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RESEARCH ARTICLE

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A RAPID AND SENSITIVE METHOD FOR ESTIMATION OF BEPOTASTINE IN HUMAN PLASMA BY LCMS/MS

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ABSTRACT

Bepotastine is a new antihistamine drug for treatment of itchy and watery eyes as well as for allergic rhinitis and chronic urticaria. Bepotastine and cetirizine (IS), extracted from the plasma by SPE method, were separated on a Zorbax SB C18 column at 50^0C using the mobile phase mixture of methanol and ammonium formate (80:20, v/v) at a flow rate of 0.8 ml/min. The analytes were detected in API 3000 Mass spectrometer in the positive electrospray ionization mode (split ratio 4:1) with multiple reactions monitoring (MRM). The MRM transitions monitored were m/z for parent ion 389.1 & daughter ion 202.1 (bepotastine), m/z for parent ion 389.1 & daughter ion 201.2 (cetirizine). A linear calibration plot of bepotastine and I.S. was achieved in the concentration ranges of 0.505 to 538.713 ng/ml and the correlation coefficient was > 0.9995. Recoveries were consistently more than 71.71%. The assay was specific, precise, accurate and reproducible.

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KEY WORDS

Bepotastine, Liquid chromatography mass spectrometry, Reverse phase column, Human plasma, Method development & validation

INTRODUCTION

Bepotastine besilate ({d-(S)-4-[4-[(4-chlorophenyl) (2-pyridyl)methoxy] piperidino} butyric acid monobenzenesulphonate), is a new second-generation antihistamine developed in Japan. It reduces the natural chemical histamine in the body which can produce allergic symptoms of itching or watery eyes. Bepotastine ophthalmic (as an eye drop) is used to treat itchy eyes caused by allergies [1 - 2]. Moreover, oral bepotastine, a histamine H_1 receptor antagonist, also suppresses some allergic inflammatory processes like allergic rhinitis and chronic urticaria [3]. A clinical trial study showed that bepotastine, cetirizine, fexofenadine, and olopatadine inhibit histamine-induced wheal-and-flare response of humans in vivo and induce a variable systemic sedative effect and impaired psychomotor activity [4 - 5]. Bepotastine was generally well tolerated in adult and paediatric patients with Allergic conditions. It was also noticed that bepotastine (20mg/day) was significantly more effective than terfenadine (120 mg/day) in patients with perennial allergic rhinitis [6]. Although a number of studies have been made to evaluate clinical efficacy and safety of bepotastine, scarcely any literature is available for its estimation [7]. We would like to report here a new method of estimation of bepotastine by LCMS/MS which is validated as per the FDA regulations. It is highly sensitive method and can be used for estimation of this drug for pharmacokinetic analysis and other studies.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol and acetonitrile (HPLC grade) were purchased from Spectrochem whereas other reagents used were of analytical grade. Millipore (USA) deionized water was used throughout the procedure.

Bepotastine Besilate was purchased from Bal Pharma Ltd., Bangalore and cetirizine dihydrochloride was from Clearsynth, Mumbai. Stock solution (1mg/ml) of cetirizine was prepared in Milli Q water. Final concentration was made using the potency and actual amount weighed. Serial dilutions were made using acetonitrile: water (1:4, v/v) to obtain a final concentration 5 µg /ml.

Stock solution (1mg/ml) of bepotastine was prepared in Milli Q water. Similar to cetirizine, final concentration was corrected based on its potency and actual amount weighed. Stock solution was serially diluted with acetonitrile: water (1:4, v/v) to obtain a concentration range of 0.025 – 27.012 µg/ml.

Quality control (QC) samples for analyte were prepared in the range of 0.025 – 20.615 g/ml using acetonitrile: water (1:4, v/v) as the diluent.

Construction of Calibration Plot

The blank plasma was spiked with bepotastine to obtain the final concentration 0.499 – 540.249 ng/ml. Of course, the final concentration was again corrected based on potency and stock weight. 50µl of internal standard (cetirizine) was added to all these bepotastine spiked plasma (200µl). Bepotastine was extracted from the plasma by solid phase extraction after adding 0.5ml of 50mM ammonium formate, vortexed for 30 sec

followed by centrifugation at 4500 RPM for 10 min. The sample was loaded onto a C18 and 1cc Cartridge preconditioned with 1ml of methanol followed by 1ml water. The cartridge was sequentially washed with 1ml of 50mM ammonium formate and 1ml of 25% methanol (twice). The analyte and the IS were eluted with 0.25ml of mobile phase (2 times). Sample was then centrifuged at 4500 RPM for 10 min. 5µl was injected to LCMS/MS for analysis.
Quality control (QC) samples, marked as LLOQC, LQC, MQC and HQC respectively, containing 0.501 ng /ml, 1.319 ng /ml, 164.918 ng /ml and 412.296 ng /ml of the drug were prepared in blank plasma.

Sample Analysis
Human blood containing K_2 EDTA as an anti-coagulant was centrifuged at 3500 rpm for 15 min at 4^0C to separate the plasma. Bepotastine was isolated from the plasma after solid phase extraction using C18 cartridges (Analchem) .

