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Spectrophotometric determination of benzydamine HCl, levamisole HCl and mebeverine HCl through ion-pair complex formation with methyl orange

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Abstract

A simple, rapid and sensitive spectrophotometric method has been proposed for the assay of benzydamine HCl (BENZ), levamisole HCl (LEV) and mebeverine HCl (MBV) in bulk and pharmaceutical formulations. The method based on the reaction of the selected drugs with methyl orange (MO) in buffered aqueous solution at pH 3.6. The formed yellow ion-pair complexes were extracted with dichloromethane and measured quantitatively with maximum absorption at 422 nm. The analytical parameters and their effects on the reported systems are investigated. The extracts are intensely colored and very stable at room temperature. The calibration graphs were linear over the concentration range of 2–10 μg ml^{-1} for BENZ, 6–24 μg ml^{-1} for LEV and 4–14 μg ml^{-1} for MBV. The stoichiometry of the reaction was found to be 1:1 in all cases and the conditional stability constant (K_f) of the complexes have been calculated. The proposed method was successfully extended to pharmaceutical preparations-tablets. Excipients used as additive in commercial formulations did not interfere in the analysis. The proposed method can be recommended for quality control and routine analysis where time, cost effectiveness and high specificity of analytical technique are of great importance.
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Keywords: Benzydamine; Levamisole; Mebeverine; Methyl orange; Ion-pair complexes; Spectrophotometry

1. Introduction

Benzydamine hydrochloride (BENZ), (1-benzyl-3-(3-dimethylamino-propoxy)-1*H*-indazole hydrochloride) is widespread therapeutic application due to its analgesic, anti-inflammatory and anti-pyretic activity, used either systemically or topically [1]. It is a white crystalline powder, very soluble in water, freely soluble in ethanol (95%), and in chloroform, practically insoluble in ether. The drug is official in the British Pharmacopoeia [2]. A survey of the literature revealed that only a single ultraviolet [3] and one visible spectrophotometric methods have been reported [4]. Other methods include amperometric biosensor [5], polarography [6], potentiometry [7,8] and high-performance liquid chromatography [9,10].

Levamisole hydrochloride (LEV), (2,3,5,6-tetrahydro-6-phenylimidazole [2,1-*b*] thiazole) is an imidazothiazole derivative. Levamisole hydrochloride is a white to almost white crystalline powder, which is almost odorless and is freely soluble in water. It is quite stable in acid aqueous media but hydrolyzes in alkaline or neutral solutions. Levamisole is a broad spectrum anthelminthic drug widely used to control internal parasites in livestock and occasionally in human medicine as an anthelminthic. It is also used for a variety of other indications including adjuvant therapy in cancer treatment [11,12]. The determination of trace levels of LEV is very necessary for the residue analysis in animal products and in clinics. Reported methods for the determination of LEV are based mainly on atomic absorption [13–15], potentiometry [16], gas chromatography [17–19], liquid chromatography [20,21], and high-performance liquid chromatography [22–25]. No visible spectrophotometric method has been reported for the estimation of LEV. Hence, it was though worthwhile to develop spectrophotometric method for the same.

Mebeverine hydrochloride (MBV), is chemically known as 3,4-dimethoxybenzoic acid 4-[ethyl-[2-(4-methoxy-phenyl)-l-methylethyl] amino] butyl ester. It is an anti-spasmodic agent, used in a variety of conditions affecting the vascular system and the gastro-intestinal and genitourinary tracts. It is mainly used as a gastrointestinal antispasmodic in conditions such as

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irritable bowel syndrome [26]. The drug and its formulations are official in B.P. [27]. Few methods have appeared in the literature for the determination of MBV in biological fluids and pharmaceutical formulations. The techniques used in this connection include potentiometry [28], thin layer chromatography [29], high-performance liquid chromatography [30–32], ultraviolet [33,34] and visible spectrophotometry [35–39].

The present study describes accurate extraction spectrophotometric method for the determination of benzydamine, levamisole and mebeverine hydrochlorides through ion-pair complex formation with methyl orange. The reaction conditions and the application of the proposed method for the determination of BENZ, LEV and MBV in their pharmaceutical dosage forms have been established. The proposed method is simple, sensitive and the complex formed is stable for more than 24 h.

