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SPECTROPHOTOMETRIC AND SPECTROFLUORIMETRIC DETERMINATION OF TRIMEBUTINE

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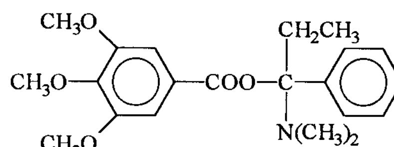
تم استحداث طريقتين تتميز كلا منهما بالحساسية والسهولة لتحليل مركب التراى مبيوتين. تعتمد الطريقة الأولى على تفاعل المركب مع اثنان من الكواشف هما البرومو فينول الأزرق والثيمول الأزرق لتكوين متراكبات مزدوج الأيون. وقد أمكن قياس الألوان الناتجة عند أطوال موجية قدرها ٤١٥ و٤٢٥ نانوميتر فى الحالة الأولى والثانية على الترتيب. وتعتمد الطريقة الثانية على تفاعل التراى مبيوتين مع كلا من حمض المالونيك والأستيك اللامائى كى يعطى نواتج يكون لها وميض يمكن قياسه عند طول موجه قدره ٤٣٠ نانوميتر وذلك بإستخدام طول موجة للإثارة قدره ٣٩٠ نانوميتر. وقد تم استخدام الطرق المقترحة لتحليل العقار فى الصورة النقية وتراوحت نسبة التقدير بين ٧-٦٠ ميكروجرام/ملى ، ٥-٣٠ ميكروجرام/ملى ، ٠,٨-٨٠ نانوجرام/ملى فى كل الحالات على التوالى. وقد تم تطبيق هذه الطرق لتحليل المستحضرات الصيدلية للتراى مبيوتين وأعطت نتائج استخراج عالية تتراوح من ٩٨,٤٪ إلى ١٠١,٥٪. وقد تم تطبيق الطريقة الثانية لتعيين المركب فى البول وأمكن تعيين العقار بنسبة استخراج تتراوح من ٩٨,٤٥٪ إلى ١٠٢,١٥٪ لتركيزات تتراوح من ٠,٠١ إلى ١,٠ ملى جرام لكل واحد ملى من البول.

Two simple, rapid and sensitive spectrophotometric and spectrofluorimetric methods have been developed for the determination of trimebutine in bulk drug, pharmaceutical preparations and in urine.

The first method depends on ion-pair complex reaction of trimebutine with two indicators, bromophenol blue (BPB) and thymol blue (TB), in methanol. Different variables affecting the reactions were studied and optimized. The coloured products of the drug with bromophenol blue and thymol blue were extracted and measured at 415 and 425 nm, respectively. Beer's law was obeyed in the concentration ranges 7-60 $\mu g\ ml^{-1}$ and 5-30 $\mu g\ ml^{-1}$ for BPB and TB respectively. The spectrofluorimetric method depends on the condensation of malonic acid and acetic anhydride under the catalytic effect of trimebutine. The condensation product gave emission light measured at 430 nm (excitation at 390 nm). Calibration range gave good correlation in the range of 0.8-80 $ng\ ml^{-1}$. The proposed methods were applied for estimation of the drug in different pharmaceutical preparations without interference from common encountered additives. Percentage recoveries ranged from 98.4% to 101.5%. The fluorimetric method was applied for the determination of drug in urine and gave good recoveries ranging from 98.45 to 102.15% for spiked concentration in the range 0.01 to 1.00 $mg\ ml^{-1}$ and the measured concentration ranged from 0.8 to 80 $ng\ ml^{-1}$.

INTRODUCTION

Trimebutine [2-dimethylamino-2-phenyl butyl 3,4,5-trimethoxy benzoate] is an antispasmodic drug, which has been reported to be effective in the treatment of bowel syndrome. This effect may be related to its action on opioid receptors in the gastro-intestinal tract.[1]



Trimebutine

Few methods were reported for determination of the drug in pure, pharmaceutical preparations and in plasma. High performance liquid chromatography with

