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Spectrophotometric Determination of Mebeverine Hydrochloride

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Abstract. Three simple, sensitive and reproducible visible spectrophotometric methods (A–C) for the determination of mebeverine hydrochloride (MVH) in bulk samples and pharmaceutical formulations are described. Methods A and B are based on the formation of ion-association complexes between the drug and fast green FCF (FGFCF, λ_{max} 625 nm) or bromothymol blue (BTB, λ_{max} 405 nm). Method C is based on the formation of a molecular complex between the drug and cobalt thiocyanate (CTC, λ_{max} 625 nm). Regression analysis of Beer's plots showed good correlation in the concentration ranges 2–40, 2–25 and 100–600 μg/ml for methods A,B and C respectively. No interference was observed from the usually existing additives in pharmaceutical formulations and the applicability of the methods was examined by analysing tablets containing MVH. Standard deviations were typically $\leqslant$ 0.75 mg per dose (RSD: 0.25–0.5%). Recoveries were 99.0–100.2%.

Key words: visible spectrophotometry, mebeverine hydrochloride, fast green FCF, bromothymol blue, cobalt thiocyanate, ion-association complex, molecular complex.

Mebeverine hydrochloride (MVH) is an antispasmodic agent, used in a variety of conditions affecting the vascular system and the gastro-intestinal and genito-urinary tracts. It is mainly used as a gastrointestinal antispasmodic in conditions such as irritable bowel syndrome [1]. It is chemically known as 3,4-dimethoxybenzoic acid 4-[ethyl-[2-(4-methoxyphenyl)-1-methylethyl] amino]butyl ester. The drug and its formulations are official in B. P. [2]. Few methods have appeared in the literature for the determination of MVH in biological fluids and pharmaceutical formulations. The techniques used in this connection include HPLC [3–5], HPTLC [6], TLC [7, 8], micro LC [9], LC [10], UV [11, 12] and visible spectrophotometry [13]. However, analytically important functional groups in MVH have not been exploited properly in developing visible spectrophotometric methods. This paper describes some attempts in this direction, with three visible spectrophotometric procedures exploiting the basic property of the tertiary amino group in the aliphatic chain of MVH [ion-association complex formation with acidic dyes such as fast green FCF (FG FCF, method A) and bromothymol blue (BTB; method B) and molecular complex formation with cobalt thiocyanate (CTC, method C)].

Experimental

Apparatus

A Systronics model 106 visible spectrophotometer, Shimadzu UV-150-02 (double-beam) spectrophotometer and an Elico LI-120 model digital pH meter were used for absorbance and pH measurements.

Reagents and Solutions

All chemicals were of analytical grade and all solutions were freshly prepared in triply distilled water.

Aqueous solutions of FGFCF (Loba, 6.18×10^{-3} *M*), hydrochloric acid (E. Merck, 1.0×10^{-2} *M*), BTB (Loba, 3.203×10^{-3} *M*) and buffer solutions of pH 2.0 (glycine-HCl) and pH 3.0 (phthalate-HCl) were prepared in the usual way.

Cobalt thiocyanate (CTC, 1.71×10^{-1} m) solution was prepared by dissolving 20 g of ammonium thiocyanate and 5 g of cobaltous nitrate in 100 ml of water and the solution was saturated with sodium chloride.

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Preparation of standard drug solution. MVH pharmaceutical grade was used as standard without further treatment. An accurately weighed 100 mg of MVH was dissolved in distilled water and made up to 100 ml with water to give a stock solution of 1 mg/ml. The stock solution was further diluted stepwise with distilled water to give the working standard solutions (100 µg/ml for methods A and B; 1 mg/ml for method C).

Preparation of the sample drug solution. Tablet powder equivalent to 100 mg of MVH was dissolved in 100 ml of water and filtered to give the stock solution of 1 mg/ml. This solution was further diluted stepwise to the requisite concentration with water and analysed as for bulk samples.