2. Experimental

2.1. Apparatus

All the absorbance spectral measurements were made using spectroscan 80 D double-beam UV/Visible spectrophotometer (Biotech Engineering Ltd. (UK), with wavelength range 190–1100 nm, spectral bandwidth 2.0 nm, with 10 mm matched quartz cells.

2.2. Reagents and solutions

All of the chemicals used were of analytical or pharmaceutical grade and used without further purification. Double distilled de-ionized water was used to prepare all solutions.

Stock solutions of pure BENZ, LEV and MBV were prepared separately by dissolving 10 mg of BENZ, MBV and 30 mg of LEV in a 100 ml calibrated flask. Working solutions of lower concentrations were freshly prepared by appropriate dilution with water.

A 0.02% (w/v) of methyl orange was prepared by dissolving the accurate weighed amount of 20 mg in 100 ml water. Series of buffer solutions of KCl–HCl (pH 1.0–2.2), NaOAc–HCl (2.2–3.6) and NaOAc–AcOH (3.4–5.6) pH were prepared by standard methods.

2.3. General recommended procedures

2.3.1. Procedure for calibration curve

Into a series of separating funnels, accurately measured aliquots of BENZ, LEV or MBV in the concentration range shown in (Table 1) were pitted out. Then, 2.5 ml of 0.02% MO, 1.0 ml of buffer solution of pH 3.6 were added and the volume was completed to 10 ml with distilled water. The ion-pairs were extracted with 10 ml of dichloromethane by shaking for 1.0 min and then, the combined dichloromethane extracts were dried over anhydrous sodium sulphate. The absorbance of yellow colored ion-pair complexes were measured within 20 min of extraction at 422 nm against reagent blank prepared in the same manner except addition of drugs.

2.3.2. Procedure for tablets

At least 10 tablets of the drugs were weight into a small dish, powdered and mixed well. A portion equivalent to 10 mg of BENZ, MBV and 30 mg of LEV were weight and dissolved in distilled water, filtered into a 100 ml calibrated flask and diluted to volume with water. Solutions of working range concentration were prepared by proper dilution of this stock solution with water and followed the above procedure for the analysis.

3. Results and discussion

Ion-pair extraction spectrophotometry has received considerable attention for quantitative estimation of many pharmaceutical compounds [40–46]. This technique depends on the reaction of a drug that has basic cationic nitrogen and an anionic dye, where a highly colored ion-pair complex is formed. BENZ, LEV and MBV reacted with an anionic dye (MO) in acidic buffer to form a yellow ion-pair complexes, which are soluble in dichloromethane. These complexes has an absorp-

Table 1
Analytical parameters and optical characteristics of the proposed method with BENZ, LEV and MBV

Parameters	BENZ	LEV	MBV
Drug aliquot (ml)	0.2–1.0	0.2–0.8	0.4–1.4
Molar absorptivity ($l\,mol^{-1}\,cm^{-1}$)	4.16×10^4	1.37×10^4	4.4×10^4
Beer's law limits ($\mu g\,ml^{-1}$)	2–10	6–24	4–14
Sandell sensitivity ($ng\,cm^{-2}$)	8.31	17.57	10.59
Correlation coefficient (r)	0.9991	0.9985	1.001
Detection limits ($\mu g\,ml^{-1}$)	0.0752	0.1452	0.0817
Quantification limits ($\mu g\,ml^{-1}$)	0.2508	0.4842	0.2725
Regression equation[a]			
Slope (b)	0.1228	0.0636	0.1130
Intercept (a)	−0.04	0.1706	−0.261
$S_{y/x}$	0.2572	0.4	0.4722
S.D. of slope (S_b)	0.0614	0.0318	0.0564
S.D. of intercept (S_a)	1.1295	1.0536	1.0539
Stoichiometric ratio	1:1	1:1	1:1
Stability constant (k_f)	4.741 ± 0.241	4.420 ± 0.223	4.722 ± 0.227

[a] $A = a + bC$, where C is the concentration in $\mu g\,ml^{-1}$.

tion maximum at 422 nm against reagent blank and hence, this wavelength was used for all subsequent measurements.