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UV detector was used for determination of intact drug alone[2] or in presence of its related impurities.[3] Also HPLC/UV technique was used for determination of the drug and its major metabolite N-monodesmethyl trimebutine in rat and human plasma.[4-5] The drug was determined in capsules[6] and in rat plasma and tissues by capillary zone electrophoreses.[7] One colorimetric method depending on the ion-pair formation between bismuth iodide and trimebutine was used for determination of the drug in dosage forms.[8] Survey of literature review revealed that there is no fluorimetric method reported for its determination. The proposed spectrophotometric method is based on the presence of tertiary amine moiety in the structure of trimebutine, which forms ion-pair complexes with two indicators, bromophenol blue and thymol blue, that can be extracted and measured colorimetrically. The fluorimetric procedure depends on the catalytical effect of the tertiary amine moiety of the compound on the condensation of malonic acid with acetic anhydride to give fluorescent product. The developed methods were applied to the determination of the drug in different pharmaceutical preparations. The Fluorimetric method was used for the determination of drug in urine. The validation parameters of the method were evaluated.

EXPERIMENTAL

Apparatus

UV-1601 PC, UV-visible spectrophotometer (Shimadzu, Japan) and spectrofluorometer SFM 23/B (Kontron, Switzerland) were used for all measurements.

Chemicals and reagents

Trimebutine (Sigma, USA) was used as a working standard as it is without further purification. Bromophenol blue and thymol blue solutions (B.D.H., England) were 0.1% w/v in methanol. Malonic acid (Merk, Hoherbruna) was prepared as 10% w/v in acetic anhydride and the reagent is stable for about 20hours.

Buffer solutions

Teorell and Stenhogen buffer solutions[9] of the pH range 2 to 5 were prepared in freshly boiled and cooled distilled water.

Methanol, ethanol, chloroform, dichloromethane and all other solvents were of analytical grade.

Pharmaceutical formulations

The following commercial formulations were subjected to the analytical procedures

- Debridat® tablets (Hoechst Marion Roussel S.A.E. Cario, Egypt) contain 100 mg of trimebutine maleate.
- Gast-Reg® suspension (Amoun Pharm. CO. S.A.F., Cario, Egypt) contains 24 mg/5ml trimebutine.
- Gast-Regular® suppositories (Amoun Pharm. CO. S.A.F.) contain 100 mg of trimebutine maleate.

Standard solutions

About 100 mg of trimebutine were weighed accurately and transferred into a 100-ml calibration flask. The drug was dissolved in methanol to obtain solution of concentration 1 mg ml^{-1} Further dilutions with methanol were made to obtain the suitable concentrations.

General procedures

Spectrophotometric method

i- Using bromophenol blue

One millilitre of the standard or sample solution of trimebutine was transferred into a 50-ml separating funnel containing 4ml of buffer pH 3.3, 1.5 ml of 0.1% w/v bromophenol blue in methanol was added. The solution was extracted well with three 8 ml quantities of chloroform. The extracts were collected in a 25 ml volumetric flask and completed to volume with chloroform. Approximately 0.1 g of anhydrous sodium sulphate was added, shaked for about 1 min and filtered, rejecting the first portion of the filtrate. The absorbance of the resulting solution was measured at 415 nm against a reagent blank treated similarly.

ii-Using thymol blue

One millilitre of the standard or sample solution of trimebutine was transferred into a 50-ml separating funnel containing 2ml of buffer pH 3.1, 1.5 ml of 0.1% w/v thymol blue was added. The procedures were completed as above and the developed yellow colour was measured at 425 nm against a reagent blank treated similarly.

Spectrofluorimetric procedure

One millilitre of standard or sample solution of trimebutine (0.01-1.0 mg) was transferred into a test tube, evaporated to dryness on a boiling water bath, 2.5 ml of 10% w/v malonic acid/acetic anhydride reagent was added and the mixture heated again in a boiling water bath for 25 min. The solution was cooled, transferred quantitatively into 25-ml standard flask with ethanol and completed to volume with ethanol. Several dilutions were made with ethanol to obtain a final concentration in the range from 0.8 to 80 ng ml^{-1}. The relative fluorescence intensity was measured at 430 nm (excitation at 390 nm) against reagent blank treated similarly.

