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Conductometric Determination of Tiemonium Methylsulfate, Alizapride Hydrochloride, Trimebutine Maleate using Rose Bengal, Ammonium Reineckate and Phosphotungstic Acid

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ABSTRACT

Simple, cost-effective, accurate and easily applicable conductometric titration was applied using three different ion-pairing reagents; rose bengal, ammonium reineckate, and phosphotungstic acid represented as method A, B and C, respectively, for determination of tiemonium methylsulfate (TIM) and alizapride hydrochloride (AL). Method A, B had been described for the determination of trimebutine maleate (TRM). The proposed methods have been successfully employed for the determination of pure and pharmaceutical dosage forms with studying the effect of common pharmaceutical excipients which did not interfere with the assay procedure. Optimized conditions including temperature, solvent, reagent concentration, and molar ratio were studied. Beer's law was obeyed in the concentration ranges of (1-20), (1.5-10), (1-12) mg/50 ml of TIM, (1-17), (1-10), (0.6-12) mg/50 ml of AL for Method A, B and C, respectively, where (1-17), (1.5-10) mg/50 ml of TRM for Method A and B, respectively. Results of analysis were validated statistically by recovery studies.

Key words: *Tiemonium, Alizapride, Trimebutine, Conductometry.*

1. INTRODUCTION

Tiemonium methylsulfate or tiemoniummetilsulfate (TIM), 4-[3-Hydroxy-3-phenyl-3-(2-thienyl)propyl]-4-methyl-morpholinium methylsulfate with molecular formula $C_{19}H_{27}NO_6S_2$=429.6, is quaternary ammonium antimuscarinics which are used in the relief of visceral spasms [1]. The drug is not included in any official pharmacopeias. From the literature survey, there were few methods had been reported for TIM determination using particle induced X-ray emission [2] and ultraviolet (UV) spectroscopic methods [3] for TIM, its dosage forms and in the presence of its degradation products [4,5]. Chromatographic methods also had been applied [6].

Alizapride hydrochloride (HCl) (AL), N-[(1-Allyl-2-pyrrolidinyl)methyl)-6-methoxy-1H-benzotriazole-5-carboxamide HCl with molecular formula: $C_{16}H_{21}N_5O_2$, HCl =351.83, is a substituted benzamide similar to metoclopramide, which is used to control nausea and vomiting associated with a variety of disorders [1]. The drug is not included in any official pharmacopeias. Chromatographic methods had been reported in literature for determination of AL using tandem mass spectrometric [7] and UV detections [8]. Stability studies were carried out for the determination of AL and its degradation products in bulk drug and pharmaceutical formulations [9]. Spectrofluorimetric method was reported to study effects of pH and solvent on the fluorescence properties of biomedically important benzamides [10].

Trimebutine maleate (TRM) is chemically known as(2RS)-2-(Dimethylamino)-2phenylbutyl3,4,5-trimethoxybenzoate(Z)-butenedioate with molecular formula $C_{26}H_{33}NO_9$ = 503.5 [11]. It has been used as an antispasmodic in gastrointestinal tract and has indicated for treatment of irritable bowel syndrome [1]. It can be determined in British Pharmacopoeia by non-aqueous titration using perchloric acid determining end-point potentiometrically [11]. The literature search revealed different techniques for the analysis of TRM. Derivative spectrophotometry [12] and different reagents used for colorimetric determination of TRM such as cobalt(II)-thiocyanate, molybdenum(V)-thiocyanate [13], bi(III)-iodide [14] and 2,3-dichloro-5,6-dicyano-p-benzoquinone, tetracyanoethylene, tetracyanoquinodimethane [15] had been applied. Electrochemical methods were also included for its quantitation, e.g., stripping voltammetry [16] and conductometry using phosphotungstic acid (PTA) [17]. Chromatographic methods were used

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involving high-performance liquid chromatography (HPLC) and capillary zone electrophoresis [18]. HPLC techniques were used for determination of pure TRM, in pharmaceutical formulations [19] and in the presence of related impurities [20].

Rose bengal (RB) is chemically known as disodium salt of 4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein, it had been used for many investigations via spectrophotometric methods through either ternary complex formations with certain antihistaminic drugs [21] or ion pair complex formation with clindamycin HCl, its dosage forms [22] and oxybuprocine HCl [23] using extractive or non-extractive procedure or both [24]. Moreover, it had been applied for the determination of barium and sulfate using novel barium polymeric membrane sensor [25]. Conductometric methods had been also reported for clindamycin HCl and macrolide antibiotics [22,26] in addition to fluorometric quantitation of pancuronium bromide in human serum [27].