Procedures

Method A: Pipette up to 2.0 ml of solution (containing 200 ± 100 µg of MVH) into a 125-ml separating funnel containing 3.0 ml of 1.0×10^{-2} *M* HCl and 0.5 ml of 6.18×10^{-3} *M* FGFCF solution. The total volume of the aqueous phase is brought to 10 ml with distilled water. A 10 ml portion of chloroform is added and the contents shaken for 2 min. The absorbance of the separated chloroform layer was measured at 625 nm against a reagent blank within 20 min. The amount of MVH present was calculated from a calibration curve prepared with 0.25–4.0 ml of 100 µg/ml standard MVH solution.

Method B: Pipette up to 2.0 ml of solution (containing 100 ± 50 µg of MVH) into a 125-ml separating funnel containing 8 ml of pH 2.0 buffer solution and 5 ml of 3.20×10^{-3} *M* BTB solution. The total volume of aqueous phase is adjusted to 15 ml in each separating funnel with water, then 10 ml of benzene are added and the contents shaken for 2 min. The two phases are allowed to separate and the absorbance of the separated benzene layer was measured after 5 min at 405 nm against a reagent blank. The amount of MVH present is calculated from a calibration curve prepared with 0.2–2.0 ml of 100 µg/ml standard MVH solution.

Method C: Pipette up to 2.0 ml of solution containing (3.0 ± 1.0 mg of MVH) into a 125-ml separating funnel containing 2 ml of pH 3.0 buffer and 5 ml of 1.71×10^{-1} *M* CTC solution. The total volume of the aqueous layer in each one is brought to 15 ml with distilled water. A 10-ml portion of benzene is added to each funnel and the contents shaken for 2 min. The absorbance of the separated benzene layer is measured after 5 min at 625 nm against a reagent blank. The amount of MVH present is calculated from a calibration curve prepared with 0.5–6.0 ml of 1 mg/ml standard MVH solution.

Results and Discussion

The optimum conditions for the development of methods A–C were established by varying the parameters one at a time and observing the effect produced on the absorbance of the coloured species [13]. For each method various organic solvents such as chloroform, methylene chloride, carbon tetrachloride, isoamyl alcohol, benzene and *n*-butanol were used for extraction of the respective coloured complex.

In order to establish the experimental conditions for method A, MVH was allowed to react with FGFCF in dilute HCl ranging from 0.008 to 0.012 *M* and the complex was extracted into the organic layer. Constant absorbance was obtained with 0.009–0.011 *M* HCl, hence 0.01 *M* HCl was used. A volume of 0.5 ml FGFCF solution was found to be optimal. Shaking time of 1–4 min produced constant absorbance, hence 2 min was chosen. Chloroform was preferred for the selective extraction of the drug-dye complexes among the water-immiscible solvents tried. A ratio of 1:1 aqueous to chloroform phases was required for efficient extraction of the coloured species. The absorption spectrum of the coloured species is shown in Fig. 1.

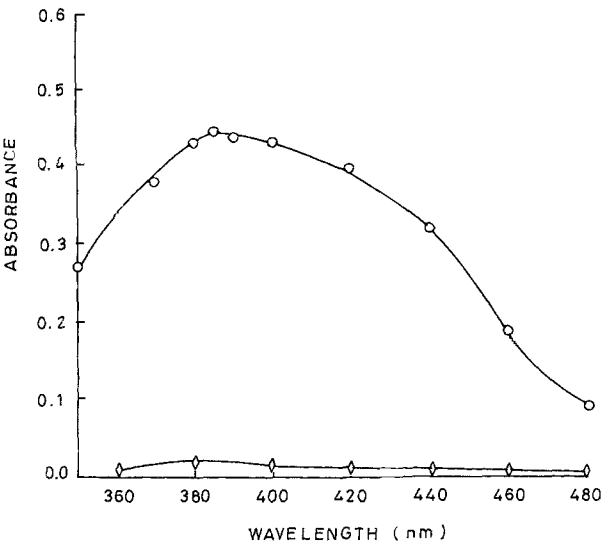

Fig. 1. Absorption spectra of MVH-FGFCF system, its reagent blank (◇---◇) and aqueous FG FCF in 0.01 *M* HCl (○—○). [MVH]: 5.365×10^{-5} *M*; [FGFCF]: 3.09×10^{-4} *M*, [aq. FGFCF]: 1.72×10^{-5} *M* and [HCl]: 0.01 *M*

