August 2008 Volume 7 Issue 8

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 7(8) 2008 [598-601]

Simultaneous RP-HPLC determination of bambuterol HCl and montelukast sodium in pharmaceutical preparations

V.V.Vaidya[1]*, G.R.Singh[1], M.B.Kekare[2], M.P.Choukekar[2]

[1]Department of Chemistry, S.P.Mandali's Ramnarain Ruia College, Matunga, Mumbai-400019, (INDIA)

[2]Department of Chemistry, Kirti M.Dungersee College, Dadar, Mumbai-400028, (INDIA)

Tel : 09322404966

E-mail : mpc26@rediffmail.com

Received: 5th July, 2008 ; Accepted: 10th July, 2008

ABSTRACT

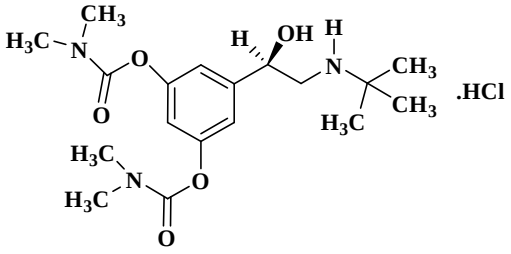
A simple, fast and precise reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of bambuterol HCl and montelukast sodium using mebeverine hcl as an internal standard. Chromatographic separation was performed on a waters symmetry C_{18} column (150mm×4.6 mm, 5μm) as stationary phase with a mobile phase comprising of 0.05% trifluoro acetic acid in water: 0.05% trifluoro acetic acid in acetonitrile (45:55 v/v), at a flow rate of 0.7mL min^{-1} and UV detection at 215nm. The Retention time of Bambuterol hcl, montelukast sodium and Mebeverine HCl were 1.270 min, 4.322 and 1.604 min respectively. The proposed method was validated for linearity, accuracy, precision, LOD, LOQ. Linearity, accuracy and precision were found to be acceptable over the ranges of 250-750μg mL^{-1} for both bambuterol HCl and montelukast sodium. It can be conveniently adopted for routine quality control analysis.

KEYWORDS

ICH Guidelines;
Validation;
Column liquid chromatography;
Pharmaceutical preparations;
Bambuterol HCl;
Montelukast sodium.

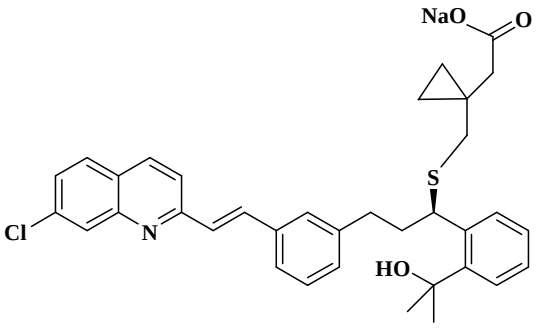
INTRODUCTION

Bambuterol HCl 5-[(CRS)-2-[(1,1-dimethylethyl) amino]-1-hydroxyethyl]-1,3-phenylene bis (dimethyl carbamate) hydrochloride. is a drug used for the treatment of asthma, breathing difficulties due to a narrowing of the airways (bronchospasm), and for chronic obstructive pulmonary disease[1-2]. Montelukast sodium is described chemically as [R-(E)]-1-[[[1-[3-[2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1methylethyl)phenyl]propyl] thio]methyl]cyclopropaneacetic acid, monosodium salt is a drug used for the prophylaxis and chronic treatment of asthma in adults[5]. The structure of both drugs is shown in figures 1 and 2. One such combination contains 5 mg of bambuterol hcl and 5 mg of montelukast sodium. The literature revealed no method was available for simultaneous determination of this drug in such pharmaceutical preparation by HPLC. Therefore an HPLC method was developed for determination of bambuterol hcl and montelukast sodium from their combined dosage form[1,2,6-10]. The method described is simple, fast, precise and accurate for simultaneous determination of bambuterol hcl and montelukast sodium from pharmaceutical preparation.



Bambuterol HCl ($C_{18}H_{29}N_3O_5$.HCl)

Figure 1: Structures of bambuterol HCl



Montelukast sodium ($C_{35}H_{35}ClNNaO_3S$)
Figure 2 : Structures of montelukast sodium

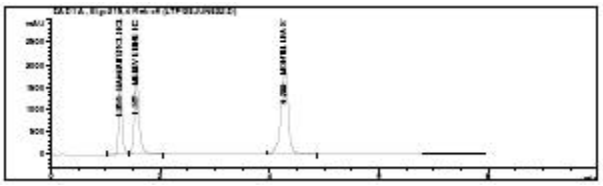


Figure 3: Chromatogram of bambuterol HCl and montelukast sodium with mebeverine HCl(internal standard) in standard preparation

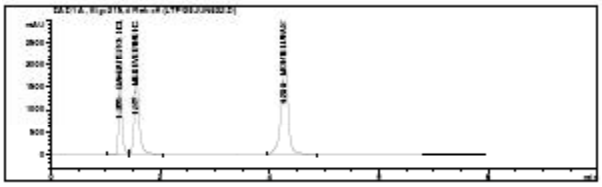


Figure 4: Chromatogram of bambuterol HCl and montelukast sodium with Mebeverine HCl (internal standard) in sample preparation

Chemicals and reagents

Standards were supplied from J.B. chemicals and pharmaceuticals, Mumbai, India.