Ammonium reineckate (Amm.Rt) salt, is chemically known as ammonium tetrathiocyanato-diamminechromate (III) monohydrate, was used for quantitative determination of many pharmaceutical compounds in its pure form and pharmaceutical preparations through applying different techniques as spectrophotometry [28]. Atomic absorption spectroscopy [28], conductometry [28-30], potentiometry [31], and ion-associate complexes-atomic emission spectrometry [32].

PTA is also known as dodeca - Tungstophosphoric acid had been commonly used in conductometric titrations of precipitation reactions for analysis of different compounds [17,33,34] as well as potentiometric determinations [35,36]. Gel filtration and HPLC analysis [37] had been also reported.

In this paper, three simple and easily applicable conductometric methods with reproducible results had been used for determination of the cited drugs using RB, Amm.Rt and PTA. The proposed methods had been also applied to the assay of these drugs in their dosage forms.

2. EXPERIMENTAL

2.1. Apparatus

JENWAY model 470 Conductivity/TDS Meter (470 201) with a cell constant of 1.09 cm^{-1}, with conductivity/temperature probe (027 298) was used.

Digital analyzer pH meter (USA) was used.

2.2. Chemicals and Reagents

All solvents and reagents were of analytical reagent grade; bi-distilled water was used throughout the work.

1. Samples were generously supplied by their respective manufactures;
   - TIM (Adwia Pharmaceutical Industries Co., Egypt). Its purity was found to be 99.90% as reported from company.
   - AL (Mina Pharm Pharmaceuticals, Cairo, Egypt). Its purity was found to be 99.98 according to the comparison method [9].
   - TRM (Amoun Pharmaceutical Industries Co., Egypt). Its purity was found to be 100.00% as reported from company.

   And were used as received without further treatment.
2. RB (Acros Organics) was prepared as 10^{-2} M aqueous solution
3. Amm.Rt (Aldrich) was prepared as 5×10^{-3} M aqueous solution by dissolving appropriate weight in boiled double distilled water
4. PTA (AnalaR) was prepared as 10^{-2} M aqueous solution.
5. Acetone (99%), ethanol (98%) and methanol (98%) (El-NasrChemical Co., Egypt) ,sodium hydroxide were used.
6. Lactose,sodium chloride, ,starch, and magnesium stearate (El-Nasr Chemical Co., Egypt).

2.3.Pharmaceutical preparations

The following available commercial formulations are subjected to analytical procedure:

1. Visceralgine® tablets, Batch No. 0513224 (Sedico Pharmaceutical company, Giza, Egypt) labeled to contain 50mg tiemonium methyl sulphate per tablet.
2. Viscera® ampoules, Batch No144766, 144162, 14462, 140673 (Amoun Pharmaceutical Co., S.A.E.El-Obour City, Cairo, Egypt) labeled to contain 5mg tiemonium methyl sulphate per 2ml.
3. Nausilex® ampoules, Batch No. EKE3378, DHE2261 (Mina Pharm Pharmaceuticals, Egypt), labeled to contain 55.8mg Alizapride hydrochloride per 2 ml.
4. Tritone® tablets, Batch No. A24405(Global Napi PharmaceuticalS, October, Egypt) labeled to contain 200mg Trimebutine maleate per tablet.

2.4.Working standard solutions

For method A: working solutions were prepared to contain 1 mg ml^{-1} by dissolving 100 mg of TIM, TRM and AL in 100 ml of 50% acetone, methanol and bi-distilled water respectively in 100 ml volumetric flask.

For method B,C: Aqueous solution of 1 mg ml^{-1} of TIM, AL and TRM were prepared by dissolving 100 mg of the pure drug in 100 ml bi-distilled water in volumetric flask.

2.5. General Procedures

suitable aliquots of working solutions containing(1-20), (1.5-10), (1-12) mg of TIM,(1-17), (1-10), (0.6-12)

mg of AL for method A,B and C respectively, where (1-17), (1.5-10) mg of TRM for method A and B respectively were transferred to 50 mL calibrated flasks.