Optimum conditions necessary for rapid and quantitative formation of colored ion-pair complexes with maximum stability and sensitivity were established by a number of preliminary experiments.

3.1. Choice of organic solvent

Different organic solvents as dichloromethane, carbon tetrachloride, chloroform and ether were tested as extractive solvents for the proposed method. Dichloromethane was preferred to other solvents for its selective and obtained highest absorbance with dichloromethane. It was also observed that only one extraction was adequate to achieve a quantitative recovery of the complexes and the shortest time to reach the equilibrium between both phases. Shaking time of 0.5–5 min provided constant absorbance and hence, 1.0 min was selected as the optimum shaking time.

3.2. Effect of pH

It was observed that the effective extraction of the complex depends on the type of buffer used and its pH. The effect of pH was studied by extracting the colored complexes in the presence of various buffers, such as KCl–HCl (pH 1.0–2.2), NaOAc–HCl (pH 1.9–4.92) and NaOAc–AcOH (pH 3.4–5.6). It was noticed that the maximum color intensity and constant absorbances were observed in NaOAc–AcOH of pH 3.6 for the three drugs, Fig. 1. The volume of buffer solution added were studied and complete color development was attained by adding 1.0 ml buffer solution of pH 3.6. For the highest color intensity and maximum absorbance, the buffer solution should be added after mixing the drug–dye solution at neutral pH.

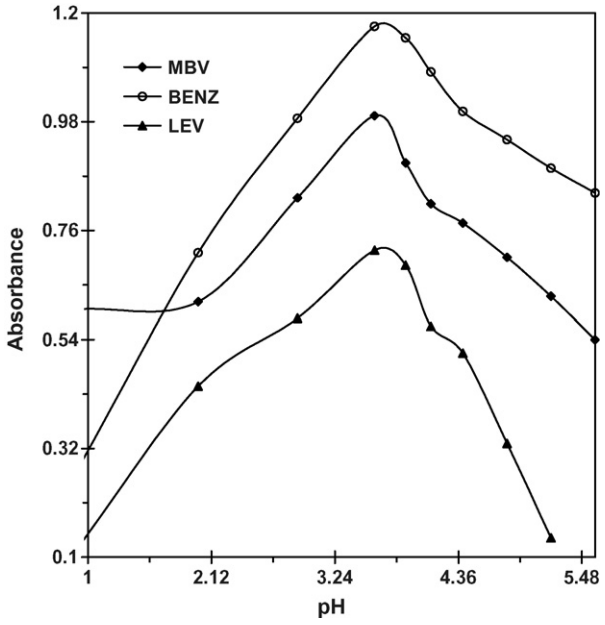


Fig. 1. Effect of pH on the absorbance of the ion-pair complexes formed with BENZ, MBV (10 μg ml^{-1}) and LEV (30 μg ml^{-1}).

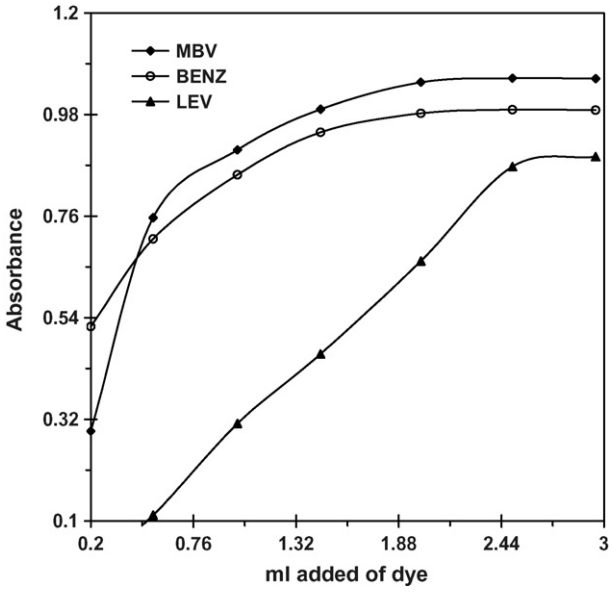


Fig. 2. Effect of ml added of 0.02% MO on the absorbance of the ion-pair complexes of BENZ, MBV (10 μg ml^{-1}) and LEV (30 μg ml^{-1}).