Dosage forms

Tablets

Twenty tablets were weighed and finely powdered. An amount of the powdered tablet equivalent to 100 mg was transferred into a 100-ml volumetric flask with 80 ml methanol. The solution was sonicated for five min and completed to the mark with methanol. The solution was filtered, the first portion of the filtrate discarded and completed as under general procedures.

Suspension

A volume of suspension equivalent to 100-mg of trimebutine was transferred into a 100-ml volumetric flask, completed to the mark with methanol. The solution was filtered, discarding the first portion of the filtrate and completed as under general procedures.

Suppository

An accurately weighed portion of Gast-Regular suppository equivalent to about 50 mg trimebutine was placed in a 50-ml beaker, melted in a water bath at 50-60°. Five millilitres of methanol was added and placed again in the water bath for five min with gentle shaking. The solution was quantitatively transferred into a 50-ml volumetric flask and completed to the mark with the same solvent. The solution was filtered and completed as under general procedures.

Determination in urine

One millilitre of standard solution, in the concentration range of 0.01 to 1.0 mg of drug was transferred into a test tube, evaporated on a water bath till dryness. One ml urine was added and shaked well on a shaker for 3 min, then transferred quantitatively into a 50-ml separator funnel with the aid of two portions each of 5-ml dichloromethane, shacked well and the organic layer was collected into a test tube. The solution was evaporated till dryness on a water bath. Malonic acid/acetic anhydride (2.5 ml of 10% w/v) was added and completed as mentioned under the general procedures.

RESULTS AND DISCUSSION

Spectrophotometric method

Ion pair extraction methods seem to be the most effective means of transferring the nitrogenous compounds from aqueous phase to organic phase. The separated ion-pair complex is determined colorimetrically.[10-20] Trimebutine as a cationic drug can interact easily and quantitatively with two dyes, bromophenol blue and thymol blue under favourable conditions forming coloured ion pairs extractable in chloroform. The wavelengthes of maximum absorption for the ion pairs are 415 and 425 nm in case of bromophenol blue and thymol blue respectively (Fig. 1).

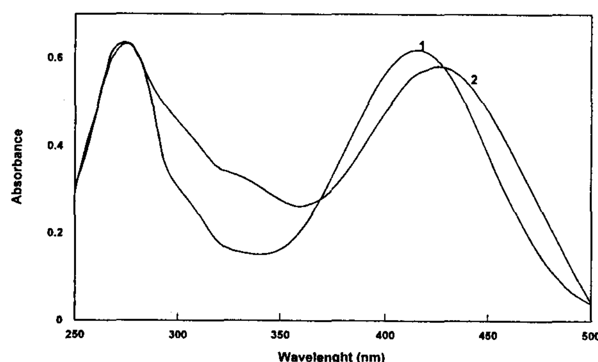


Fig. 1: Absorption spectra of ion-pair complexes of (1) trimebutine (30 µg/ml) and bromophenol blue and (2) trimebutine (20 µg/ml) and thymol blue.

Optimization of reaction conditions

Various parameters such as pH of buffer, volume of buffer, volume of dye solutions and type of solvent for extraction were studied for their effect on intensity and stability of the developed colour.

Effect of buffer pH

The effect of pH was studied and the most suitable pH in case of bromophenol blue is 3.3 and in case of thymol blue is 3.1 (Fig. 2). The optimum volume of the buffer in case of BPB is 4ml and in case of TB is 2 ml. The significant decrease in the absorbance at little or excess volume of buffer may be explained on the basis of incorrect ratio between methanol and water which may lead to the incomplete extraction of the complex with chloroform.

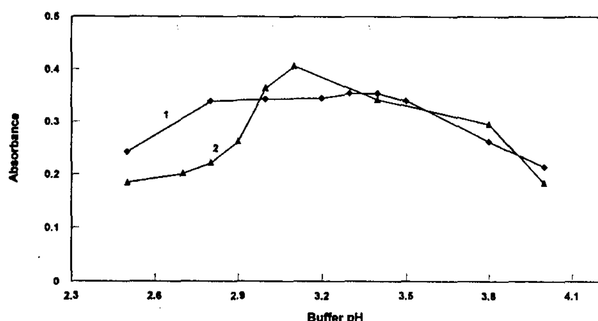


Fig. 2: Effect of buffer pH on the absorbance of ion-pair complexes of trimebutine (30 µg/ml) with bromophenol blue (1) and trimebutine (20 µg/ml) with thymol blue (2).