To ascertain the optimum conditions for method B, the drug was allowed to react with BTB in aqueous solution buffered to pH 1.0–3.0 and the complex formed was extracted into a benzene layer for measurement. Constant absorbance was obtained over the pH range 1.8–2.2, hence pH 2.0 was used. A 5-ml portion of BTB solution was found to be optimal. Constant absorbance was obtained for shaking periods between 1–4 min, hence 2 min was selected. Benzene was chosen for its selective extraction of the drug-dye complex among the water immiscible solvents tested. A ratio of 1.5:1 aqueous to benzene layers was required for efficient extraction of the coloured species. The absorption spectrum of the coloured species is shown in Fig. 2.

In order to establish the optimum experimental conditions for method C, the drug was allowed to react with CTC in aqueous solution buffered to pH 2.0–4.0 and the molecular complex formed was extracted into benzene for measurement. Constant absorbance was obtained over the pH range 2.8–3.2, hence pH 3.0 was

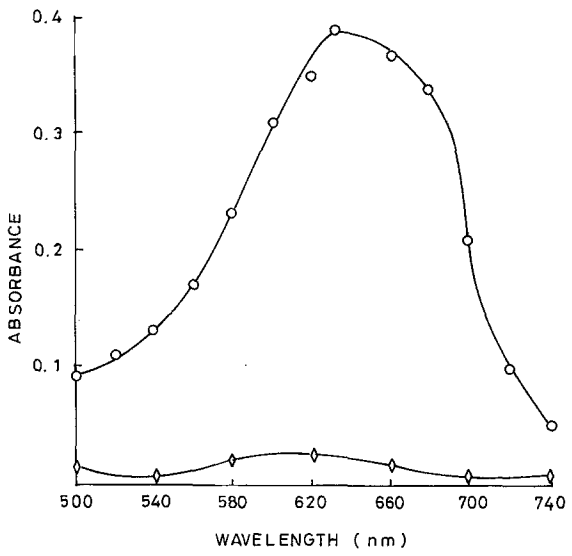

Fig. 2. Absorption spectra of MVH-BTB System; its reagent blank (○—○) and aqueous BTB in pH 2.0 buffer (◇—◇). [MVH]: $2.79 \times 10^{-5} M$, [BTB]: $1.602 \times 10^{-3} M$, and [aq. BTB]: $4.805 \times 10^{-5} M$.

used. A 5-ml portion of CTC solution was found to be optimal. Constant absorbance was obtained for shaking periods between 1–4 min, hence 2 min was selected. Benzene was preferred for the selective extraction of the drug-dye complex among the water-immiscible solvents. A ratio of 1.5:1 aqueous to benzene phases was required for efficient extraction of the coloured species. The absorption spectrum of the coloured species is shown in Fig. 3.

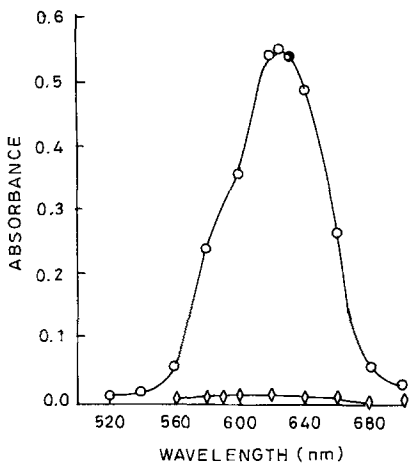

Fig. 3. Absorption spectra of MVH-CTC system (○—○) and its reagent blank (◇—◇). [MVH]: $8.58 \times 10^{-4} M$; [CTC]: $1.6 \times 10^{-3} M$

Analytical Data

The Beer's law limits, molar absorptivity, Sandell's sensitivity, regression equation and correlation coefficients obtained by least squares treatment of these results are given in Table 1. The precision of each method was tested by analysing six replicate samples containing 300, 150 and 400 μg of bulk drug for methods A, B and C, respectively. The relative standard deviations and the percent ranges of error at 95% confidence level are given in Table 1.