In each flask, volume was made up to the mark using bi-distilled water except in method A 50% acetone, methanol were used for TIM and TRM respectively. The content of each calibrated flask was transferred to a beaker and the conductivity cell was immersed in the sample solution. 10^{-2} M RB, 5×10^{-3} M Amm.Rt and 10^{-2} M PTA were used as titrants. The conductance reading was taken subsequent to each addition of titrant after stirring for 2 min and corrected for dilution effects by means of the following equation, assuming that conductivity is a linear function of dilution:

$$\Omega{-}1 \text{ correct} = \Omega{-}1 \text{ obs } [v1+v2/v1] \quad (1)$$

Where, Ω−1 correct is the corrected electrolytic conductivity, Ω−1 obs is the observed electrolytic conductivity, v1 is the initial volume and v2 is the volume of reagent added.

A graph of corrected conductivity versus the volume of added titrant was constructed, and end-point was determined graphically at the intersection of two lines. Each procedure takes about 20-35 min in all (Table 1).

2.3. Procedures for Pharmaceutical Preparations

2.3.1. Procedures for tablets

2.3.1.1. Method A, B, C

About 10 tablets of analyzed drugs were weighed, grounded into fine powder then amounts equivalent to 25 mg for TIM, TRM were accurately weighed and extracted with methanol for TIM and bidistilled water for TRM, followed by filtration after good shaking, washing the residue several times and complete to final volume with the same solvent in 25 ml volumetric flasks. In the case of methanol, filtrates of TIM were evaporated and residue of drug was dissolved with bidistilled water (Method B, C) and 50% acetone (Method A). Volumes were completed to the mark with the same solvent in 25 ml volumetric flasks.

2.4. Procedures for Ampoules

2.4.1. Viscera ampoule

2.4.1.1. Method A, B

A value of 10 ml of viscera ampoules containing 25 mg of TIM salt were quantitatively transferred into 60 ml separating funnel, add 5 ml saturated NaOH solution to get base free which was extracted with 3 × 15 ml portions of chloroform after shaking well for 2 min each time. Evaporate the extracted organic solvent, the residue was dissolved in 0.5 ml of 1 M H_2SO_4 and complete to 25 ml with 50% acetone (Method A), bi-distilled water (Method B) in the 25 ml volumetric flasks after adjustment pH using H_2SO_4 and NaOH till equalize pH of pure drug solution.

2.4.1.2. Method C

About 10 ml of viscera ampoules containing 25 mg of TIM salt were quantitatively transferred into 25 ml volumetric flask completed to final volume with bi-distilled water.

2.4.2. Nausilex ampoule

2.4.2.1. Method A, B, C

0.9 ml of Nausilex ampoule containing 25 mg of AL salt were quantitatively transferred into 25 ml volumetric flask completed to final volume with bi-distilled water.

The general procedure was followed, and recovery experiments were performed by standard addition technique as an additional check on the accuracy of the proposed method.

3. RESULT AND DISCUSSION

3.1. Method Development

The principle of conductometric titration is based on the fact that during the titration, one of the ions is replaced by the other and these two ions which differ in the mobility and its total concentration causing difference in the conductivity of the solution during the titration before and after the equivalence point, so it can be used successfully in quantitative determination of certain drugs. The equivalence point can be determined using the conventional

Table 1: Analytical performance data of conductometric procedures.

Parameter	TIM			AL			TRM	
	Method A	Method B	Method C	Method A	Method B	Method C	Method A	Method B
Optimum concentrated, mg/50 ml	1-20	1.5-10	1-12	1-17	1-10	0.6-12	1-17	1.5-10
Type of solvent	50% acetone-water	bi-distilled water	bi-distilled water	bi-distilled water	bi-distilled water	bi-distilled water	methanol	bi-distilled water
Reagent's concentration (M)	10^{-2}	5×10^{-3}	10^{-2}	10^{-2}	5×10^{-3}	10^{-2}	10^{-2}	5×10^{-3}
Molar ratio (drug: titrant)	1:1	1:1	3:1	1:1	1:1	3:1	1:1	1:1

TIM=Tiemonium methylsulfate, AL=Alizapride hydrochloride, TRM=Trimebutine maleate

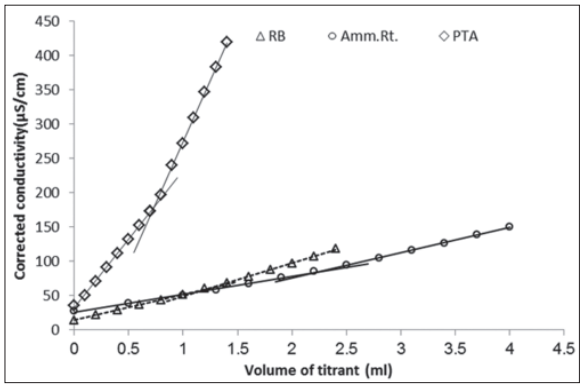

Figure 1: Conductometric titration curves of 5 mg TIE using (5×10^{-3} M) ammonium reineckate, 10^{-2} M rose bengal and 10 mg tiemonium methylsulfate (TIE) titrated with 10^{-2} M phosphotungstic acid.

