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Original Article

SIMULTANEOUS QUANTITATIVE ESTIMATION OF MEBEVERINE HYDROCHLORIDE AND CHLORDIAZEPOXIDE IN CAPSULES USING SPECTROPHOTOMETRY

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ABSTRACT

Objective: To develop a simple and cheap UV spectrophotometric method for the simultaneous quantitative estimation of Mebeverine hydrochloride and Chlordiazepoxide in MEVA C Capsules and validate as per ICH guidelines.

Methods: The optimized method uses triethylammonium phosphate buffer (pH 4.0) as a solvent for the estimation of assay of Mebeverine hydrochloride and Chlordiazepoxide in capsules at detection wavelengths of 263 nm and 245 nm respectively.

Results: The developed method exhibited linearity in the range of 7.5-22.5µg/ml for Mebeverine hydrochlorideand 2.5-7.5µg/ml for Chlordiazepoxide. Precision for Mebeverine hydrochloride and Chlordiazepoxide is exemplified by relative standard deviation of 0.589% and 0.332 respectively. Percentage Mean recovery for Mebeverine hydrochloride and Chlordiazepoxide was found to be in the range of 98-102, during accuracy studies. The limit of detection (LOD) for Mebeverine hydrochloride and Chlordiazepoxide was found to be 528ng/ml and 192ng/ml respectively, while limit of quantitiation (LOQ) for Mebeverine hydrochloride and Chlordiazepoxide was found to be 1.6µg/ml and 583ng/ml respectively.

Conclusion: A simple and a cheap UV spectrophotometric method were developed and validated for the simultaneous quantitative estimation of Mebeverine hydrochloride and Chlordiazepoxide in capsules as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

Keywords: UV, Mebeverine hydrochloride, Chlordiazepoxide, Method development, Validation.

INTRODUCTION

Mebeverine hydrochloride (Figure 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].

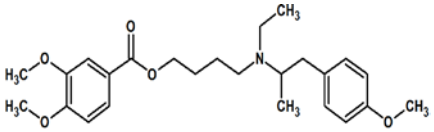

Fig. 1: Structure of Mebeverine hydrochloride

Chlordiazepoxide **(Figure 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(methylamino)-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 stereospecific 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).

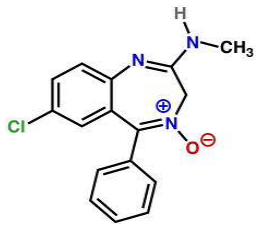

Fig. 2: Structure of Chlordiazepoxide

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 UV spectrophotometric method development and validation for the simultaneous quantitative estimation of Mebeverine and Chlordiazepoxide as drug combination in pharmaceutical dosage forms[24]. Hence we have explored in developing a new, accurate, precise and linear UV spectrophotometric method for the quantitative estimation of Mebeverine and Chlordiazepoxide in MEVA C capsules and validate as per ICH guidelines.

MATERIALS AND METHODS

Materials

Instrument

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 weighing analytical balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101) and a sonicator (sonica, model 2200 MH) were used in this study.

Chemicals and Reagents

Analytically pure samples of Mebeverine hydrochloride 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, Hyderabad, India with labelled amount 135mg of Mebeverine hydrochloride and 5mg of Chlordiazepoxide. Triethylamine (AR Grade) and 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.

Methods

Solvent

Solvent used is prepared by adding 5 ml of triethylamine to 1000 ml of distilled water and later pH was adjusted to 4.0 using 30% v/v of ortho phosphoric acid in water.

Selection of suitable detection wavelength

Suitable wavelength for Mebeverine hydrochloride and Chlordiazepoxide for the total experiment was determined by recording UV spectrums in the range of 200-400 nm and suitable wavelength for Mebeverine hydrochloride and Chlordiazepoxide was considered as 263 nm and 245 nm respectively. (Figure 3 and 4).

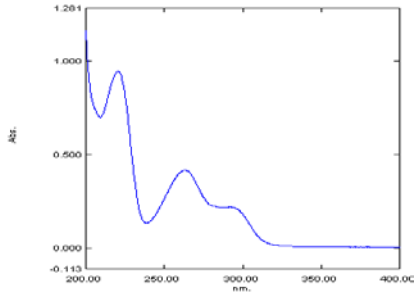

Fig. 3: UV spectrum of standard Mebeverine hydrochloride.

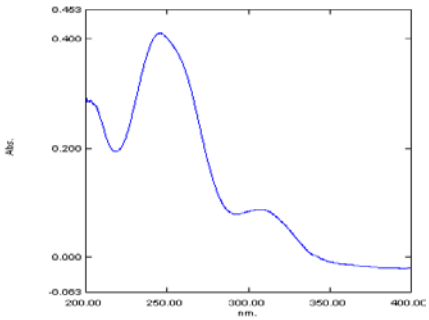

Fig. 4: UV spectrum of standard Chlordiazepoxide.