The commonly used excipients and additives in the formulations of drugs such as talc (up to 350-fold excess (m/m) compared with the MVH), starch (250-fold), boric acid (150-fold), stearic acid (100-fold), cetyl alcohol [25-fold], glyceryl monostearate (40-fold), sodium lauryl sulphate (30-fold); propyl paraben (20-fold) and glycerine (50-fold) did not interfere in the determination of MVH by using proposed procedures. These methods were applied for the estimation of MVH in pharmaceutical formulations and compared with the B. P. reference method by means of *F*- and *t*-tests (Table 2). As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the preanalysed formulations. These results are also summarised in Table 2. The results indicate that the proposed procedures do not differ significantly from reference method.

Chemistry of the Coloured Species

In methods A and B, the formation of the coloured complex is based on the basic nature of the drug (MVH), which under specified experimental conditions forms ion-association complexes with certain acidic dyes (FGFCF or BTB) which are extractable into organic phases (chloroform for method A and benzene for method B). The stoichiometric ratio of mebeverine to FGFCF or BTB was determined with the slope ratio method and found to be 1:1. The quantitative measure of the effect of complexation on acid-base equilibrium is most likely to be interpretable in terms of electronic, steric and other effects of complexing. The drug MVH (1 mole) and the oppositely charged form of the dye (1 mole) behave as a single unit, being held together by electrostatic attraction.

In method C, the drug (MVH) forms a co-ordinate complex with CTC reagent. Cobalt thiocyanate (CTC), which results from the combination of ammonium thiocyanate with cobaltous nitrate, has been proved to be a valuable reagent for the detection and determination of tertiary amino compounds and drugs [14, 15]. The coloured complex formed between the drug and CTC was quantitatively extractable into benzene from

Table 1. Optical characteristics, precision and accuracy of the proposed methods for MVH

Parameters	A	B	C
λ_{max} (nm)	625	405	625
Beer's law limits (μg/ml, C)	2.0–40.0	2.0–25.0	100–600
Molar absorptivity (1/mole/cm)$^{\neq}$	9.32×10^3	1.67×10^4	6.06×10^2
Sandell's sensitivity ($\mu g/cm^2$/0.001 absorbance unit)	0.05	0.0286	0.769
Optimum photometric range (μg/ml)	10.0–35.0	5.0–22.0	200–500
Regression equation ($Y = a + bC$)			
Slope (b)	0.0199	0.0354	0.0013
Intercept (a)	0.00416	−0.005	0.00001
Correlation coefficient (r)	0.9999	0.9998	0.9999
RSD* (%)	0.64	1.15	1.45
% Range of error (confidence limits)			
0.05 level	±0.54	±0.97	±1.2
0.01 level	±0.80	±1.43	±1.8

* $n = 6$.
$\neq$ Refer to organic phase.

Table 2. Analysis of pharmaceutical formulations by proposed and reference methods

Formulation	Labelled amount (mg)	Amount found by proposed methods (mg)*			Amount found by reference method [2] (mg)	Recovery by the proposed methods** %		
		A	B	C		A	B	C
Tablets								
I	135	134.9 ± 0.54 $t = 0.53, F = 1.65$	134.7 ± 0.72 $t = 1.09, F = 2.94$	134.8 ± 0.38 $t = 0.36, F = 2.25$	134.5 ± 0.42	99.4	99.3	99.7
II	135	135.4 ± 0.49 $t = 0.71, F = 1.26$	135.3 ± 0.46 $t = 1.27, F = 1.46$	134.7 ± 0.46 $t = 0.24, F = 1.43$	134.4 ± 0.55	100.0	100.2	99.5
III	135	135.2 ± 0.46 $t = 1.23, F = 1.38$	134.6 ± 0.34 $t = 0.28, F = 2.52$	134.4 ± 0.49 $t = 0.71, F = 1.21$	134.7 ± 0.54	99.4	99.4	99.7