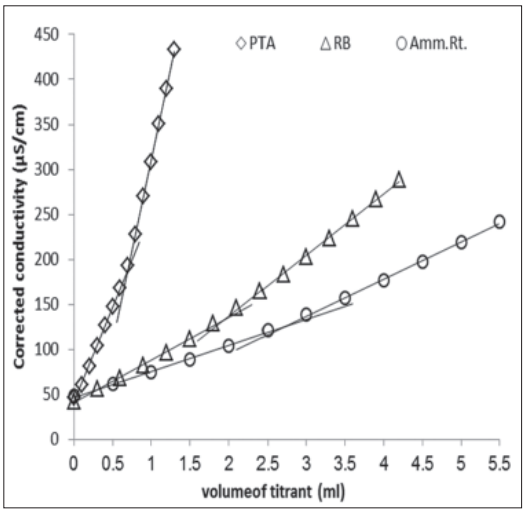

Figure 2: Conductometric titration curves of 5 mg alizapride hydrochloride using (5×10^{-3} M) ammonium reineckate and 7 mg alizapride hydrochloride titrated with 10^{-2} M phosphotungstic acid and rose bengal.

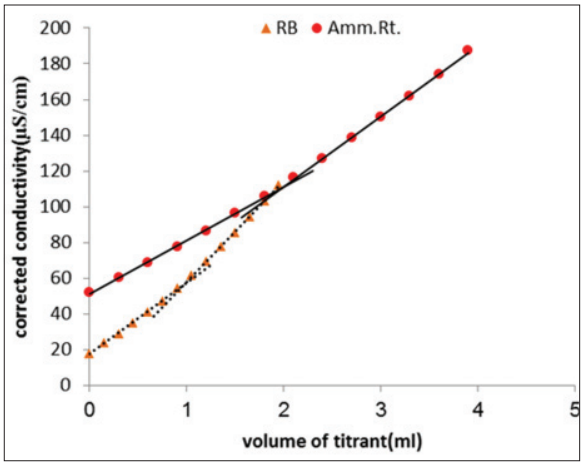

Figure 3: Conductometric titration curves of 5 mg trimebutine maleate using (5×10^{-3} M) ammonium reineckate and 10^{-2} M rose bengal.

procedure for locating the endpoint which is the intersection point of two straight lines obtained graphically by plotting corrected conductivity as a function of the added titrant volume. From equivalence point which measures the volume of titrant used for complete reaction, the amount of the drug can be calculated according to the following equation:

Amount of drug (mg) = VMR/N

Where, V is the volume of titrant (ml), M is molecular weight of drug, R is the molar concentration of titrant and N is the number of moles of titrant consumed by one mole of drug. 1 ml of 10^{-2} mol L^{-1} RB, 5×10^{-3} mol L^{-1} Amm.Rt, 10^{-2} PTA is theoretically equivalent to 4.29, 2.15, 12.89 mg of TIM or 3.52, 1.76, 10.55 mg of AL or 5.04, 2.52 mg of TRM. Where the shape of the titration curves not only rely on the total conductance of the species present in solution but also other factors such as ion-pair association, dielectric constant, solvation, viscosity and proton transfer can be affected. In this study; three reagents, RB, Amm.Rt. and PTA, were found to react with the cited drugs forming stable ion pairs with different aqueous solubility. The ion pair was either sparingly soluble as TIM, AL, TRM that were able to form precipitates with Amm.RT and heteropoly acids as PTA; or soluble like ion-associates between RB dye and the cited quaternary ammonium drugs so conductometric titration was successfully used for determination of end points in precipitation reactions and highly colored complex formation simply without any need to buffer or surfactant. The representative titration curves are shown in Figures 1-3. During titration, pink complexes of drug-RB and pink precipitates of drug-Amm.Rt or white precipitates of drug-PTA were formed leading to regular rise in conductance up to the equivalence point where a sudden change in the slope occurs corresponds to the excess of titrant (the first branch of the curve descending the second one).