Preparation of stock and working standard solution for mebeverine hydrochloride

10mg of Mebeverine hydrochloride was accurately weighed and taken in 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 the standard stock solution (100µg/ml). 1.5 ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 15µg/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 the standard stock solution (100µg/ml). 0.5 ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 5µg/ml, treated as working standard, 100% target concentration.

Preparation of stock and working sample solution for mebeverine hydrochloride

Ten capsules were opened and only white powders in it were weighed separately 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. 0.11 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 15µg/ml.

Preparation of stock and working sample solution for chlordiazepoxide

Ten capsules were opened and only tablets in it were weighed separately and the average weight was determined. Ten tablets present in ten capsules were grinded in a pestle and mortar and 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 50µg/ml. 1 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 5 µg/ml.

RESULTS AND DISCUSSION

Method development

Various buffers were explored (inorganic and organic based) as solvents, including Potassium dihydrogen orthophosphate, triethylammonium phosphate and ammonium acetate buffers varying pH in the ranges of 2-7. Mebeverine hydrochloride and Chlordiazepoxide were found to be soluble and stable for minimum of 1 hour at room temperature using pH 4.0 triethylammonium phosphate buffer and hence this buffer was used for the determination of suitable detection wavelength and working concentration of both drugs. In order to test the applicability of the developed method to a commercial formulation, MEVA C was studied at working concentration. Assay at working concentration for both the samples in the formulation was in acceptance limits (98-102%) during extraction of drugs in the samples using the solvent for 10 minutes. The protocol affords reproducible quantification of the drugs in the samples ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control. Hence the method is optimized. Figures 5 and 6 represent UV spectrums of the formulation, mebeverine hydrochloride and chlodiazepoxide respectively.

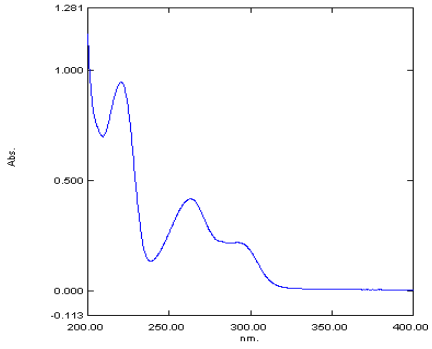

Fig. 5: UV spectrum of Mebeverine hydrochloride (white powder) of the sample.

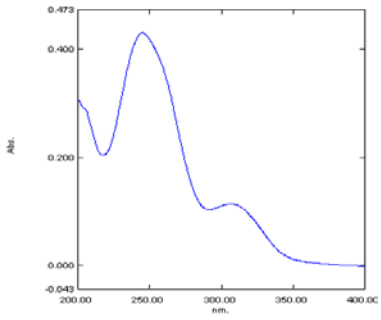

Fig. 6: UV spectrum of Chlordiazepoxide (tablet) of the sample.

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 quantitiation (LOQ).

Precision

System precision

Six replicate recording of absorbances for both the drugs at working concentration showed % RSD (Relative Standard Deviation) less than 2, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in Table 1.

Method precision

Method precision was determined by performing assay of both the drugs in the formulation under the test of repeatability (Intra day precision) at working concentration.

Repeatability (Intra day precision)

Six replicate recording of absorbances for both the samples in the formulation from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for 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 2).

Table 1: System precision results (UV absorbance) of Mebeverine and Chlordiazepoxide

n	Mebeverine hydrochloride 263 nm	Chlordiazepoxide 245 nm
1	0.407	0.431
2	0.411	0.430
3	0.413	0.428
4	0.409	0.430
5	0.412	0.432
Average	0.410	0.4302
SD	0.0024	0.0014
% RSD	0.585	0.3254

Table 2: Intra day precision results of Mebeverine hydrochloride and Chlordiazepoxide

n	Mebeverine HCl (263 nm)	Chlordiazepoxide 245 nm
	% Assay	% Assay
1	100.79	99.00
2	99.81	99.23
3	99.32	99.69
4	100.29	99.23
5	99.56	99.77
Average	99.95	99.38
S. D.	0.589	0.330
% RSD	0.589	0.332

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 absorbance for both the drugs. The results show an excellent correlation between absorbance and concentration level within the concentration range of 7.5-22.5µg/ml for Mebeverine hydrochloride and 2.5-7.5µg/ml for Chlodiazepoxide (Tables 3 and 4). 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.