3.3. Effect of dye concentration

The effect of the dye concentration on the intensity of the color developed at the selected wavelength and constant drugs concentration was tested using different volumes of methyl orange (0.5–3 ml). It was observed that 2.5 ml of 0.02% MO was necessary for maximum color development of the ion-pair complexes. After this volume, the absorbance remains constant by increasing the volume of the reagent. The effect of the reagent concentration on the absorbance is shown in Fig. 2.

3.4. Composition of the ion-pair complexes

The composition of ion-pair complexes was established by applying Job's method of continuous variations. The method is simple and widely used for elucidating the composition of complexes and is based on the variation of both the drug and the reagent (MO) of equal molar concentrations, keeping the total volume of the drug and the reagent constant. The plot reached a maximum value at a mole fraction of 0.5 (Fig. 3), which indicated that a 1:1 (drug:MO) ion-pairs are formed through the electrostatic attraction between positive protonated drugs and methyl orange anions. The suggested mechanism for the reaction product of BENZ–MO ion-pair complex formation for example, is given in Scheme 1.

3.5. Stability of the ion-pair complexes

The stability of the ion-pair complexes formed between the studied drugs and MO was evaluated. Although the ion-pairs were obtained instantaneously, constant absorbance readings were obtained after not less than 20 min of standing at room temperature (25 ± 2 °C). Ion-pairs were stable for at least 24 h without any change in color intensity or in λ_{max}.

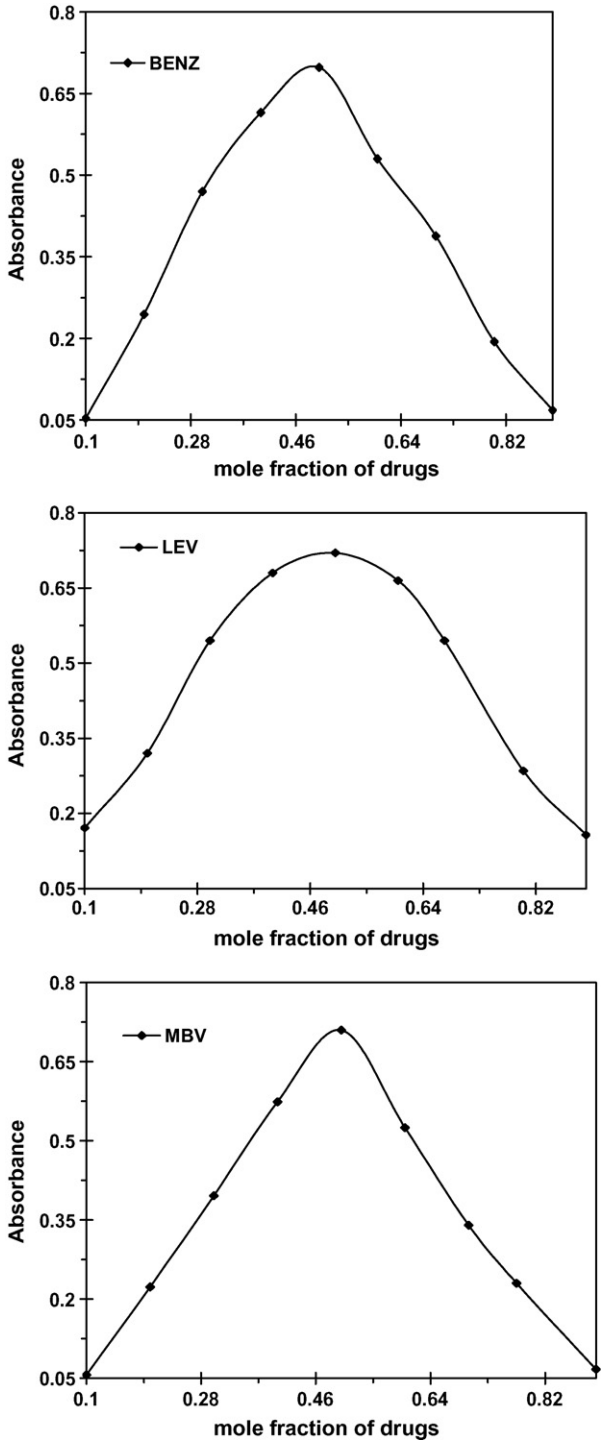


Fig. 3. Continuous variation plots for the ion-pair complexes of BENZ, MBV (2.8×10^{-4} M) and LEV (1.0×10^{-3} M).