TIE CH_4SO_4+RB $COO^-Na^+ \rightarrow [TIE\ H]^+\ [RB\ COO]^- + Na^+ + CH_3SO_4^-$

Studying the influence of some variables on the reaction has been studied to establish the optimum conditions for performing the titration with successful results in a quantitative manner (higher conductance and most sharp end point) as in the following preliminary experiments:

3.1.1. Effect of solvent

Titrations using different solvents for both drugs and reagents such as (bi-distilled water, methanol, 50% v/v methanol-water, 50% v/v ethanol-water

Table 2: Assay results for determination of the cited drugs (authentic) with RB.

Parameters	Method A								
	TIM			AL			TRM		
	Taken (mg)	Found (mg)	Recovery* %	Taken (mg)	Found (mg)	Recovery* %	Taken (mg)	Found (mg)	Recovery* %
	1	1.013	101.30	1	0.985	98.50	1	0.992	99.20
	2	2.013	100.65	3	2.990	99.67	2	2.029	101.45
	3	2.990	99.67	5	4.996	99.92	3	3.051	101.70
	5	5.051	101.02	7	7.070	101.00	5	5.016	100.32
	10	9.892	98.92	10	9.992	99.92	7	7.121	101.73
	17	16.927	99.57	17	17.099	100.58	10	10.100	101.00
	20	19.995	99.98				12	12.035	100.29
							17	17.031	100.18
Mean±SD	100.16±0.860			99.93±0.858			100.73±0.889		
N	7			6			8		
RSD	0.859			0.859			0.882		
SE	0.325			0.351			0.314		
Variance	0.740			0.737			0.790		

*Average of three different determinations. SE=Standard error, RSD=Relative standard deviation, SD=Standard deviation, TIM=Tiemonium methylsulfate, AL=Alizapride hydrochloride, TRM=Trimebutine maleate, RB=Rose bengal

Table 3: Assay results for determination of the cited drugs (authentic) with Amm.Rt.

Parameters	Method B								
	TIM			AL			TRM		
	Taken (mg)	Found (mg)	Recovery* %	Taken (mg)	Found (mg)	Recovery* %	Taken (mg)	Found (mg)	Recovery* %
	1.5	1.504	100.27	1	1.003	100.30	1.5	1.484	98.93
	2.5	2.534	101.36	2	1.972	98.60	2.5	2.517	100.68
	3	3.008	100.27	3	2.991	99.70	3	2.997	99.90
	4	3.996	99.90	5	5.031	100.62	4	3.978	99.45
	5	5.069	101.38	7	7.037	100.53	5	5.036	100.72
	7	6.959	99.41	10	10.116	101.16	7	7.052	100.74
	10	10.008	100.08				10	10.072	100.72
Mean±SD	100.38±0.735			100.15±0.896			100.16±0.743		
N	7			6			7		
RSD	0.732			0.894			0.742		
SE	0.278			0.366			0.281		
Variance	0.540			0.802			0.552		

*Average of three different determinations. SE=Standard error, RSD=Relative standard deviation, SD=Standard deviation, TIM=Tiemonium methylsulfate, AL=Alizapride hydrochloride, TRM=Trimebutine maleate, Amm.Rt=Ammonium reineckate

and 50% v/v acetone-water, for Method A, B, C in addition to ethanol and acetone for Method B in the case of TRM. Aqueous medium was the most suitable for Methods A (only AL), B and C. Whereas in 50% v/v acetone-water for TIM and methanol for TRM were used in Method A, sharpest end points were detected. The dilution of drug solution to 50 ml will be used for all experiment processes.

3.1.2. Reagent's concentration

Ranges from 9×10^{-3} to 3×10^{-2}, 10^{-2} to 2×10^{-3} and 3×10^{-2} to 10^{-3} molar solutions of RB, Amm.Rt and PTA, respectively, were investigated. The reagent

Table 4: Assay results for determination of the cited drugs (authentic) with PTA.

Parameters	Method C					
	TIM			AL		
	Taken (mg)	Found (mg)	Recovery* %	Taken (mg)	Found (mg)	Recovery* %
	1	0.979	97.90	0.6	0.597	99.50
	2	1.984	99.20	1	1.000	100.00
	3	2.974	99.13	2	2.038	101.90
	5	5.025	100.50	4	4.064	101.60
	7	6.961	99.44	5	5.073	101.46
	10	10.051	100.51	7	7.073	101.04
	12	11.984	99.87	10	10.137	101.37
				12	12.253	102.11
Mean±SD		99.51±0.908			101.12±0.916	
N		7			8	
RSD		0.912			0.906	
SE		0.343			0.324	
Variance		0.824			0.840	

*Average of three different determinations. SE=Standard error, RSD=Relative standard deviation, SD=Standard deviation, TIM=Tiemonium methylsulfate, AL=Alizapride hydrochloride, TRM=Trimebutine maleate, PTA=Phosphotungestic acid

Table 5: Statistical analysis of results obtained by the proposed methods compared with reported and official methods.