Table 3: Calibration data for Mebeverine hydrochloride (263 nm)

% Level	Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3
50	07.50	0.204	0.210	0.209
75	11.25	0.282	0.286	0.278
100	15.00	0.413	0.410	0.412
125	18.75	0.495	0.490	0.489
150	22.50	0.598	0.591	0.588
Regression equation		y=0.026x-0.002	y=0.025x+0.011	y=0.025x+0.0076
Regression coefficient		0.994	0.995	0.991

Table 4: Calibration data for Chlordiazepoxide (245 nm)

% Level	Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3
50	2.5	0.209	0.214	0.209
75	3.75	0.327	0.322	0.325
100	5	0.432	0.430	0.435
125	6.25	0.527	0.531	0.536
150	7.5	0.630	0.645	0.653
Regression equation		y=0.083x-0.0101	y=0.085x-0.0098	y=0.086x-0.001
Regression coefficient		0.999	0.997	0.999

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 and %RSD between recoveries are calculated as shown in Table 5. The accepted limits of mean recovery are 98% -102% and %RSD not more than 2% and all observed data were within the required range, which indicates good recovery values and hence the accuracy of the method developed.

Table 5: Results of Accuracy studies for Mebeverine hydrochloride and Chlordiazepoxide

Concentration level (%)	% Mean Recovery Mebeverine HCl	% Mean Recovery Chlordiazepoxide
50	100.67	98.97
100	99.95	99.38
150	101.02	99.78

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is concluded that the method is robust as it is found that the % RSD is less than 2 regarding the determination of assays of both the drugs in the formulation, despite deliberate variations done concerning pH ± 0.2 and detection wavelength ± 2 nm (Tables 6 and 7).

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 = 3.3 x N / B and LOQ = 10x N / B where N is the standard deviation of intercepts of calibration plots and B is the average of the slopes of the corresponding calibration plot.

Table 6: Robustness results of Mebeverine hydrochloride sample

Variation parameter	Variation	% mean Assay	%RSD
pH(± 0.2)	2.8	100.02	0.588
	3.0	99.65	0.643
	3.2	98.99	0.410
Wave length (± 2 nm)			
	261	100.7	0.956
	263	101.2	1.045
	265	99.54	1.120

Table 7: Robustness results of Chlordiazepoxide sample

Variation parameter	Variation	% mean Assay	%RSD
pH(± 0.2)	2.8	99.63	0.342
	3.0	99.36	0.998
	3.2	100.76	0.682
Wave length (± 2 nm)	243	98.63	0.482
	245	99.2	0.396
	247	100.7	0.421

The limit of detection (LOD) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 528ng/ml and 192ng/ml respectively, while limit of quantitiation (LOQ) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 1.6µg/ml and 583ng/ml respectively. Optical characteristics and validation parameters results are summarized in Table 8.

Table 8: Optical characteristics and validation parameters

Parameters	Mebeverine hydrochloride	Chlordiazepoxide
Detection wavelength (nm)	263	245
Beer's Law limits (µg/ml)	7.5-22.5	2.5-7.5
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.1	0.01
Regression equation (y = mx+c)	(y y=0.025x+0.0055	Y=0.084x+0.0069
Correlation coefficient (r^2)	0.993	0.999
Slope (m)	0.025	0.084
Intercept (c)	0.0055	0.0069
(% RSD) System precision	0.585	0.325
(% RSD) Intra-day precision	0.589	0.332
(% RSD) Inter-day precision	≤2	≤2
Accuracy (% Mean Recovery)		
50 % Level	100.67	98.97
100 % Level	99.95	99.38
150 % Level	101.02	99.78
LOD (µg/ml)	0.528	0.192
LOQ (µg/ml)	1.6	0.583
Robustness		
pH(± 0.2) (% RSD)	≤2	≤2
Wavelength (± 2 nm) (% RSD)	≤2	≤2

CONCLUSION

A cheap and a rapid UV spectrophotometric method was developed and validated for the quantitative estimation of Mebeverine hydrochloride and Chlordiazepoxide in capsules as per ICH guidelines. The developed method exhibited linearity in the range of 7.5-22.5µg/ml for Mebeverine hydrochloride and 2.5-7.5µg/ml for Chlordiazepoxide.

The precision for Mebeverine hydrochloride and Chlordiazepoxide is exemplified by relative standard deviation of 0.589% and 0.322 respectively.

Percentage Mean recovery for Mebeverine hydrochloride and Chlordiazepoxide 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 528ng/ml and 192ng/ml respectively, while limit of quantitiation (LOQ) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 1.6µg/ml and 583ng/ml respectively. Accordingly it is concluded that the developed UV spectrophotometric method is accurate, precise, linear, rugged and robust and therefore the method can be used for the routine analysis of Mebeverine hydrochloride and Chlordiazepoxide in capsules in various pharmaceutical industries.

CONFLICT OF INTERESTS

Declared None

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