and auto-sampler stability (for 48 hours at 4°C).The results

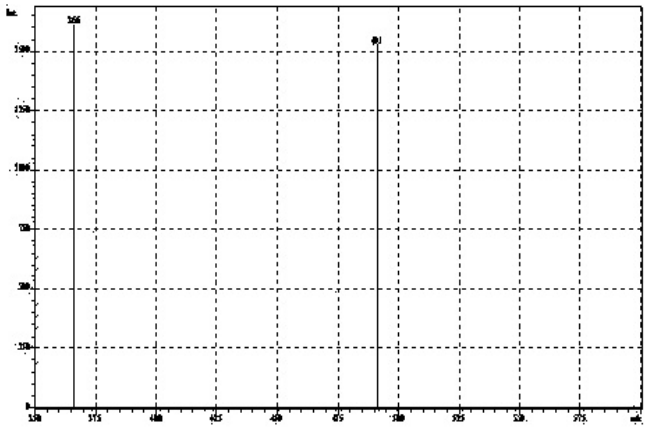

Figure 1: Mass spectrum of drug and internal standard in negative ion mode.

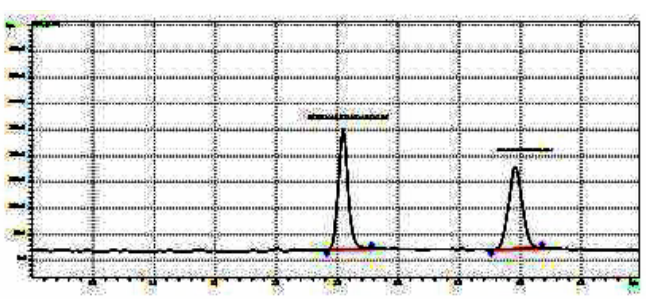

Figure 2: Chromatogram for drug and internal standard.

Freeze and Thaw	**LQC**	**MQC**	**HQC**
Concentration (ng/mL)	50.000	250.000	1000.000
Cycle 1	47.621	243.025	976.789
Cycle 2	42.339	273.978	953.351
Cycle 3	41.120	226.444	941.214
Mean (N = 3)	43.6933	235.8157	957.1180
S.D (+/-)	3.4556	8.4994	18.0842
CV (%)	7.91	3.60	1.89
% Nominal	87.39	94.33	95.71
Short Term Plasma at Room Temperature	**LQC**	**MQC**	**HQC**
Concentration (ng/mL)	50.000	250.000	1000.000
After 1 hr	43.674	234.781	961.975
After 2 hr	45.123	257.336	942.312
After 3 hr	37.978	223.025	982.167
Mean (N = 3)	42.2583	238.3807	962.1513
S.D (+/-)	3.4556	17.43644	19.928.9
CV (%)	8.49	7.31	2.07
% Nominal	84.52	95.35	96.22
Long Term Plasma Sample at – 70° C	**LQC**	**MQC**	**HQC**
Nominal Concentration (ng/mL)	50.000	250.000	1000.000
After 1 Weeks	38.125	241.267	925.139
After 2 Weeks	46.789	252.325	917.025
After 4 Weeks	39.781	248.026	943.976
Mean (N = 3)	41.5650	247.2060	928.7133
S.D (+/-)	4.59926	5.57442	13.82646
CV (%)	11.07	2.25	1.49
% Nominal	83.13	98.88	92.87

Table 1: Precision studies.

QC levels	Mean Recovery (%) In plasma Sample(N = 6)	CV (%)	Mean Recovery(%)in mobilephase(N = 6)	CV (%)	Relative recovery (%)
LQC	96.00	1.05	97.30	1.62	99.66
MQC	98.40	1.27	99.10	2.19	99.29
HQC	98.70	0.88	98.40	2.51	100.30

Table 2: Recovery studies.

Freeze and Thaw	**LQC**	**MQC**	**HQC**
Concentration(ng/mL)	50.000	250.000	1000.000
Cycle 1	47.621	243.025	976.789
Cycle 2	42.339	273.978	953.351
Cycle 3	41.120	226.444	941.214
Mean (N = 3)	43.6933	235.8157	957.1180
S.D (+/-)	3.4556	8.4994	18.0842
CV (%)	7.91	3.60	1.89
% Nominal	87.39	94.33	95.71
Short Term Plasma at Room Temperature	**LQC**	**MQC**	**HQC**
Concentration (ng/mL)	50.000	250.000	1000.000
After 1 hr	43.674	234.781	961.975
After 2 hr	45.123	257.336	942.312
After 3 hr	37.978	223.025	982.167
Mean (N = 3)	42.2583	238.3807	962.1513
S.D (+/-)	3.4556	17.43644	19.928.9
CV (%)	8.49	7.31	2.07
% Nominal	84.52	95.35	96.22
Long Term Plasma Sample at – 70° C	**LQC**	**MQC**	**HQC**
Nominal Concentration (ng/mL)	50.000	250.000	1000.000
After 1 Weeks	38.125	241.267	925.139

After 2 Weeks	46.789	252.325	917.025
After 4 Weeks	39.781	248.026	943.976
Mean (N = 3)	41.5650	247.2060	928.7133
S.D (+/-)	4.59926	5.57442	13.82646
CV (%)	11.07	2.25	1.49
% Nominal	83.13	98.88	92.87

Table 3: Stability studies.

show that Barnidipine containing plasma samples can be stored for one month without degradation of Barnidipine in frozen state. The results of short-term storage at room temperature stability and freeze thaw cycles indicated no degradation of Barnidipine in plasma as well as in sample solution therefore plasma samples in light protected containers could be handled without special precautions. The results of stability data are given in Table 3.

Conclusion

A simple, rapid, selective method in chromatographic condition and extraction is more precise, sensitive and also compatible for the analysis of Barnidipine in human plasma with good recovery by using LCMS. The method was developed and validated, which was found to have good in stability.The minimum sample requirement makes this method highly suitable for pharmacokinetic studies.

References

1. Martindale (2005) The complete drug reference 34: 863-866.
2. Toshiharu Sakai, Toshio Teramura, Hideaki Okamiya, Osamu Inagakiz (1997) Cardiovascular Drug Reviews 15: 273-290.
3. Giuseppina Ioele, Filomena Oliverio, Inmaculada Andreu, Michele De Luca, Miguel A Miranda, et al. (2010) Different photodegradation behavior of barnidipine under natural and forced irradiation. Journal of Photochemistry and Photobiology A: Chemistry 215: 205-213.
4. Pawula M, Watson D, Teramura T, Watanabe T, Higuchi S, et al. (1998) Journal of Chromatography B: Biomedical Sciences and Applications 719: 113-123.
5. ICH Harmonized Tripartite Guideline (1994) Text on validation of analytical procedures: 1-8.