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Original Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS QUANTITATIVE ESTIMATION OF MEBEVERINE HYDROCHLORIDE AND CHLORDIAZEPOXIDE IN CAPSULES

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ABSTRACT

Objective: To develop an accurate, precise and linear Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for simultaneous quantitative estimation of Mebeverine hydrochloride and Chlordiazepoxide in MEVA C capsules and validate as per ICH guidelines.

Methods: The optimized method uses a reverse phase column, Enable Make C18G (250 X 4.6 mm; 5µ), a mobile phase of triethylammonium phosphate buffer (pH 2.5): acetonitrile in the proportion of 60:40 v/v, flow rate of 1.0 ml/min and a detection wavelength of 240 nm using a UV detector.

Results: The developed method resulted in Mebeverine eluting at 5.8 min and Chlordiazepoxide at 3.9 min. Mebeverine exhibited linearity in the range 250-750µg/ml, while Chlordiazepoxide exhibited linearity in the range 9.25-27.75µg/ml. The precision is exemplified by relative standard deviations of 1.45% for Mebeverine and 1.58% for Chlordiazepoxide. Percentage Mean recoveries were found to be in the range of 98-102, during accuracy studies. The limit of detection (LOD) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 91.67µg/ml and 2.23µg/ml respectively, while limit of quantitation (LOQ) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 277.81µg/ml and 6.77µg/ml respectively.

Conclusion: A simple, accurate, precise, linear and rapid RP-HPLC method was developed for simultaneous quantitative estimation of Mebeverine hydrochloride and Chlordiazepoxide in MEVA C capsules and validated as per ICH guidelines. Hence it can be used for the routine analysis of Mebeverine and Chlordiazepoxide in capusles in various pharmaceutical industries.

Keywords: RP-HPLC, Mebeverine, Chlordiazepoxide, Method development, Validation.

INTRODUCTION

Mebeverine hydrochloride (**fig. 1**) is a white crystalline powder having a molecular formula $C_{25}H_{35}NO_5HCl$, molecular weight 466 and melting point 105-107ºC. It is freely soluble in water and ethanol (96%), while practically insoluble in diethyl ether[1]. IUPAC name of Mebeverine hydrochloride is 3,4-Dimethoxybenzoic acid 4-[ethyl[2-(4-methoxy phenyl)-1-methylethyl]amino]-butyl ester. It is a direct antispasmodic acting mainly on the smooth muscles of the gastrointestinal tract and particularly effective against the colonic spasm[2]. Mebeverine hydrochloride is widely used as a relaxant agent for the treatment of gastrointestinal spasmodic disorders such as irritable bowel syndrome [3].

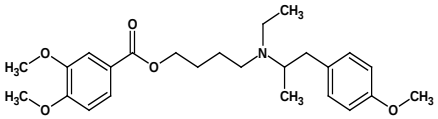

Fig. 1: Structure of Mebeverine hydrochloride

Chlordiazepoxide (fig. 2) is a first among the class of benzodiazepines to be used clinically as anti-anxiety drug [4]. Chlordiazepoxide is a white crystalline powder possessing solubility in water, whose IUPAC name is (7-chloro-2(methyl amino)-5-phenyl-3-H-1,4 benzodiazepine 4-oxide). Chlordiazepoxide mainly acts on limbic system and ascending reticular formation in the central nervous system. It binds to stereo specific benzodiazepine binding sites on GABA receptor complexes at several sites within the central nervous system including the limbic system and reticular formation. The binding will facilitates GABA mediated chloride channel opening and produce hyperpolarisation. This will increase the concentration of inhibitory neurotransmitter GABA and chloride ions in the CNS and decreases firing rate of neurons [5]. Mebeverine hydrochloride (135mg) and Chlordiazepoxide (5mg) is commercially available as capsules (trade name: MEVA C).