3.6. Conditional stability constants (K_f)

The conditional stability constants (K_f) of the ion-pair complexes were calculated from continuous variation data using the following formula [47]:

$$K_f = \frac{A/A_m}{[1 - A/A_m]^{n+1} C_D^n}$$

where A and A_m are the observed maximum absorbance and the absorbance value of all the drugs present is associated, respectively. C_M is the molar concentration corresponding to the maximum in absorbance and n is the stoichiometric constant with which dye ion associates with drugs. Using this equation, the stability constants were found to be equal to 4.741 ± 0.241, 4.420 ± 0.223 and 4.722 ± 0.227 for BENZ, LEV and MBV, respectively.

3.7. Effect of interferences

In order to evaluate the selectivity of the proposed method for the analysis pharmaceutical formulations, the effect of the presence of excipients and additives, which can occur in real samples were investigated. It was found that the presence of the common excipients of tablets such as talc, starch, gelatin, glucose, sulfate, acetate, phosphate and magnesium stearate did not interfere with the determination of the studied drugs at the levels normally found in dosage forms.

3.8. Linearity and range

The Beer's law range, molar absorptivity, Sandell's sensitivity, regression equation, slope, intercept and correlation coefficient determined for each drug are given in Table 1. A linear relationship was found between the absorbance and the concentration of each drug in the range of 2–10 μg ml^{-1} for BENZ, 6–24 μg ml^{-1} for LEV and 4–14 μg ml^{-1} for MBV. The correlation coefficients were 0.9985–1.001 indicating good linearity.

3.9. Detection and quantification limits

The detection limit (LOD) for the proposed method was calculated using the following equation [48]:

$$\mathrm{LOD} = \frac{3s}{k}$$

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the drug and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance were found to be 0.0752 μg ml^{-1} for BENZ, 0.1452 μg ml^{-1} for LEV and 0.0817 μg ml^{-1} for MBV.

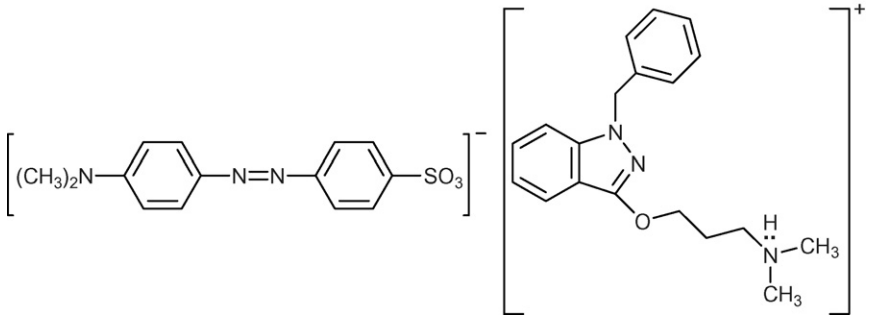
The limits of quantification, LOQ, defined as [49]:

$$\mathrm{LOQ} = \frac{10s}{k}$$

According to this equation, the limits of quantification were found to be 0.2508 μg ml^{-1} for BENZ, 0.4842 μg ml^{-1} for LEV and 0.2725 μg ml^{-1} for MBV.

3.10. Validation of the method

The validity of the method for the analysis of selected drugs in its pure form and in its pharmaceutical formulations were



Scheme 1. Suggested mechanism of BENZ-MO ion-pair complex formation.

