Spectrophotometric Determination of Mebeverine hydrochloride in pharmaceutical preparation via Ion Association Reaction

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Abstract. We developed colorimetric method to determine Mebeverine hydrochloride. Briefly, the principle of our method is the formation of ion associated complex between the drug and phenol red indicator. The spectrum of the complex was measured with maximum absorption at 396 nm – Beers law is followed over the range over 2 – 25 µg/ml with molar absorptivity of (7.57×10^4 L/Mol. Cm), a sandal sensitivity of (0.00615 µg. Cm^-2), and the relative standard deviation RSD % (3.3974%), mean recovery was found to be in the range (96-99 %), and correlation coefficient of (R^2=0.9941). However, the method accurately and successfully was performed to determine Mebeverine hydrochloride in bulk and pharmaceutical preparations.

1. Introduction.
Mebeverine hydrochloride(MBV) is a white crystalline powder, molecular weight 466 g/Mol, molecular formula C_{25}H_{35}NO_{5}.HCl Fig (1). MBV is freely soluble in water and 96% ethanol, but it is insoluble in di ethyl ether (1). The IUPAC name of Mebeverine hydro Chloride is 3/4- Dimethoxy benzoic acid (ethyl 2, 4 methoxy 4-phenyl -1-methyl ethyl) amino – butyl ester. MBV widely uses as a relaxant and antispasmodic medication for the gastrointestinal tract especially colonic spasm and irritable bowel syndrome (2, 3).

Figure 1. Represents the chemical structure of Mebeverine hydrochloride.

Actually there are several analytical methods including spectrophotometric (4-10), Construction Spectroscopy (11), HPLC (12-19) ion selective Electrodes (20), Carbon Paste Electrode (21) to determine the pharmaceutical dosage of MBV. Therefore, we designed our experiment to develop colorimetric method, which characterized as easier, faster and more accurate to measure the dose of MBVHCL in bulk and pharmaceutical preparation.

2. Experimental parts.
2.1. Apparatus.
We used Shimadzo model 1800, which has a double beam UV visible spectrophotometer, and two matched quartz cells that allow 1 cm light path.

2.2. Material and reagent.
We made sure that all the chemical we used were analytical grade and did not need further purification. Mebeverine HCl was provided from SDI –Iraq. Distilled H2O was used throughout.
- **Stock solution of** Mebeverine HCl MBV was prepared by transfer (100 mg) of the drug in 100 mL volumetric flask, dissolved in small amount of DW and make up to the volume with same Solvent.
- **Buffer Solution of 2.3 pH:** was prepared by dissolving (1.287 gm Na OH) in dH2O and finish the volume to 250 ml with dH2O, and (3.4206 gm KH2PO4) was dissolving in (D. W) and make up to 250 ml in volumetric flask with same solvent. The pH was adjusted with small amount of 1 M HCl. **Phenol red** to get (3.203x10^-3 M), we dissolved 0.056 gram of phenol red in 25 ml ethanol and finish the volume with dH2O to 250 ml in volumetric flask.

2.3. *Recommended steps.*
Transfer a suitable concentration 2µg/ml equal to 0.2 ml of a standard solution of MBV into a separating funnel (size equal to 125 ml) containing 8 ml of buffer solution (PH=2.3) and 5 ml of 3.203x10^-3 M of phenol red. The above solution, shaking well for 2 minutes, then 10 ml of Chloroform was added to shake the contents for 2 min to allow the two aqueous phases to separate. We removed the organic layer and kept in 10ml volumetric flask. Then we diluted the mixture to the mark with chloroform. A separated Chloroform layer was standing for 5 minutes and measured at 396nm against a reagent blank.

2.4. *Procedure for pharmaceutical preparations.*
Ten pills were weighted and grinded to get the medication in powder shape. We dissolved 100 mg of the powder form in 100 ml dH2O in 100 ml volumetric flask to get 100 µg /ml concentration. The solution was stirred for 10 min then filtered through the filter paper #5 to place the solution new volumetric flask (the size equal 100 ml), then we finished the volume with dH2O to 100 ml.

3. *Results and Discussion.*
In order to get the sensitive and the stabile product number of factors that influence of formation complex were carefully studied. The spectrum graph of the colored reaction compound was measured against the blank reagent (absorbance at λmax 396 nm) see figure 2.

![Figure 2](attachment:image.png)

**Figure 2.** Absorption spectra of the colored compound \((2.14 \times 10^{-5} \text{ M})\) of MBV with phenol red \((3.2 \times 10^{-3}\text{M})\).
3.1. **Optimization of the Experimental condition.**

We optimized the reaction conditions by measuring various parameters that effect on the absorption intensity of the reaction complex.

3.2. **Effect of phenol red concentration.**

Various concentration of the phenol red solution was added to fixed amount of MBV and was found that 5 mL of $3.203 \times 10^{-3}$ M of phenol red was the final concentration which could give us full intensity with a minimum blank value of the observance. And this consider to be optimum for measuring the concentration range between (0.2 - 25 μg/ml) of the drug as in figure 4.

**Figure 3.** The spectrum of 8 μg/ml of Mebeverine HCl.

**Figure 4.** Effect of different volume of $3.203 \times 10^{-3}$ M phenol red on absorption of the complex between MBV and phenol red.
3.3. Study temperature effect.
The temperature effect on the observance of the formed compound using water bath was studied and found that 25 °C gave the highest absorption at $\lambda_{\text{max}}$ 396 nm, therefore it is recommended that the colored compound should be carried out at 25 °C as in figure 5.