Chromatography
The drug was separated on a Zorbax SB C18, 100 mm x 4.6 mm column with particle size 3.5 µ□(Agilent) using the binary pump [pump A - Methanol: pump B – 2mM Ammonium Formate; 80:20] at a flow rate of 0.8 ml/min in Shimadzu UFLC Prominence attached to API 3000 Mass spectrometer (Applied Biosystems, USA) with an ESI interface. The column oven temperature was maintained at 50^0C and the run time was 3 min. The analytes were detected on mass spectrometer operating in the positive electrospray ionization mode (split ratio 4:1) with multiple reactions monitoring (MRM). The MRM transitions monitored were m/z for parent ion 389.1 & daughter ion 202.1 (bepotastine) and m/z for parent ion 389.1 & daughter ion 201.2 (cetirizine) with a dwell time of 200 msec. Data were acquired and processed with Analyst software 1.4.1.

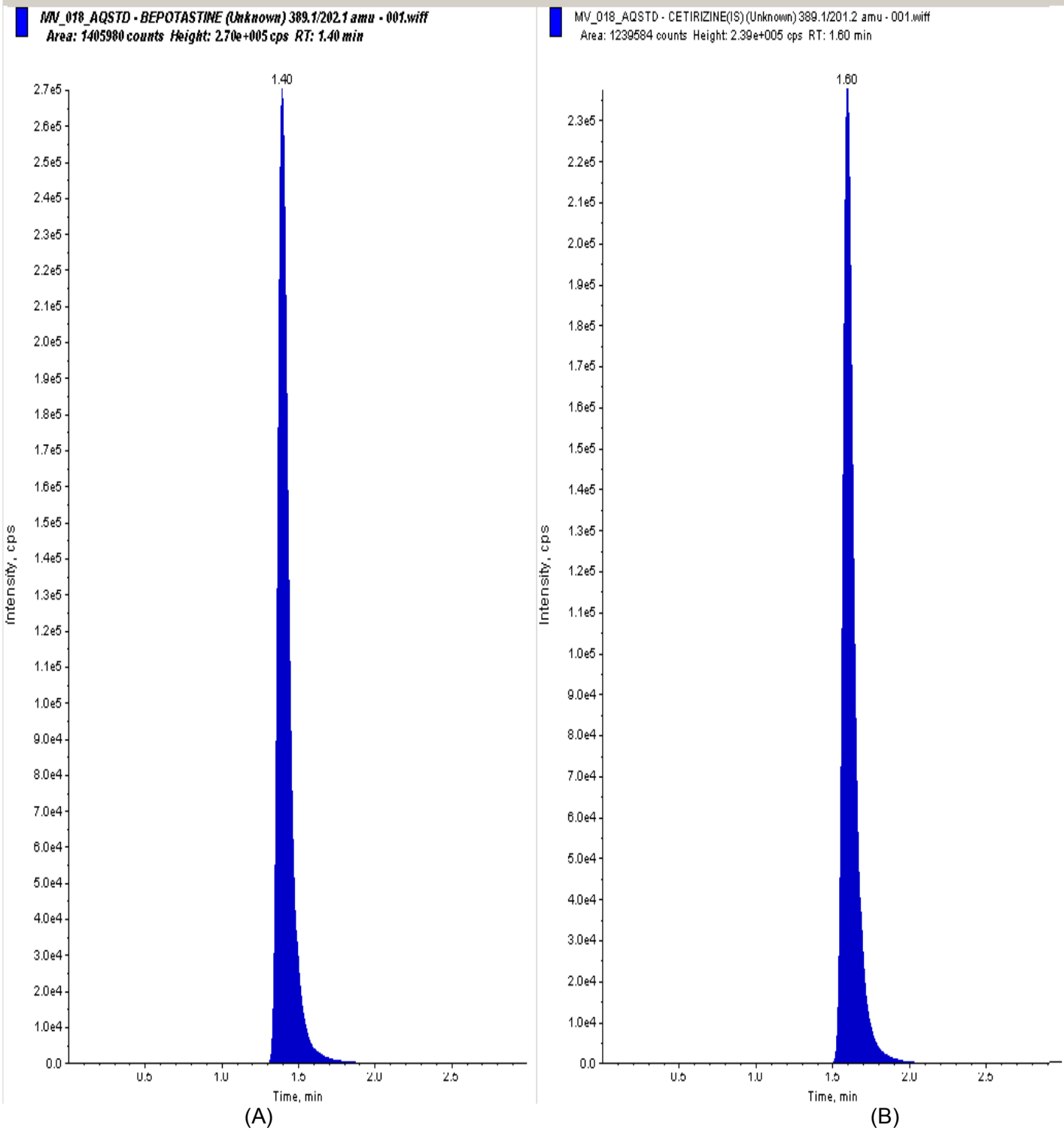
RESULT & DISCUSSION

Specificity
The retention times for bepotastine and the internal standard were 1.40 min and 1.60 min, respectively (Fig.1). No interfering peaks were observed in the blank at the retention times corresponding to drug and I.S. indicating that the procedure is specific to bepotastine. Similarly, no matrix effect was found while analyzing the human plasma samples, calibration standards and QC samples (Table 1).