Effect of volume of 0.1% dyes

The optimum volume of 0.1% dyes used was also selected and 1.5 ml was found the most suitable volume of both dyes (Fig. 3).

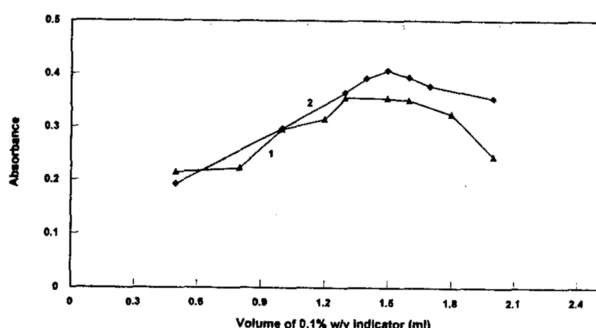


Fig. 3: Effect of volume of 0.1% w/v indicator on the absorbance of trimebutine with (1) bromophenol blue, (2) thymol blue.

Effect of diluting solvent

Different solvents such as, chloroform, dichloromethane and dichloroethane were studied for their effect on intensity, stability of colour and efficacy of extraction. Chloroform was selected because of its slightly high efficiency and considerable lower extraction ability for the reagent blank. The developed ion-pair complex between trimebutine and bromophenol blue was stable for about 40 min while that between drug and thymol blue was stable for at least 2 h.

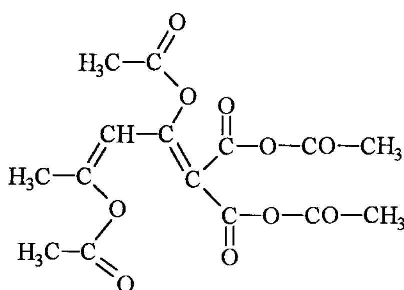
From the structure of the drug which containing one tertiary amine moiety and from the previously reported similar work using the same acidic indicators with drugs containing one tertiary amine moiety,[21] it was suggested that the stoichiometry of the reaction with these two dyes is 1:1 ratio. Accordingly, in the present work the suggested stoichiometry of the reaction is 1:1 ratio.

Spectrofluorimetric procedure

The reactions between anhydride and tertiary amines have been reported to give highly coloured and fluorescent products.[22-26] Thomas[27] used the malonic acid/acetic anhydride system for the fluorimetric determination o alkaloids containing tertiary amines.

In the present work the malonic acid/acetic anhydride reagent reacts under the catalytic effect of trimebutine as a tertiary amine to give a condensation product with high relative fluorescence intensity. The excitation and emission spectra of trimebutine with malonic acid/acetic anhydride appear in Figure 4. It was noted that the condensation product has two excitation maxima at 325 and 390 nm, so we selected the longer wavelength 390 nm and the emission wavelength is 430 nm.

The mechanism of the overall fluorogenic reaction has not yet been elucidated but it seems that the final product results from the base catalysed condenstation of mixed anhydride.[22,28] Groth and Wallerburg[28] have postulated a structure for the mixed anhydride of malonic acid and acetic anhydride, and suggested that the condensation product is as below:



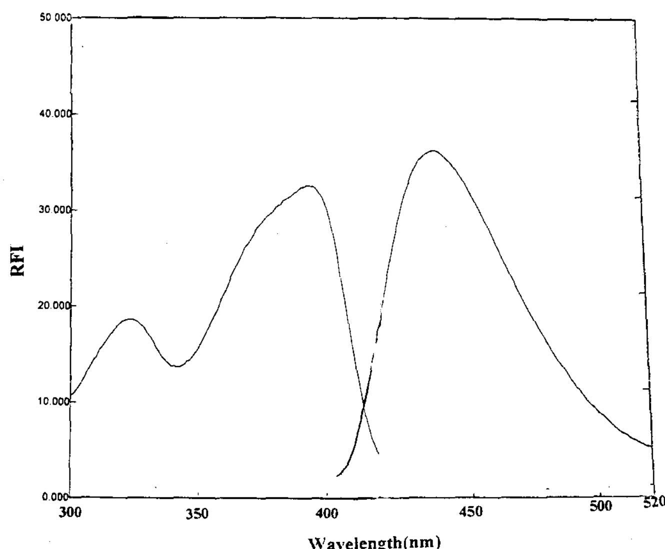


Fig. 4: Fluorecence spectra of the condensation product of malonic acid/acetic anhydride in presence of trimebutine (35 ng ml^{-1}).

