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Original Article

# UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS QUANTITATIVE ESTIMATION OF MEBEVERINE HYDROCHLORIDE AND CHLORDIAZEPOXIDE IN CAPSULES

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**ABSTRACT**

**Objective:** To develop a simple and cheap UV spectrophotometric method for the simultaneous quantitative estimation of Mebeverine hydrochloride (135mg) and Chlordiazepoxide (5mg) in MEVA C Capsules and validate as per ICH guidelines.

**Methods:** The optimized method uses a diluent 100% Triethylammonium phosphate buffer (pH 3.0) for the estimation of assay of Mebeverine hydrochloride and Chlordiazepoxide in Capsules which are analyzed at a detection wavelength of 260nm.

**Results:** The developed method exhibited linearity in the range of 10-40µg/ml for Mebeverine hydrochloride and 2.5-10µg/ml for Chlordiazepoxide. The precision for Mebeverine hydrochloride and Chlordiazepoxide is exemplified by relative standard deviation of 0.499% and 1.75 respectively. Percentage Mean recovery for Mebeverine hydrochloride and Chlordiazepoxide were found to be in the range of 98102, during accuracy studies. The limit of detection (LOD) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 321ng/ml and 3.7ng/ml respectively, while limit of quantitiation (LOQ) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 973ng/ml and 11.2ng/ml respectively.

**Conclusion:** A simple and a cheap UV spectrophotometric method was 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 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]-butylester. 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].

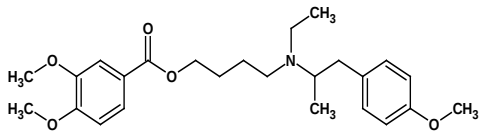

**Fig. 1: Structure of Mebeverine hydrochloride**

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 hardly any 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. 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 analytical weighing 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.

**Method**

**Solvent**

Solvent used is prepared by adding 5ml of triethyl amine to 1000 ml of distilled water and later pH was adjusted to 3.0 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 both the drugs selected was 260 nm (**Figure 3 and 4**).

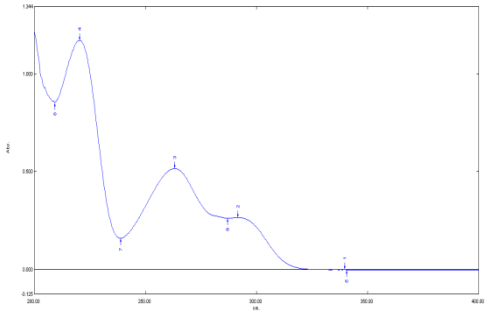

**Fig. 3: UV spectrum of Mebeverine hydrochloride.**

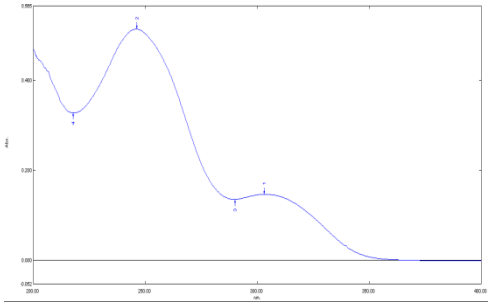

**Fig. 4: UV spectrum of Chlordiazepoxide.**

**Preparation of stock and working standard solution for Mebeverine hydrochloride**

10mg of Mebeverine hydrochloride was accurately weighed and taken in 100ml clean and dry volumetric flask containing 80ml of solvent and then the solution was made up to the mark using the solvent. This is considered as standard stock solution (100µg/ml). 2ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 20µg/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 100ml clean and dry volumetric flask containing 80ml of solvent and then the solution was made up to the mark using the solvent. This is considered as standard stock solution (100µg/ml). 0.5ml 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 powder was weighed separately and the average weight was determined. The average weight weighed from the ten tablets was transferred to a 100 ml volumetric flask containing 100ml 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. 0.1481 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 20µg/ml.

**Preparation of stock and working sample solution for Chlordiazepoxide**

Ten capsules were opened and only tablet was weighed separately and the average weight was determined. Ten tablets were grinded in a pestle and mortar and the average weight was transferred to a 100 ml volumetric flask containing 100ml 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 solvents were explored, 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 3.0 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 at 260 nm 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.

**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 [24] 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 absorbance at 260nm 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 tests of (i) repeatability (Intra day precision) and (ii) Intermediate precision (Inter day precision) performed during 3 consecutive days by three different analysts, at working concentration.