![Figure 5. Effect of Temperature absorption spectra of MBV.](image)

3.4. Study time effect on the absorption intensity.
Interesting the color intensity reached a maximum absorption after Mebeverine has been reacted with phenol red at 20 min. Therefore, 20 min development time was chosen for further experiment. The colored compound was studied of test 2 hour. The results obtained are shown in figure 6.

![Figure 6. Represents the effect of time on the absorption intensity of MBV. HCL](image)
3.5. Calibration Graph Study time effect on the absorption intensity.

A linear calibration graph was obtained when employing the condition describe above for determination of MBV which shows that Beer’s law obeyed to the concentration range (0.2-25) μg/ml with a correlation coefficient (0.9955). The molar absorptivity of the formed compound was found to be $1.87 \times 10^4$ L/Mol.Cm and a standard sensitivity of 0.0615 μg.cm$^{-2}$. The result that we obtained is shown in figure 7.

![Graph showing calibration curve to measure MBV.HCL according to Beer’s law.](image)

Figure 7. represents calibration curve to measure MBV.HCL according to Beer’s law.

3.6. The stoichiometry of the MBV.HCL and Phenol red reaction product.

We followed the mole ratio and Jobs method to test the nature of MBV.HCL and phenol red reaction products. Interesting about 2:1 reaction product was formed between the drug and the reagent (see figure 8 A and B). Schematic figure 1 may explain the mechanism of dye formation in MBV.HCL and phenol red reaction.

![Scheme 1. Represents the mechanism of MBV- Ph. Red ion-pair formation.](image)
3.7. Method of evaluating the stability of the ion pair complex.

We tasted the stability of the complex that formed between MBV and phenol red. Although the constant observance readings were obtained at short time 20 min and 25°C. The formed complex was constant for at least two hours without observance intensity changing or in $\lambda_{\text{max}}$. The conditional constant was calculated according to literature (22), and found to $1.315 \times 10^7$, and this supported the stability of the forming complex.

3.8. Steps that followed to choose the Organic solvent.

A different of organic solvents as chloroform, carbon tetrachloride, dichloromethane, dichloromethane and ether were tested to choose the suitable solvent to give a high absorbance. Chloroform was the optimal choice in comparing other solvent types for its highest absorbance feature. It was found that one extracted is adequate to obtain high retrieval of the complex and short time to touch the equilibrium between the two phases. Furthermore, the two minutes shaking time provided the stable observation.

3.9. Evaluation of the proposed method.

Evaluation the precision and accuracy of the proposed method.

The result shows that proposed method was accurate according the values of the recovery with the range of (96.74-99.39) when the three levels of concentration were measured also the low values of R.S.D% indicated that the method was high precise (0.479-1.986) %.

**Figure 8.** A mole fraction of drug and B represented the mole ratio.
3.10. **Interference studies.**

The results showed that no interferences were found in the presence of 40 μg/ml for each excipient (Glucose, Lactose, Starch, Sucrose, Magnesium Stearate, Talk, Aacia) with these concentration different concentrations of the drug up to (2,10,20) µg/ml in the determination of MBV.

**Table 1.** Represents the concentration of some materials that interfered with the pharmaceutical preparation of the drug.

| Concentration of MBV. µg/ml | Mean Recovery (%) |
|-----------------------------|-------------------|
| 2                           | 97.5903           |
| 10                          | 96.1445           |
| 20                          | 99.3975           |

| N | Concentration In µg/mL | Found  | R.S.D% | Recovery (%) |
|---|------------------------|--------|--------|--------------|
| 1 | 8                      | 7.7000 | 0.479  | 96.75        |
| 2 | 16                     | 15.6263| 0.608  | 97.66        |
| 3 | 20                     | 19.5180| 1.989  | 97.59        |

3.11. **Detection and quantification limits.**

LOD refers to the limit of detection while LOQ refers to the limit of quantification. LOD= 3SD/K and LOQ= 10SD/K, where SD means the standard deviation of replicate determination values under the same conditions in the absent of the drug and K is the slop of the calibration graph (22). According to these forms, the LOD equal to 0.213ug/ml and LOQ equal to 0.213 ug/ml.

3.12. **Analytical Applications.**

The results of our proposed modified method suggested that the proposed method is easier, accurate and précised as compare to the other measuring methods. The low value of (R. S. D %), which means relative standard division indicated good reproducibility and precision. The mean of percent recoveries obtained were in the range of (97.18-99.55) indicating high accuracy of the suggested method.

**Table 2.** The obtained results from the application of the proposed method.

| Company | Claimed | Found | R.S.D% | Recovery (%) |
|---------|---------|-------|--------|--------------|
| Duspatalin | 135mg   | 131.2 | 1.3212 | 97.18        |
| EVACOL  | 135mg   | 133.5 | 1.4900 | 98.88        |
| MEVA    | 135mg   | 134.4 | 1.3800 | 99.55        |

4. **Conclusion.**

The proposed method shows a good accuracy, sensitive for estimation of MBV in bulk and pharmaceutical preparation. The proposed has some advantage like fast determination of the drug in
its pure and in pharmaceutical preparation also the uses of 25 c made the proposed method simple and not require heating. The wide ranges of the linearity of the method gave a good application for the pharmaceutical preparation. The absent of the effect of the interference materials in the reaction of the MBV.HCL and phenol red reaction, which suggests that the method is beneficial for routine analysis tests and quality control assay of the drug in pure materials and in tablet forms.

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