Table 2
Evaluation of accuracy and precision of the proposed method

Drug	Found ($\mu g\,ml^{-1}$)	Recovery ± S.D.	R.S.D.[a] (%)	S.E.[b] (%)
BENZ	4	$99.99 \pm 3.22 \times 10^{-3}$	0.7118	0.144
	5	$100.08 \pm 3.88 \times 10^{-3}$	0.6643	0.173
	6	$100.09 \pm 3.4 \times 10^{-3}$	0.4914	0.152
	7	$99.99 \pm 3.13 \times 10^{-3}$	0.3874	0.139
	8	$100.02 \pm 3.87 \times 10^{-3}$	0.4042	0.173
LEV	9	$100.13 \pm 4.26 \times 10^{-3}$	1.211	0.191
	12	$99.91 \pm 3.97 \times 10^{-3}$	0.6380	0.178
	15	$100.06 \pm 5.74 \times 10^{-3}$	0.7083	0.257
	18	$99.99 \pm 7.11 \times 10^{-3}$	0.7715	0.318
	21	$100.00 \pm 6.97 \times 10^{-3}$	0.5917	0.304
MBV	4	$100.27 \pm 2.93 \times 10^{-3}$	0.2663	0.131
	6	$100.12 \pm 3.94 \times 10^{-3}$	0.8933	0.176
	8	$99.94 \pm 5.36 \times 10^{-3}$	0.8628	0.240
	10	$100.01 \pm 4.91 \times 10^{-3}$	0.5514	0.220
	12	99.99 ± 0.0148	1.345	0.662

[a] Relative standard deviation for six determinations.
[b] Standard error.

examined by analyzing the sample using the proposed method. In order to determine the accuracy and precision of the proposed method, solution containing five different concentrations of the studied drugs were prepared and analyzed in six replicates. The analytical results obtained for this investigation are summarized in Table 2. The low values of percent relative standard deviation (R.S.D.%) indicate good precision and reproducibility of the proposed method. The average percent recoveries obtained were quantitative (99.91–100.27), indicating good accuracy of the method. The validity of the method was evaluated by statistical evaluation of the regression lines. It was found that the standard deviation of the residuals ($S_{y/x}$) is 0.2572, 0.4 and 0.4722, the standard deviation of the intercept (S_a) is 1.1295, 1.0536 and 1.0539 and standard deviation of the slope (S_b) is 0.0614, 0.0318 and 0.0564 for BENZ, LEV and MBV, respectively. The small values of the figures point out the low scattering of the points of the calibration curves.

3.11. Tablets analysis

The proposed method was successfully applied to the determination of BENZ, LEV and MBV in their commercially tablets. The applicability of the proposed method for the assay of the cited drugs in pharmaceutical formulations was examined by analyzing various formulations and the results were tabulated in Table 3. The results were reproducible with low R.S.D. values. The average percent recoveries obtained were quantitative (99.90–100.45), indicating good accuracy of the method. The

Table 3
Analysis of pharmaceutical formulations by proposed method

Drug	Commerical formulations analyzed	Supplier	Label claim (mg/tablet)	Amount found (mg)[a]			Recovery (%)	R.S.D. (%)
				BENZ	LEV	MBV		
Benzydamine	Tantum verde P	EIPICO	3.0	2.99			99.96	0.5825
Levamisole	Katrex	Kahira	40		40.18		100.45	1.1983
Mebeverine	Duspatalin	Pharco	135			134.86	99.90	0.9694

[a] Average of six determinations.

results of analysis of the commercial tablets and the recovery study of drugs suggested that there is no interference from any excipients (such as talc, starch, gelatin, glucose, sulfate, acetate, phosphate and magnesium stearate), which are present in tablets.

4. Conclusion

The proposed method make use of simple reagent, which an ordinary analytical laboratory can afford. The method is sufficiently sensitive to permit determination even down to $2.0\,\mu g\,ml^{-1}$. The proposed method is highly reliable owing to the stability of the dye and ion-pair complexes, which are ultimately measured.

The proposed method is simple, precise, accurate and convenient. Therefore, it can be useful for routine analyses and quality control assay of the examined drugs in raw material and in tablets without fear of interference caused by the excipients expected to be present in tablets. This is for the first time that spectrophotometric method is being reported for the assay of levamisole in pure form and also in its pharmaceutical formulation.

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