Drug	Proposed methods			Comparison method
	Method A	Method B	Method C	
TIM				
Mean±SD	100.16±0.860	100.38±0.735	99.51±0.908	100.22±0.964
Variance	0.740	0.540	0.824	0.930
N	7	7	7	6
Student's t-test	0.119 (2.201)*	0.34 (2.201)*	1.129 (2.201)*	-
F-test	1.257 (4.39)*	1.722 (4.39)*	1.366 (4.39)*	-
AL				
Mean±SD	99.93±0.858	100.15±0.896	101.12±0.916	99.98±0.836
Variance	0.737	0.802	0.840	0.699
N	6	6	8	3
Student's t-test	0.083 (2.365)*	0.274 (2.365)*	1.873 (2.262)*	-
F-test	1.054 (5.79)*	1.147 (5.79)*	1.202 (4.74) *	-
TRM				
Mean±SD	100.73±0.889	100.16±0.743	-	99.91±0.585
Variance	0.790	0.552	-	0.342
N	8	7	-	3
Student's t-test	1.458 (2.262)*	0.512 (2.306)*	-	-
F-test	2.310 (4.74)*	1.614 (5.14)*	-	-

*The corresponding theoretical values for t- and F-tests at P=0.05. SD=Standard deviation, TIM=Tiemonium methylsulfate, AL=Alizapride hydrochloride, TRM=Trimebutine maleate

concentration in each titration must be not <10 times that of the drug solution in order to minimize the dilution effect on the conductivity through the titration. The optimum concentrations of the reagents were 10^{-2}, 5×10^{-3} and 10^{-2} M of RB, Amm.Rt and PTA respectively in the titration of the studied drugs to

achieve highly constant and stable reading after 2 min of mixing.

3.1.3. Effect of temperature

Raising the temperature to 40°C, has no effect on the reaction since conductivity cell could be affected by elevated temperature. Thus, experiments were performed at room temperature.

3.1.4. Molar ratio

Prepare equimolar 10^{-2} M bidistilled water solutions of the three cited drugs and the three used titrants, transfer certain volume of each drug to a beaker, and the general procedure was followed for determination of the drug-titrant ratio for each reaction. It is confirmed that ratios of drugs to RB, Amm.Rt. were (1:1) while (1:3) were the ratios of PTA to TIM and AL.

3.2. Methods Validation

Validations of the proposed methods were carried out via statistical analysis of the data obtained from its application on the drug in the pure form and in their formulations. Under the optimum conditions described, satisfactory results were obtained for pure drug as shown in Tables 2-4 with good recovery values and standard deviations.

Recovery % = (Calculated end point/Theoretical end point) × 100.

3.2.1. Accuracy and precision

3.2.1.1. Accuracy

The accuracy of the proposed methods was ascertained by determining pure samples of the cited drugs with reported methods. Statistical analysis of

Table 6: Precision data for the determination of cited drugs by the proposed methods.

