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Simultaneous RP-HPLC determination of mebeverine HCl and chlorodiazepoxide in pharmaceutical preparations

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ABSTRACT

A simple, fast and precise reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of mebeverine hcl and chlorodiazepoxide using methyl paraben as an internal standard. Chromatographic separation was performed on a waters symmetry C_{18} column (150mm3.9 mm, 5µm) as stationary phase with a mobile phase comprising of 0.5% orthophosphoric acid in water : acetonitrile (50:50 v/v), at a flow rate of 0.7mL min^{-1} and UV detection at 216nm. The Retention time of Mebeverine hcl, chlorodiazepoxide and methyl paraben were 3.067 min, 1.524 and 4.238 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 337.5-1012.5µg mL^{-1} for mebeverine hcl and 12.5-37.5µg mL^{-1} for chlorodiazepoxide. It can be conveniently adopted for routine quality control analysis.

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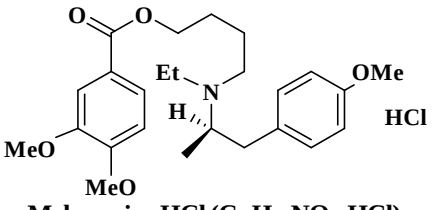
KEYWORDS

ICH guidelines;
Validation;
Column liquid chromatography;
Pharmaceutical preparations;
Mebeverine HCl;
Chlorodiazepoxide.

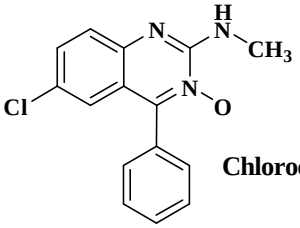
INTRODUCTION

Mebeverine HCl 4-[Ethyl(4-methoxy-α-methyl phenethyl)amino] butyl veratrate is antispasmodic agent with direct action on the smooth muscle of the gastrointestinal tract. It relieves the abdominal pain and cramps[1]. Chlorodiazepoxide is described chemically as 7-chloro-N-methyl-5-phenyl-3H-1,4 benzadiazepine -2-amine 4 oxide is a drug used in treating anxiety, insomnia, agitation, seizures, and muscle spasms[2]. The structure of both drugs is shown in figures 1 and 2. One such combination contains 135 mg of mebeverine HCl and 5 mg of chlorodiazepoxide. 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



Mebeverine HCl ($C_{25}H_{35}NO_5$, HCl)

Figure 1: Structures of mebeverine HCl



Chlorodiazepoxide ($C_{16}H_{14}ClN_3O$)

Figure 2: Structures of chlorodiazepoxide

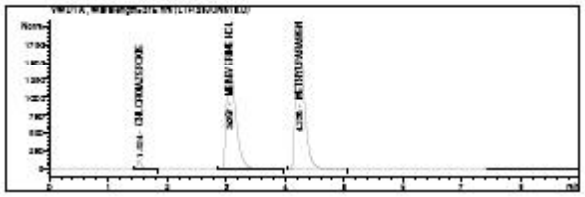


Figure 3: Chromatogram of mebeverine HCl and chloro diazepoxide with methyl paraben (internal standard) in standard preparation

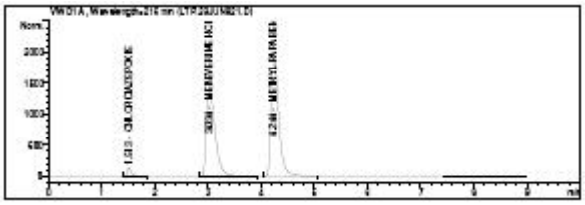


Figure 4: Chromatogram of mebeverine HCl and chloro diazepoxide with methyl paraben (internal standard) in sample preparation

for determination of mebeverine HCl and chloro diazepoxide from their combined dosage form[1,2,5-10]. The method described is simple, fast, precise and accurate for simultaneous determination of mebeverine HCl and chlorodiazepoxide from pharmaceutical preparation.

Chemicals and reagents

Standards were supplied from Nicholas Piramal (India) ltd, Mumbai, India.