Table 1
Matrix Effect for estimation of bepotastine

Aqueous LQC Analyte (Area count) (n=6)	Post spiked LQC analyte (Area count) (n=6)	Percentage matrix Effect (n=6)
11732	11823	100.78

Figure-1
Chromatograms of Bepotastine (A) and Cetirizine (B)



Linearity of the Calibration Plot

A calibration plot of bepotastine and I.S. showed that the calibration was linear in the concentration ranges of 0.505 to 538.713 ng/ml and the correlation coefficient was > 0.9995. Not only the range of linearity obtained in this method is wider but also its sensitivity is almost double of the previous reported method. Moreover, this method has been developed in LCMS/MS API 3000 and the sensitivity may even increase further if it is carried out in latest model. Inter day and Intra day precision and accuracy were determined by replicate analysis of LLOQC, LQC, MQC and HQC samples and the mean RSD for inter (n=24) and intra (n=18) day assay reproducibility were 5.26 and 5.73 respectively, which are within acceptable limit (Table 2).

Table 2

Results from determination of the accuracy and precision of analysis of bepotastine in the quality-control samples

	LOQC			LQC			MQC			HQC		
	Actual conc. (ng/ml)	Estimated conc. (ng/ml)	% RSD	Actual conc. (ng/ml)	Estimated conc. (ng/ml)	% RSD	Actual conc. (ng/ml)	Estimated conc. (ng/ml)	% RSD	Actual conc. (ng/ml)	Estimated conc. (ng/ml)	% RSD
Intraday	0.507	0.579	8.00	1.318	1.28945	4.341	164.740	171.325	5.03	411.849	414.292	5.560
Interday	0.507	0.527	12.80	1.318	1.246	3.630	164.740	162.991	1.81	411.849	399.167	2.830

Recovery

- Absolute recovery percentage was determined by comparing the peak area of bepotastine obtained by injecting 6 extracted samples of LQC, MQC and HQC sample with the peak obtained by injection of standard solutions of the same concentration. Mean percentage recovery was 71.71 (Table 3).

Table 3.

Recovery of bepotastine from biological matrix

LQC			MQC			HQC		
Unextracted area (n=6)	Extracted area (n=6)	Mean percentage recovery	Unextracted area (n=6)	Extracted area (n=6)	Mean percentage recovery	Unextracted area (n=6)	Extracted area (n=6)	Mean percentage recovery
12129	8944	74.77	1390901	994476	71.50	3314313	2282513	68.87

Stability:

Short – Term/bench - top stability

To check whether the sample is stable during analysis, six aliquots of LQC & HQC samples were thawed and kept at room temperature for 6 hours, which has been decided based on the time required for analysis. The samples were then processed and analyzed as mentioned above. No significant differences were noticed when these results were compared with those obtained from the freshly spiked samples indicating that the analyte was stable at room temperature (Table 4).

Table 4
Stability of bepotastine

Stability check Procedure	LQC Actual conc. (ng/ml)	LQC Avg. conc. Found± SD (n=6) (ng/ml)	HQC Actual conc. (ng/ml)	HQC Avg. conc. Found± SD (n=6) (ng/ml)
Bench Top (hrs)	1.318	1.284 ± 0.030	411.849	378.905 ± 2.449
Freeze Thaw (after 3 Cycles at -70^{0}C)	1.318	1.227 ± 0.055	411.849	354.207 ± 12.166
Auto sampler at 5^o C (after 42 hrs)	1.318	1.263 ± 0.079	411.849	372.165 ± 2.797

Auto sampler stability

The stability of the processed samples in the auto sampler during analysis was determined by using six aliquots of LQC, MQC & HQC samples. The stability of drug and IS were assessed for 42 hours, the expected run time for batches of validation samples. The results were then compared with that of freshly spiked samples. No significant difference in the results indicated that the bepotastine and IS are stable for at least 42 hour in the auto sampler (Table 4).

Freeze – Thaw stability

Analyte stability was determined after three freeze – thaw cycles for six aliquots of each of the LQC and HQC. The samples were stored below – 20^{0}C for 24h and then allowed to thaw at room temperature unassisted. After complete thawing, the samples were stored at same temperature for 12h. The freeze – thaw cycle was repeated twice before analyzing the samples. Comparison of the results with the fresh QC samples indicated no differences (Table 4).

CONCLUSION

This is the first reported and validated method for the estimation of bepotastine in human plasma by LCMS/MS API 3000. The assay was specific, accurate, precise and reproducible (inter- and intra-day precisions R.S.D. 5.26 and 5.73 respectively) for six replicates of low, medium, and high quality control samples. The main advantages of this method are its sensitivity, simplicity, reproducibility and rapidity. The method is suitable for pharmacokinetic studies for wide range of dosages of bepotastine in human plasma as per the regulatory authorities.

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