Optimization of variables

All parameters were optimized.

Effect of volume of reagent

The effect of volume of 10% malonic acid/acetic anhydride (0.5-3.0 ml) on relative fluorescence intensity was studied. The relative fluorescence intensity increased with the volume of reagent till 2 ml and remained approximately constant with further increase in the volume, so 2.5 ml was selected as a suitable volume of the reagent.

Effect of heating time

The effect of time of heating, on a boiling water bath was also studied and was found that 25 min is the most optimum heating time to obtain the highest relative fluorescence intensity.

Effect of diluting solvent

Different diluting solvents were studied water, ethanol, methanol, acetonitril, dimethylformamide (DMF), chloroform, carbon tetrachloride and dichlormethane (Table 1). Ethanol was found to give the highest fluorescence intensity and most stable products, this may be explained by the high solubility of the condensation products in ethanol. The condensation product was found to be stable for 40 min so measurements must be done within this time.

Table 1: Effect of solvnets on the relative fluorescence intensity of trimebutine with malonic acid/acetic anhydride.

Solvent	Relative fluorescence intensity
Ethanol	42.9
Methanol	27.1
Acetonitril	39.0
DMF	17.0
Water	10.0
Chloroform	26.7
Carbon tetrachloride	9.0
Dichloromethane	11.9

Method validation

Calibration data

Absorbance recorded with increasing amount of trimebutine show that the absorbance increased linearly with the increase in concentration. The regression equations are, $A = 0.040 + 0.0102\ C$ with $r = 0.9988$ over the concentration range 7-60 μg ml^{-1} for drug with bromophenol blue and $A = 0.0062 + 0.0194\ C$ with $r = 0.9991$ over a concentration range of 5-30 μg ml^{-1} for drug with thymol blue. For the spectrofluorimetric method the regression equation was $RFI = 2.3111 + 0.9472\ C$ with $r = 0.9999$ over a concentration range of 0.8 to 80 ng ml^{-1}. All these results were recorded in Table 2.

Limits of quantification (LOQ)

The lower quantitative limit was calculated by the equation $LOQ = 10\ \sigma/s$,[29] where σ is the standard deviation of the intercept and s is the slope. The lower quantitative limits with each of BPB, TB and MAA reagent were 6.57 μg ml^{-1}, 4.81 μg ml^{-1} and 0.49 ng ml^{-1}, respectively (Table 2).

Table 2: Calibration data of trimebutine by different methods.

Method	Calibration range	R	R^2	Intercept ± SD	Slope ± SD	LOD	LOQ
1-BPB	7-60 ($\mu g\ ml^{-1}$)	0.9994	0.9988	0.0401±0.0067	0.0102±0.0002	1.97	6.57
2-TB	5-30 ($\mu g\ ml^{-1}$)	0.9991	0.9983	0.0062±0.0093	0.0194±0.0005	1.44	4.81
3-Fluor.	0.8-80 ($ng\ ml^{-1}$)	0.9999	0.9999	2.3111±0.0464	0.9472±0.0009	0.14	0.49

BPB = bromophenol blue, TB = thymol blue.

Table 3: Determination of trimebutine in different formulations by different proposed methods and a reported method.

Formulation	Label Claim	% recovery ± SD			
		BPB	TB	Fluorimetric method	Reported method[8]
Debridate tablet	100/tablet	98.8 (±0.725) F= 4.223 t= 1.838	101.5 (±1.649) F= 1.227 t= 1.703	99.5 (±1.949) F= 1.771 t= 0.476	99.9 (±1.489)
Gast-Reg® suspension	125/5 ml	100.7 (±1.621) F= 2.277 t= 1.268	99.5 (±1.703) F= 2.516 t= 0.154	100.9 (±1.105) F= 1.055 t= 1.799	99.7 (±1.074)
Gast-regulator®	100/supp.	98.4 (±1.298) F= 1.779 t= 0.087	100.2 (±1.971) F= 1.295 t= 1.608	100.2 (±2.257) F= 1.699 t= 1.312	98.5 (±1.732)

*Theoretical values of F and t at 95% confidence limit are 5.05 and 2.228.
**Average of six determinations.