**Table 1: System precision results of Mebeverine hydrochloride and Chlordiazepoxide.**

| N | Mebeverine hydrochloride | Chlordiazepoxide |
|---|---|---|
| 1 | 0.539 | 0.458 |
| 2 | 0.543 | 0.471 |
| 3 | 0.542 | 0.448 |
| 4 | 0.550 | 0.455 |
| 5 | 0.551 | 0.458 |
| 6 | 0.536 | 0.464 |
| Average | 0.543 | 0.459 |
| SD | 0.0073 | 0.0078 |
| % RSD | 1.34 | 1.699 |

**Repeatability (Intra day precision)**

Six replicate recording of absorbance at 260nm 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 2: Intra day precision results of Mebeverine hydrochloride and Chlordiazepoxide**

| n | Mebeverine HCl | Chlordiazepoxide |
|---|---|---|
| | **% Assay** | **% Assay** |
| 1 | 98.3 | 98.1 |
| 2 | 98.6 | 98.4 |
| 3 | 98.01 | 99.2 |
| 4 | 98.7 | 100.1 |
| 5 | 99 | 101.8 |
| 6 | 99.4 | 102.3 |
| **Average** | **98.66** | **99.98** |
| **S.D.** | **0.493** | **1.75** |
| **% RSD** | **0.499** | **1.75** |

**Intermediate Precision (Inter day precision / Ruggedness)**

Six replicate recording of absorbance at 260nm for both the samples in the formulation from the same homogeneous mixture at working concentration on three consecutive days by three different analysts showed % RSD less than 2 concerning % assay for the drugs within and between days, which indicate the method developed is inter day precise / rugged (**Table 3)**.

**Table 3: Inter day precision results of Mebeverine hydrochloride and Chlordiazepoxide.**

| n | Mebeverine hydrochloride | | | Chlordiazepoxide | | |
|---|---|---|---|---|---|---|
| | **Day 1** | **Day 2** | **Day 3** | **Day 1** | **Day 2 Day 3** | |
| 1 | 98.3 | 99.8 | 100.44 | 98.1 | 100.44 | 99.48 |
| 2 | 98.6 | 99.01 | 101.1 | 98.4 | 101.1 | 99.32 |
| 3 | 98.01 | 101.3 | 100.3 | 99.2 | 100.3 | 98.3 |
| 4 | 98.7 | 101 | 100.6 | 100.1 | 100.6 | 100.3 |
| 5 | 99 | 100 | 100.2 | 101.8 | 100.2 | 100.4 |
| 6 | 99.4 | 101 | 99.8 | 102.35 | 99.8 | 99.55 |
| Average | 98.66 | 100.35 | 100.4 | 99.98 | 100.4 | 99.55 |
| SD | 0.493 | 0.89 | 0.555 | 1.75 | 0.555 | 0.681 |
| % RSD | 0.499 | 0.88 | 0.433 | 1.75 | 0.433 | 0.7622 |

**Table 4: Calibration data for Mebeverine hydrochloride**

| % Level | Concentration (µg/ml) | Absorbance 1 | Absorbance 2 | Absorbance 3 |
|---|---|---|---|---|
| 50 | 10 | 0.3207 | 0.2738 | 0.3155 |
| 75 | 15 | 0.4197 | 0.3697 | 0.4117 |
| 100 | 20 | 0.5684 | 0.5239 | 0.5657 |
| 125 | 25 | 0.6964 | 0.6501 | 0.684 |
| 150 | 30 | 0.8481 | 0.8137 | 0.8325 |
| 175 | 35 | 1.0010 | 0.9494 | 0.9837 |
| 200 | 40 | 1.095 | 1.0444 | 1.0785 |
| **Regression equation** | | y=0.269x+0.0336 | y=0.268x-0.0118 | y=0.264x+0.0347 |
| **Regression coefficient** | | 0.996 | 0.996 | 0.9968 |

**Table 5: Calibration data for Chlordiazepoxide**

| % Level | Concentration (µg/ml) | Absorbance 1 | Absorbance 2 | Absorbance 3 |
|---|---|---|---|---|
| 50 | 2.5 | 0.209 | 0.215 | 0.212 |
| 75 | 3.75 | 0.325 | 0.330 | 0.337 |
| 100 | 5 | 0.435 | 0.442 | 0.447 |
| 125 | 6.25 | 0.536 | 0.537 | 0.538 |
| 150 | 7.5 | 0.653 | 0.651 | 0.654 |
| 175 | 8.75 | 0.755 | 0.797 | 0.798 |
| 200 | 10 | 0.880 | 0.879 | 0.882 |
| **Regression equation** | | y=0.883x-0.0101 | y=0.895x-0.0098 | y=0.896x-0.00796 |
| **Regression coefficient** | | 0.999 | 0.997 | 0.997 |