Method	Intra-day				Inter-day		
	Added (mg)	Found±SE (mg)	RSD %	Er %	Found±SE (mg)	RSD %	Er %
Method A							
TIM	2	2.01±0.441	0.761	0.33	2.00±0.569	0.983	0.203
	3	3.00±0.401	0.693	0.11	3.02±0.509	0.876	0.675
	5	5.05±0.133	0.229	0.93	5.04±0.231	0.397	0.80
AL	3	3.00±0.568	0.983	0.01	3.01±0.509	0.879	0.33
	5	5.05±0.529	0.907	1.00	5.03±0.353	0.608	0.53
	7	7.12±0.082	0.140	1.71	7.08±0.190	0.326	1.19
TRM	2	1.99±0.500	0.870	−0.50	2.00±0.577	1.000	0.00
	3	3.01±0.484	0.835	0.44	3.03±0.401	0.688	0.89
	5	4.95±0.546	0.955	−1.07	4.98±0.546	0.948	−0.33
Method B							
TIM	5	5.040±0.573	0.985	0.81	5.040±0.573	0.985	0.81
	7	7.045±0.614	1.057	0.64	7.023±0.532	0.918	0.33
	10	10.030±0.217	0.374	0.30	10.058±0.259	0.446	0.58
AL	2	1.984±0.583	1.019	−0.82	1.984±0.583	1.019	−0.82
	3	3.026±0.588	1.009	0.88	3.009±0.589	1.017	0.29
	7	7.054±0.248	0.426	0.78	7.054±0.251	0.432	0.78
TRM	3	2.984±0.422	0.735	-0.52	2.984±0.422	0.735	−0.52
	5	5.061±0.500	0.856	1.22	5.061±0.500	0.856	1.22
	7	6.926±0.362	0.634	−1.05	7.004±0.386	0.668	0.06
Method C							
TIM	5	5.047±0.433	0.744	0.93	5.000±0.500	0.866	0.00
	7	7.002±0.581	1.006	0.02	7.002±0.581	1.006	0.02
	10	10.008±0.427	0.738	0.08	10.008±0.427	0.738	0.08
AL	5	5.089±0.227	0.386	1.77	5.051±0.379	0.650	1.01
	7	7.108±0.252	0.430	1.55	7.091±0.252	0.432	1.30
	10	10.171±0.337	0.573	1.71	10.137±0.000	0.00	1.37

TIM=Tiemonium methylsulfate, AL=Alizapride hydrochloride, TRM=Trimebutine maleate, SE=Standard error, RSD=Relative standard deviation

Table 7: Analysis of the cited drugs by Method A in the presence of some common excipients.

Excipients added (mg/50 ml)	Recovery* %							
	TIM			AL			TRM	
	Method A (5 mg/50 ml)	Method B (5 mg/50 ml)	Method C (7 mg/50 ml)	Method A (7 mg/50 ml)	Method B (5 mg/50 ml)	Method C (7 mg/50 ml)	Method A (5 mg/50 ml)	Method B (5 mg/50 ml)
Lactose (10 mg)	99.60	99.64	101.29	-	-	-	98.20	98.20
Starch (5 mg)	99.60	98.78	101.29	-	-	-	98.20	99.80
Magnesium stearate (5 mg)	98.00	97.80	101.29	-	-	-	98.20	99.71
NaCl (10 mg)	101.40	98.80	99.44	101.00	100.28	101.00	99.80	101.40

TIM=Tiemonium methylsulfate, AL=Alizapride hydrochloride, TRM=Trimebutine maleate, *Mean of three different experiments.

Table 8: Application of standard addition technique for determination of the cited drugs through reaction with RB.

TIM						AL			TRM		
Visceralgine® tablets			Viscera® ampoules			Nausilex® ampoules			Tritone® tablets		
Taken (mg)	Added (mg)	Recovery* %	Taken (mg)	Added (mg)	Recovery* %	Taken (mg)	Added (mg)	Recovery* %	Taken (mg)	Added (mg)	Recovery* %
2		99.20	2		100.65	3		100.03	2		100.70
	2	101.15		3	101.10		1.5	100.93		2	101.20
	3	101.07		4	99.33		2	100.25		3	99.03
	5	100.34		5	100.86		3	98.63		5	100.02
	6	99.87		6	100.08		4	98.60		6	99.88
	10	100.12		7	99.16		5	100.12		7	99.14
							7	100.01		8	100.60
Mean±SD	100.51±0.573		100.11±0.876			99.76±0.941			99.98±0.834		
N	5		5			0.941			6		
SE	0.256		0.392			0.384			0.341		
V	0.329		0.767			0.885			0.696		

*Mean of three different experiments. SE=Standard error, RSD=Relative standard deviation, SD=Standard deviation, TIM=Tiemonium methylsulfate, AL=Alizapride hydrochloride, TRM=Trimebutine maleate, RB=Rose bengal

Table 9: Application of standard addition technique for determination of the cited drugs through reaction with Amm.Rt.