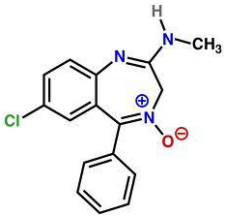

Fig. 2: Structure of Chlordiazepoxide

A detailed literature survey reveals that there exists literature concerning analytical method development and validation for individual drugs Mebeverine [1,3,6-7] and Chlordiazepoxide [8-13] in various matrices. Also analytical methods are reported for Mebeverine with other drug combinations [2,14-19] and similarly Chlordiazepoxide with other drug combinations[4,20-23]. While there is only one literature reported on RP-HPLC method development and validation for the simultaneous quantitative estimation of Mebeverine and Chlordiazepoxide in pharmaceutical dosage forms [24]. Hence we have explored in developing a new, accurate, precise and linear RP-HPLC method for the simultaneous quantitative estimation of Mebeverine and Chlordiazepoxide in MEVA C capsules and validate as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure sample of Mebeverine and Chlordiazepoxide with purities greater than 99% were obtained as gift samples from Chandra Labs, Hyderabad, India and tablet formulation [MEVA C] was procured from Medplus pharmacy, Hyderabad, India with labelled amount 5mg and 135mg of Chlordiazepoxide and Mebeverine respectively. Acetonitrile (HPLC grade) was obtained from Sigma aldrich (Hyderabad, India), water (HPLC grade),

Triethylamine (AR grade), ortho phosphoric acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India), 0.45µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on Shimadzu LC-20AD Prominence Liquid Chromatograph comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Enable C18G (250 X 4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "Lab solutions lite" software. A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance. An electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH) and UV-Visible Spectrophotometer (Shimadzu UV-1800 series, software-UV probe version 2.42) were used in this study.

Methods

Selection of Wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrums in the range of 200-400 nm for individual drug solutions of Chlordiazepoxide and Mebeverine. Suitable wavelength selected for simultaneous estimation is 240 nm (fig. 3-4).

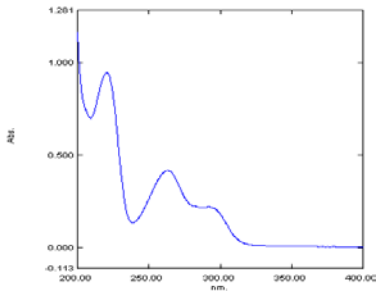

Fig. 3: UV spectrum of standard Mebeverine hydrochloride

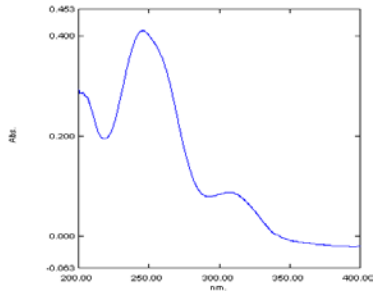

Fig. 4: UV spectrum of standard Chlordiazepoxide

Preparation of stock and working standard solution for Mebeverine hydrochloride

10mg of Mebeverine hydrochloride was accurately weighed and taken in 10 ml clean and dry volumetric flask containing 8 ml of solvent and then the solution was made up to the mark using the solvent. This is considered as standard stock solution (1000µg/ml). 5 ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 500µg/ml, treated as working standard, 100% target concentration.

Preparation of stock and working standard solution for Chlordiazepoxide

10mg of Chlordiazepoxide was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 80 ml of solvent and then the solution was made up to the mark using the solvent. This is considered as standard stock solution (100µg/ml). 1.85 ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 18.5µg/ml, treated as working standard, 100% target concentration.

Preparation of stock and working sample solution

Ten capsules were opened having white powder with tablet. Grinded totally uniformly and the average weight was determined. The average weight was transferred to a 100 ml volumetric flask containing 100 ml diluent and then stirred for 10 minutes, followed by filtration through 0.45µ nylon membrane filter to get sample stock solution of 1.35mg/ml of Mebeverine hydrochloride and 50µg/ml. 3.7 ml of the above stock solution was pipetted out and made up to 10 ml to get working sample solution equivalent to a concentration of working standard of 500µg/ml for Mebeverine hydrochloride and 18.5µg/ml for Chlordiazepoxide.

RESULTS AND DISCUSSION

Method development

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i. e. resolution factor (Rs) between peaks, tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Mebeverine at 5.8 min and chlordiazepoxide at 3.9 min. Fig. **5** and **6** represent chromatograms of blank solution and mixture of standard solution respectively. The total run time is 8 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*Rt*), number of theoretical plates (*N*), peak resolution (Rs) and peak Tailing factor (T) was evaluated for six replicate injections of the standards at the working concentration. The results given in table 1 were within acceptable limits.