Limits of detection (LOD)

Limit of detection is the lowest concentration that can be distinguished from the noise level, can be calculated by the equation LOD = 3 σ/s.[29] The LOD with BPB, TB and MAA reagent were 1.97 $\mu g\ ml^{-1}$, 1.44 $\mu g\ ml^{-1}$ and 0.14 $ng\ ml^{-1}$ respectively (Table 2).

Precision

The precision of a method is defined as the closeness of agreement between independent test results obtained under prescribed conditions. The relative standard deviation for trimebutine using bromophenol blue and thymol blue were 1.015 and 1.700 for the concentration of 10 $\mu g\ ml^{-1}$ where n= 4. The RSD using fluorimetric method was 2.225 for the concentration of 0.8ng ml^{-1} and 0.252 for the concentration of 80ng ml^{-1} where n in both cases equal to 6.

Accuracy and recoveries

Applying the proposed spectrophotometric and spectrofluorimetric procedures for the analysis of commercially available dosage forms validated the accuracy of the proposed methods. Table 3 shows the mean recoveries (98.4-101.5) with standard deviations (0.725-2.257). This indicates an excellent concordance between experimental and nominal values. The performance of the current method was jugged

by comparing with another reported method.[8] According to the variable ratio test (F-test) and t-test, the calculated values of F and t testes in Table 3 indicated that there is no significance difference between the proposed and reported method[8] with respect to precision and accuracy.

Specificity

According to the results obtained by the recovery experiment, the proposed methods are able to access the analyte in the presence of common excipients and hence the proposed methods can be considered specific.

Selectivity

Selectivity is the ability to separate the analyte from degradation products, metabolites and co-administered drugs.[30] The selectivity of the colorimetric ion-pair method was evaluated by testing benzyl alcohol, 3,4,5-trimethoxybenzoic acid and methyl-3,4,5-trimethoxybenzoate which are the related impurities of drug. All these impurities have no effect on the determination of the drug by ion-pair method. This is because; the ion-pair reaction depends on the presence of tertiary amine. Also the presence of primary, secondary and quaternary amines don't interfere with the determination of the drug by this method so N-monodesmethyl derivative, which is the main metabolite,[4] does not interfere with the determination of the intact drug i.e this ion-pair method is suitable as a stability indicating assay.

Application to pharmaceuticals

The three developed procedures for trimebutine were applied to three different pharmaceutical preparations tablets, suspension and suppository. Table 3 shows the results. The excellent recoveries (98.4-101.5) indicate the absence of interference from frequently encountered excipients or additives.

Determination in urine

The extraction of the drug from urine by dichlormethane and its determination by the proposed spectrofluorimetric method gave high recovery in the range of 98.45 to 102.15% for the determination of concentration range of 0.01-1.00 mg ml^{-1} (Table 4). These recoveries indicate the proper separation of the drug from urine and the absence of interference from its constituents. So the proposed method is suitable for determination of trimebutine in urine and also may be in other biological fluids.

Conclusion

The developed colorimetric and fluorimetric methods are simple, rapid, selective, sensitive and suitable for routine analysis of trimebutine in pure and pharmaceutical preparations. Fluorimetric method is the most sensitive and its lower detection limit makes it suitable for determination of the drug in urine and may be in other biological fluids.

Table 4: Determination of trimebutine in spiked urine samples using standard addition method.

Spiked conc. (mg ml^{-1} urine)	Expected conc. after dilution (ng ml^{-1})	Measured conc. after dilution (ng ml^{-1})	% Recovery	SD*
0.01	0.80	0.80	100.33	1.94
0.05	4.00	4.09	102.15	0.42
0.10	8.00	7.96	99.50	3.40
1.00	80.00	78.76	98.45	4.04

*Standard deviation for five determinations.

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