Montair plus 5-5 tablet manufactured by Cipla, India was procured from the market. Acetonitrile, orthophosphoric acid and trifluoro acetic acid were from Qualigens. Double distilled water was employed throughout the work. All dilutions were performed in standard volumetric flasks.

EXPERIMENTAL

Method development and optimization of chromatographic conditions

To develop a suitable LC method for the analysis of Bambuterol hcl and montelukast sodium in their combined dosage form, different mobile phases were tried. The criteria employed for selecting the mobile phase for the analyses of the drugs were cost involve, time required for the analysis, better separation of drugs. Chromatographic separation was preformed with Agilent 1100 series High performance liquid chromatography having HPLC isocratic pump, equipped with auto sampler and a photo-diode array detector. The uv spectrum of bambuterol HCl and montelukast sodium was scanned on photo diode array detector for selecting the working wavelength. Peak purity of bambuterol hcl and montelukast sodium was checked using photo diode array detector. Chromatograms and data were recorded by means of chemstation software. Waters symmetry C_{18} column (150mm × 4.6 mm, 5µm particle) was used for the analysis. The mobile phase comprising of 0.05% trifluoro acetic acid in water and 0.05% trifluoro acetic acid in acetonitrile (45:55 v/v). The system was run at a flow rate of 0.7mL min^{-1}, 5µL of sample was injected in the chromatographic system and detection wavelength was set at 215nm for simultaneous determination of bambuterol hcl and montelukast sodium. A typical HPLC chromatogram for simultaneous determination of bambuterol hcl and montelukast sodium from pharmaceutical formulation is shown in figures 3 and 4.

Preparation of standard stock solutions

The stock solution of bambuterol hcl (5000µg mL^{-1}) was prepared by dissolving 250.2 mg of bambuterol hcl (99.8%) in mix of water:acetonitrile: orthophosphoric acid (50:50:1) in a standard 50 mL volumetric flask (solution A). The stock solution of montelukast sodium (5000µg mL^{-1}) was prepared by dissolving 250.0 mg of bambuterol hcl (99.9%) in mix of water:acetonitrile: orthophosphoric acid (50:50:1) in a standard 50 mL volumetric flask (solution B). Internal standard (mebeverine HCl) stock solution (5000 µg mL^{-1}) was prepared by dissolving 249.8 mg of mebeverine HCl (99.9%) in mix of water:acetonitrile: orthophosphoric acid (50:50:1) in a standard 50 mL volumetric flask (solution C).

Working standard solution

Transferred 10.0 mL of each stock solution A, solution B and solution C to a 100 mL volumetric flask and diluted up to the mark with water:acetonitrile: or

thophosphoric acid (50:50:1).

Sample preparation

Twenty tablets were weighed and their average weight was calculated. The tablets were crushed into a homogeneous powder and a quantity equivalent to ten tablet was transferred in a 100mL volumetric flask dissolved in water:acetonitrile: orthophosphoric acid (50:50:1), 10 mL of solution c (internal standard) was added to it and filtered through Whatman no. 41 filter paper.

RESULTS AND DISCUSSION

System suitability

System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out[3-4]. System suitability tests were performed as per the USP 31 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 5µL standard solutions of bambuterol HCl and montelukast sodium of strengths 500µg mL^{-1} using mebeverine hcl as an internal standard. This was repeated five times. The RSD values of bambuterol hcl and montelukast sodium was 0.11% and 0.09% respectively. The RSD values was found to be satisfactory and meeting the requirements of USP 31 (RSD less than 2.0 %). Theoretical plates, resolution, tailing factor were determined and are presented in TABLE 1.