**Linearity**

Standard solutions of Mebeverine hydrochloride and Chlor diaz epoxide at different concentrations level (50%, 75%, 100%, 125%, 150%, 175% and 200%) were prepared in triplicates. Calibration curves were constructed by plotting the concentration level versus corresponding absorbance at 260nm for both the drugs. The results show an excellent correlation between absorbance and concentration level within the concentration range of 10-40µg/ml for Mebeverine hydrochloride and 2.5-10µg/ml for Chlodiazepoxide (**Tables 4 and 5).** The correlation coefficients were greater than 0.995 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 and %RSD between recoveries are calculated as shown in **Table 6.** 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 6: Results of Accuracy studies for Mebeverine hydrochloride and Chlordiazepoxide**

| Concentration level (%) | % Mean recovery mebeverine HCl | % Mean recovery chlordiazepoxide |
|---|---|---|
| 50 | 99.5 | 99.5 |
| 100 | 98.7 | 99.8 |
| 150 | 100.6 | 99.06 |

**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 for both the drugs concerning % assay despite deliberate variations done concerning pH ± 0.2 and detection wavelength ± 2nm (**Tables 7 and 8**).

**Table 7: Robustness results of Mebeverine hydrochloride sample**

| Variation parameter | Variation | % mean Assay | %RSD |
|---|---|---|---|
| pH(± 0.2) | 2.8 | 99.7 | 1.23 |
| | 3.0 | 98.5 | 0.620 |
| | 3.2 | 98.5 | 0.410 |
| Wave length (± 2nm) | 258 | 100.5 | 1.85 |
| | 260 | 100.2 | 1.868 |
| | 262 | 100.2 | 1.616 |

**Table 8: Robustness results of Chlordiazepoxide sample**

| Variation parameter | Variation | % mean Assay | %RSD |
|---|---|---|---|
| pH(± 0.2) | 2.8 | 99.06 | 0.764 |
| | 3.0 | 98.8 | 0.703 |
| | 3.2 | 100.9 | 0.198 |
| Wave length (± 2nm) | 258 | 99.4 | 0.713 |
| | 260 | 99.2 | 1.321 |
| | 262 | 100.3 | 1.611 |

**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). The limit of detection (LOD) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 321ng/ml and 3.7ng/ml respectively, while limit of quantitiation (LOQ) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 973ng/ml and 11.2ng/ml respectively. Optical characteristics and validation parameters results are summarized in **Table 9**.

**Table 9: Optical characteristics and validation parameters of Mebeverine hydrochloride and Chlordiazepoxide**

| Parameters | Mebeverine hydrochloride | Chlordiazepoxide |
|---|---|---|
| Detection wavelength (nm) | 260 | 260 |
| Beer's Law limits (µg/ml) | 10-40 | 2.5-10 |
| Regression equation (y = mx+c) | (y y=0.267x+0.0188 | Y=0.891x-0.0092 |
| Correlation coefficient ($r^2$) | 0.996 | 0.997 |
| Slope (m) | 0.267 | 0.891 |
| Intercept (c) | 0.0188 | -0.0092 |
| (% RSD) System precision | 1.34 | 1.699 |
| (% RSD) Intra-day precision | 0.499 | 1.75 |
| (% RSD) Inter-day precision | ≤2 | ≤2 |
| Accuracy (% Mean Recovery) | | |
| 50 % Level | 99.5 | 99.5 |
| 100 % Level | 98.7 | 99.8 |
| 150 % Level | 100.6 | 99.06 |
| LOD (µg/ml) | 0.321 | 0.0037 |
| LOQ (µg/ml) | 0.973 | 0.0112 |
| Robustness | | |
| pH(± 0.2) (% RSD) | ≤2 | ≤2 |
| Wavelength (± 2nm) (% 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 10-40µg/ml for Mebeverine hydrochloride and 2.5-10µg/ml for Chlordiazepoxide. The precision for Mebeverine hydrochloride and Chlordiazepoxide is exemplified by relative standard deviation of 0.499% and 1.75 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 321ng/ml and 3.7ng/ml respectively, while limit of quantitiation (LOQ) for Mebeverine hydrochloride and Chlordiazepoxide were found to be 973ng/ml

and 11.2ng/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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