Formulation containing mebeverine HCl 135 mg and chlorodiazepoxide 5 mg was procured from the market. Acetonitrile and orthophosphoric 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 Mebeverine HCl and chlorodiazepoxide 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 mebeverine HCl and chlorodiazepoxide was scanned on photo diode array detector for selecting the working wavelength. Peak purity of mebeverine HCl and chlorodiazepoxide was checked using photo diode array detector. Chromatograms and data were recorded by means of chemstation software. Waters symmetry C_{18} column (150mm×3.9 mm, 5µm particle) was used for the analysis. The mobile phase comprising of 0.5% ortho-phosphoric acid in water and acetonitrile (50:50 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 216 nm for simultaneous determination of mebeverine HCl and chlorodiazepoxide. A typical HPLC chromatogram for simultaneous determination of mebeverine HCl and chlorodiazepoxide from pharmaceutical formulation is shown in figures 3 and 4.

Preparation of standard stock solutions

The stock solution of mebeverine HCl (6750µg mL^{-1}) was prepared by dissolving 337 mg of mebeverine HCl (99.9%) in mix of water: acetonitrile (50:50) in a standard 50 mL volumetric flask (solution A). The stock solution of chlorodiazepoxide (250µg mL^{-1}) was prepared by dissolving 24.9 mg of chlorodiazepoxide (99.9%) in mix of water: acetonitrile (50:50) in a standard 100 mL volumetric flask (solution B). Internal standard (methyl paraben) stock solution (6750µg mL^{-1}) was prepared by dissolving 337.2 mg of methyl paraben (99.9%) in mix of water: acetonitrile (50:50) 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 (50:50).

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 one tablet was transferred in a 200mL volumetric flask dissolved in water: acetonitrile (50:50), 20 mL of solution

Full Paper

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 containing 675 µg mL^{-1} of mebeverine HCl and 25 µg mL^{-1} of chlorodiazepoxide using methyl paraben as an internal standard. This was repeated five times. The RSD values of mebeverine HCl and chlorodiazepoxide was 0.08% 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	Chlorodiazepoxide	Mebeverine HCl	Methyl paraben
Resolution	-	7.282	4.207
Tailing factor	1.605	1.706	1.591
Theoretical plates	2441	1666	4384

TABLE 2: Results of linearity

Analyte	Slope	Intercept	Correlation coefficient (r^2) (n=7)
Mebeverine HCl	23.35	117.43	0.9999
Chlorodiazepoxide	26.98	-8.43	0.9998

TABLE 3: Results of assay experiment

	Mebeverine HCl	Chlorodiazepoxide
Drug found in mg/tablet (mean)	135.07	5.04
Mean %	99.94	99.89
RSD %	0.11	0.14

Linearity

Linearity was evaluated by analysis of working standard solutions of mebeverine HCl and chlorodiazepoxide of seven different concentrations[3-4]. The range of linearity was from 337.5-1012.5µg mL^{-1} for mebeverine HCl and 12.5-37.5µg mL^{-1} chlorodiazepoxide. 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 mebeverine HCl and chlorodiazepoxide 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 mebeverine HCl and chlorodiazepoxide were experimentally determined by six injections of each drug. The LOD of mebeverine HCl and chlorodiazepoxide was found to be 0.1µg mL^{-1} and 0.1µg mL^{-1} respectively. The LOQ of mebeverine HCl and chlorodiazepoxide was found to be 0.3µg mL^{-1} and 0.4µg 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 deviation was less than 2.0%. Method precision was determined from results from ten independent determinations at 100% of the test concentrations of mebeverine HCl and chlorodiazepoxide in the product. The RSD was found

TABLE 4: Accuracy of the method

Analyte	Initial conc.(ppm)	Conc. added (ppm)	Total conc. (ppm)	Conc. Found (ppm)	RSD (%) n= 3	Recovery (%)
Mebeverine HCl	675	0	675	674.92	0.08	99.99
	675	67.5	742.5	742.52	0.11	100.00
	675	135	810	810.35	0.27	100.04
	675	202.5	877.5	877.62	0.08	100.01
Chlorodiazepoxide	25	0	25	25.01	0.54	100.05
	25	2.5	27.5	27.49	0.15	99.95
	25	5.0	30	29.97	0.34	99.90
	25	7.5	32.5	32.53	0.19	99.94

to be 0.11% and 0.14%. 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 percentage 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 mebeverine HCl and chlorodiazepoxide.

DISCUSSION AND CONCLUSION

Several mobile phases such as water-methanol, water-acetonitrile in different ratios were tried but good peak shape and good resolution between Mebeverine HCl, Chlorodiazepoxide and methyl paraben 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 mebeverine HCl and chlorodiazepoxide from pharmaceutical preparations.

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