* Average ± standard deviation of 6 determinations; the t- and F-values refer to comparision of the proposed method with the reference method. Theoretical values at 95% confidence limits $t = 2.57$, $F = 5.05$.
** After adding 10 mg of pure MVH to the preanalysed pharmaceutical formulations, each value is the average of three determinations.

the aqueous phase and it was observed that the drug, cobalt and thiocyanate were in the ratio of 2:1:4.

Conclusions

The order of λ_{max} values among the proposed methods and a reference method (R) in the determination of MVH is A = C > B > R. The higher λ_{max} of the proposed methods is a decisive advantage since the interference from the associated ingredients should be far less at these higher wavelengths. The sensitivity order of the method is R > B > A > C.

Methods A and B have the advantage that they can be applied to the determination of individual components in a multi-component mixture. This aspect of spectrophotometric analysis is of major interest in pharmaceutical analysis, since it offers distinct possibilities in the assay of a particular component in a complex dosage formulation. Method C is selective to the estimation of compounds containing an aliphatic tertiary amino group.

The proposed methods are simple, selective and sensitive for the determination of MVH, and provide a wide choice depending upon the needs of the specific situation.

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References

[1] J. E. F. Reynolds, *Martindale, The Extra Pharmacopoeia, 29th Ed.*, The Pharmaceutical Press, London, 1989.
[2] *British Pharmacopoeia, Vols I & II*, Her Majesty's Stationery Office, London, 1988.

[3] M. De Smet, D. L. Massart, *J. Chromatogr* **1987**, *410*, 77.
[4] A. A. Al-Angary, S. H. Khidr., S. S. Abd-Elhady, M. A. Bayomi, G. M. Mahrous, *Anal. Lett.* **1992**, *25*, 1251.
[5] T. Hamoir, B. Bourguignon, D. L. Massart, H. Hindriks, *J. Chromatogr.* **1993**, *633*, 43.
[6] J. A. De Schutter, G. Van der Weken, W. Van den Bossche, P. De Moerloose, *Chromatographia* **1985**, *20*, 739.
[7] G. Musumarra, G. Scarlata, G. Crima, G. Romano Guido, S. Palazzo, S. Clementi, G. Giulietti, *J. Chromatogr.* **1985**, *350*, 151.
[8] H. Schuetz, A. Pielmeyer, G. Weiler, *Aerztl. Lab.* **1990**, *36*, 113.
[9] J. D. Pinkston, C. J. Venkatramani, L. J. Tulich, D. J. Bowling, K. R. Wehmeyer *J. Chromatogr.* **1993**, *622*, 209.
[10] T. Daldrup, P. Michalke, W. Bochme, *Chromatogr. Newsl.* **1982**, *10*, 1.
[11] M. M. Bedair, M. A. Korany, M. A. Ebdel Hay, A. A. Gazy, *Analyst* **1990**, *115*, 449.
[12] M. E. Hassan, A. A. Gazy, M. M. Bedair, *Drug Dev. Ind. Pharm.* **1995**, *21*, 633.
[13] D. L. Massart, B. G. M. Vandeginste, S. N. Deming, Y. Michotte, L. Kaufman, *Chemometrics, A Text Book*, Elsevier, Amsterdam, 1988.
[14] R. G. Bhatkar, S. K. Chodankar, *Indian J. Pharm. Sci.* **1980**, *42*, 145.
[15] S. S. Zaropakar, R. V. Rele V. J. Doshi, *Indian Drugs* **1987**, *24*, 560.

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