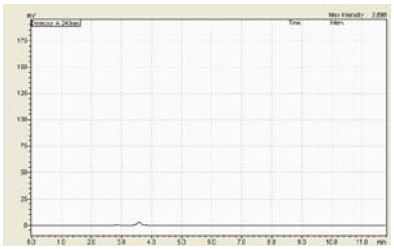

Fig. 5: Typical Chromatogram of Blank solution

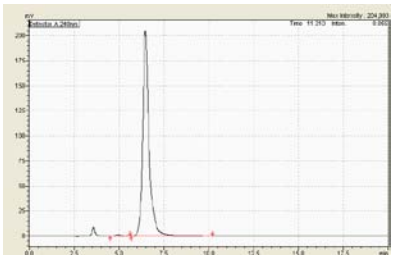

Fig. 6: Typical chromatogram of Mebeverine standard solution

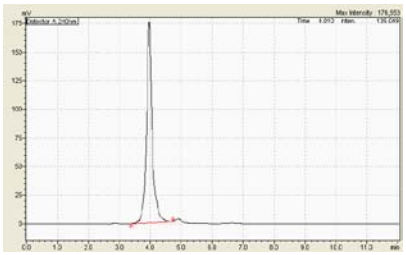

Fig. 7: Typical chromatogram of Chlordiazepoxide standard solution

Table 1: System suitability studies results.

Parameters	Acceptance Limits	Mebeverine	Chlordiazepoxide
Retention time (min)	-	5.8	3.9
Resolution factor (Rs)	Not less Than 2	4.028	
Number Of Theoretical plates (N)	Not less Than 2000	2754	2738
Tailing factor (T)	Not More Than 2	1.5	1.1

In order to test the applicability of the developed method to a commercial formulation, 'MEVA C' capsules were chromatographed at working concentration and it is shown in fig. 8. The sample peaks were identified by comparing the relative retention times with the standard drugs (fig. 6-7). System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and each drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible quantification of the two drugs with an error less than 10%, which is the standard level in any pharmaceutical quality control.

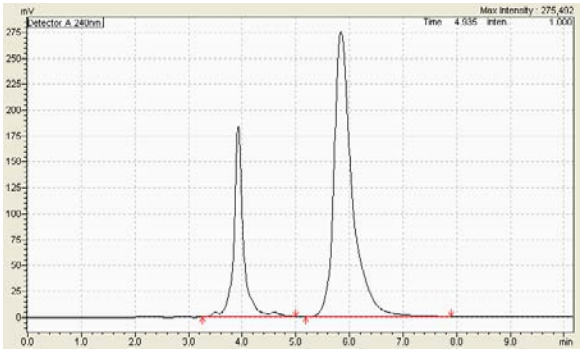

Fig. 8: Typical chromatogram of the sample

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. UV spectrophotometric method developed was validated according to International Conference on Harmonization (ICH) guidelines [25] for validation of analytical procedures. The method was validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ ruggedness, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

Specificity

Fig. 5-8 for blank, standard drug solutions and sample chromatogram reveal that the peaks obtained in the standard solutions and sample solution at working concentrations are only because of the drugs as blank has no peak at the retention time of Mebeverine and Chlordiazepoxide standards. Accordingly it can be concluded that, the method developed is said to be specific.

Precision

System precision

Six replicate injections of the standard solutions at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning peak area for both the drugs, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in table 2.

Method precision

Method precision was determined by performing the assay of the sample under the test of repeatability (intraday precision) at working concentrations.

Repeatability (Intraday precision)

Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for both the drugs which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 3).