TIM						AL			TRM		
Visceralgine® tablets			Viscera® ampoules			Nausilex® ampoules			Tritone® tablets		
Taken (mg)	Added (mg)	Recovery* %	Taken (mg)	Added (mg)	Recovery* %	Taken (mg)	Added (mg)	Recovery* %	Taken (mg)	Added (mg)	Recovery* %
3		100.27	3		102.43	2		100.25	3		99.90
	1.5	100.20		2.5	100.51		1	98.59		2	97.95
	2	99.85		3	100.20		1.5	99.67		2.5	100.20
	2.5	99.60		4	99.75		2	98.50		3	100.33
	3	100.27		5	100.08		2.5	99.92		3.5	100.37
	4	99.88		6	100.23		3	99.10		4	100.40
	5	101.36					6	99.97		5	100.46
Mean±SD	100.19±0.623		100.15±0.276			99.29±0.655			99.95±0.984		
N	6		5			6			6		
SE	0.254		0.123			0.268			0.402		
V	0.388		0.076			0.429			0.969		

*Mean of three different experiments. SE=Standard error, SD=Standard deviation, TIM=Tiemonium methylsulfate, AL=Alizapride hydrochloride, TRM=Trimebutine maleate, Amm.Rt=Ammonium reineckate

Table 10: Application of standard addition technique for determination of the cited drugs through reaction with PTA.

Visceralgine® tablets			Viscera® ampoules			Nausilex® ampoules		
Taken (mg)	**Added (mg)**	**Recovery* %**	**Taken (mg)**	**Added (mg)**	**Recovery* %**	**Taken (mg)**	**Added (mg)**	**Recovery* %**
2		100.50	2		101.80	2		100.80
	1	99.20		3	101.27		1	100.00
	2	99.20		4	100.52		2	99.75
	3	100.67		5	99.00		5	100.84
	4	99.68		6	99.17		6	100.70
	5	99.40		7	101.57		7	100.57
	6	100.08		8	101.00		8	101.13
	8	99.13		9	100.51		9	102.34
Mean±SD	99.62±0.571		100.43±0.998			100.76±0.845		
N	7		7			7		
SE	0.216		0.377			0.319		
V	0.327		0.997			0.714		

*Mean of three different experiments. SE=Standard error, SD=Standard deviation, TIM=Tiemonium methylsulfate, AL=Alizapride hydrochloride, TRM=Trimebutine maleate, PTA=Phosphotungstic acid

the results obtained by the proposed and comparison methods [3,9,11] showed that the calculated values did not exceed the theoretical ones which indicated no significant differences found between the proposed methods and comparison methods. Statistical comparison of the results was performed using Student's t-test and variance ratio F-test at 95% confidence level (Table 5).

3.2.1.2. Precision

- Intra-day precision: To determine intra-day precision (repeatability) of the proposed methods, solutions containing three different concentrations(within working ranges) of each drug in its pure form were prepared and analyzed by proposed methods on three successive times in the same day. The values of relative standard deviation were calculated, and percentages relative error (Er %) of the suggested methods were also calculated using the following equation:

$$Er\% = [(found - added)/added] \times 100$$

- Inter-day precision: · To establish inter-day precision (intermediate), three experimental replicates including three different concentrations (within working ranges) of the cited drugs were carried out using proposed methods over period of 3-day.

Intra-day and inter-day precisions and accuracy results were summarized in Table 6 indicating the validity and applicability of the proposed methods and the reproducibility of the results.

3.2.2. Selectivity

To study selectivity of the proposed methods, interference liabilities were performed to explore the effect of common excipients that might be added during formulations. Under the experimental condition employed, to known amounts of the drugs, the common excipients as magnesium stearate (5 mg), starch (5 mg), lactose (10 mg), sodium chloride (10 mg) were added and analyzed for Method A, B and C. The analysis of these laboratory-prepared samples was performed using the general recommended procedures. Results showed no interferences, but sodium chloride caused high conductance at the beginning of titration and showed attenuation in the inflection but did not affect the end point which may cause a problem with viscera ampoule especially in Method A, B. Hence, in this work, the problem was solved by extraction of the drug free base using chloroform to prevent the effect of sodium chloride (Table 7).

3.3. Analytical Applications

The proposed methods were successfully applied to the assay of the studied drugs in their pharmaceutical formulations using the standard addition technique. Satisfactory results obtained for the recoveries of the drugs were in good agreement with the label claim and proved the suitability of the proposed methods. The percentage recoveries of the pure drugs using the proposed methods shown in Tables 8-10 are in good agreement with those obtained with the comparison methods.

4. CONCLUSION

Results demonstrated the usefulness of the three reagents in the conductometric determination of the

cited drugs. The data given previously reveal that the proposed methods are simple and easily applicable to analysis of drugs in their pharmaceutical dosage forms with good accuracy and precision as an alternative to the more complex and expensive methods. Moreover, they doesnot require various elaborate treatment and they permit the analysis of the components of precipitation reactions and highly colored complexes without any need to buffers or surfactants.

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