TABLE 1 : Result of system suitability

Parameters	Bambuterol HCl	Mebeverine HCl (IS)	Montelukast sodium
Resolution	-	2.642	8.51
Tailing factor	1.401	1.264	1.468
Theoretical plates	2118	2046	5723

TABLE 2: Results of linearity

Analyte	Slope	Intercept	Correlation coefficient (r^2) (n=7)
Bambuterol hcl	14.21	27.71	0.9998
Montelukast sodium	34.35	11.43	0.9999

TABLE 3: Results of assay experiment

	Bambuterol HCl	Montelukast sodium
Drug found in mg/tablet (mean)	4.99	5.00
Mean %	99.86	99.92
RSD %	0.47	0.20

Linearity

Linearity was evaluated by analysis of working standard solutions of bambuterol hcl and montelukast sodium of seven different concentrations[3-4]. The range of linearity was from 100-300µg mL^{-1} for both bambuterol hcl and montelukast sodium. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained for the bambuterol HCl and montelukast sodium is represented in TABLE 2. The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration.

Limit of detection and limits of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively[3,4]. The LOD and LOQ of bambuterol hcl and montelukast sodium were experimentally determined by six injections of each drug. The LOD of bambuterol hcl and montelukast sodium was found to be 0.2µg mL^{-1} and 0.1µg mL^{-1} respectively. The LOQ of bambuterol HCl and montelukast sodium was found to be 0.4µg mL^{-1} and 0.2µg mL^{-1} respectively.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions[3-4]. The relative standard deviations was less than 2%. Method precision was determined from results from ten independent determinations at 100% of the test concentrations of bambuterol hcl and montelukast sodium in the product. The RSD was found to be 0.49. Refer TABLE 3.

Accuracy

To study accuracy of the method, recovery experiment was carried out by applying the standard addition method. A known quantity of drug substance corresponding to 100%, 110%, 120% and 130% of the label claim of drug was added, to determine if there are positive or negative interferences from excipients present in the formulation[4]. Each set of addition was repeated three times .The accuracy was expressed as the per

TABLE 4 : Accuracy of the method

Analyte	Initial conc. (ppm)	Conc. added (ppm)	Total conc.(ppm)	Conc. found(ppm)	RSD (%) n= 3	Recovery (%)	% Bias
Bambuterol HCl	500	0	500	499	0.16	99.80	+0.20
	500	50	550	549.84	0.09	99.97	+0.03
	500	100	600	601.08	0.11	100.18	-0.18
	500	150	650	649.96	0.07	99.99	+0.01
Montelukast sodium	500	0	500	500	0.14	100.00	+0.00
	500	50	550	551.06	0.06	100.19	-0.19
	500	100	600	600.84	0.19	100.14	-0.14
	500	150	650	649.91	0.0.3	99.99	+0.01

centage of analytes recovered by the assay. TABLE 4 lists the recoveries of the drug from a series of spiked concentrations. The results indicate the method is highly accurate for simultaneous determination of bambuterol hcl and montelukast sodium.

DISCUSSION AND CONCLUSION

Several mobile phases such as water-methanol, water-acetonitrile using orthophosphoric acid buffer in different ratios were tried but good peak shape and good resolution between Bambuterol hcl, Montelukast sodium and Mebeverine HCl was observed using the mobile phase mentioned in chromatographic conditions. The method after being completely validated showed satisfactory data for all the method validation parameters. The method was found to be specific. The low values of %RSD for Method precision suggested that the method is precise. Linearity evaluated for the analyte peak showed a good linear response over a wide range of concentration. The linearity, precision, accuracy of the method proves that the method is specific, accurate, easily reproducible and can be used for simultaneous determination of bambuterol hcl and montelukast sodium from pharmaceutical preparations.

REFERENCES

[1] Brithish Pharmacopea, **1**, 222-223 **(2008)**.

[2] European Pharmacopea 6.0, **2**, 1251-1252 **(2008)**.

[3] L.R.Snyder, J.J.Kirland, J.L.Glajch; 'Practical HPLC Method Development', 2nd edition, John Wiley and Sons, Inc., U.S.A., **(1997)**.

[4] ICH; Validation of Analytical Procedures: Methodology, ICH Harmonized Tripartite Guidelines, **(1997)**.

[5] Merck and Co.Ivana Gazic, Anita Bosak, Goran Sinko, Vladimir Vinkovic, Zrinka Kovarik; 'The Merck Index', 12th Edn., Anal.Bioanal.Chem., 25 **(2005)**.

[6] Ibrahim A.Alsarra; Saudi Pharmaceutical Journal, **12(4)**, **(2004)**.

[7] L.Liu, H.Cheng, J.J.Zhao, J.D.Rogers; J.Pharm. Biomed.Anal., **15**, 631-8 **(1997)**.

[8] H.Ochiai, N.Uchiyama, T.Takano, K.Hara, T. Kamei; J.Chromatogr.B.Biomed.Appl., **713**, 409-14 **(1998)**.

[9] T.Radhakrishna, A.Narasaraju, M.Ramakrishna, A.Satyanarayana; J.Pharm.Biomed.Anal ., **31**, 359-68 **(2003)**.