Table 2: System precision results of Mebeverine and Chlordiazepoxide

S. No.	Mebeverine	Chlordiazepoxide
1	5669077.55	2314885
2	5601566.47	2294885
3	5674688.8	2281105
4	5643246.25	2321465
5	5674139.47	2289484
6	5614483.05	2306987
Average	5646200.26	2301468.5
STD	32007.6535	15568.938
%RSD	0.52249571	0.6764784

Table 3: Intraday precision results of Mebeverine hydrochloride and Chlordiazepoxide

S. No.	Mebeverine HCl % Assay	Chlordiazepoxide % Assay
1	99.7	98.1
2	101.4	98.7
3	98.4	102.2
4	99.9	101.5
5	101.6	100
6	98.2	99.8
Average	99.9	100.1
S. D.	1.455427	1.5833392
% RSD	1.457358	1.5822551

Linearity

Standard solutions of Mebeverine hydrochloride and Chlordiazepoxide at different concentrations level (50%, 75%, 100%, 125%, and 150%) were prepared in triplicates. Calibration curves were constructed by plotting the concentration level versus corresponding peak areas for both the drugs. The results show an excellent correlation between peak areas and concentratiosn level within the concentration range of 250-750µg/ml for Mebeverin and 9.25-27.75µg/ml for Chlodiazepoxide (Tables 4-5). The correlation coefficients were greater than 0.99 for both the drugs, which meet the method validation acceptance criteria and hence the method is said to be linear for both the drugs.

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of both the drugs in the formulation at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery is calculated as showed in table 6**.** The accepted limits of mean recovery are 98% -102% and all observed data were within the required range, which indicates good recovery values and hence the accuracy of the method developed.

Table 4: Calibration data for Mebeverine

% Level	Concentration (µg/ml)	Peak Area 1	Peak Area 2	Peak Area 3
50	250	2882243	2654097	2609042
75	375	4628074	4558396	4161541
100	500	6091505	6213558	5351755
125	625	7812321	8084952	6513541
150	750	9462067	9510845	8116525
Regression equation		y=13075x-362316	y=13792x-69165	y=10693.5x+3694
Regression coefficient		0.999	0.997	0.996

Table 5: Calibration data for Chlordiazepoxide

% Level	Concentration (µg/ml)	Peak Area 1	Peak Area 2	Peak Area 3
50	9.25	1188889	1019916	956907
75	13.87	1791164	1664627	1550466
100	18.5	2614885	2406932	2281105
125	23.12	3260272	3150272	2873943
150	27.75	4156826	4026826	3565136
Regression equation		y=160111x-359331	y=162152x-545786	y=141405x-370208
Regression coefficient		0.996	0.997	0.999

Table 6: Results of Accuracy studies for Mebeverine hydrochloride and Chlordiazepoxide

Concentration level (%)	% Mean recovery mebeverine HCl	% Mean recovery chlordiazepoxide
50	99.53	99.52
100	99.4	99.93
150	99.08	99.07

Sensitivity

The sensitivity of measurement of Mebeverine hydrochloride and Chlordiazepoxide by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and limit of detection (LOD). LOQ and LOD were calculated by the use of the equations LOD = 3.3σ/S and LOQ = 10σ/S where σ is the standard deviation of intercepts of calibration plots and S is the average of the slopes of the corresponding calibration plot.

The limit of detection (LOD) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 91.67µg/ml and 2.23µg/ml respectively, while limit of quantitation (LOQ) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 277.81µg/ml and 6.77µg/ml respectively.

CONCLUSION

A reverse phase HPLC isocratic method developed has been validated as per ICH guidelines in terms of specificity, accuracy, precision, linearity, limit of detection and limit of quantitation, for for simultaneous quantitative estimation of Mebeverine hydrochloride and Chlordiazepoxide in MEVA C Capsules. The developed method resulted in Mebeverine eluting at 5.8 min and Chlordiazepoxide at 3.9 min. Mebeverine exhibited linearity in the range 250-750µg/ml, while Chlordiazepoxide exhibited linearity in the range 9.25-27.75µg/ml.

The precision is exemplified by relative standard deviations of 1.45% for Mebeverine and 1.58% for Chlordiazepoxide. Percentage Mean recoveries were found to be in the range of 98-102, during accuracy studies. The limit of detection (LOD) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 91.67µg/ml and 2.23µg/ml respectively, while limit of quantitation (LOQ) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 277.81µg/ml and 6.77µg/ml respectively.

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CONFLICT OF INTERESTS

Declared None

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