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Stability indicating atomic absorption, acid-dye spectrophotometric and conductometric methods for the determination of trospium chloride

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Abstract Three precise, accurate and sensitive methods were developed for the determination of Trospiumchloride in presence of its degradation product (acid and alkaline). Method (A) was atomic absorption spectroscopic (AAS), it is based on the formation of ion-pair precipitate complex (1:1) with ammonium reineckate. The precipitate and the filtrate were used to determine the concentration of the drug by direct and indirect way respectively at 357.9 nm. Method (B) was based on the formation of ion-pair association complex (1:1) with bromocresol purple (BCP), bromophenol blue (BPB) and thymol blue (TB). The coloured products were measured at 412.0±2.0 nm. Method (C) was based on the conductometric determination of Trospium chloride by titration with ammonium reneickateat 20°C and the conductance of the solution is measured as a function of the volume of titrant. Linearity ranges for method (A) were 8.0–120.0 µg/mL and 10.0–120.0 µg/mL for the direct and indirect procedures, respectively. In case of method (B) the linearity ranges were 3.0–20.0 µg/mL for BCP, 2.0–25.0 µg/mL for BPB and 5.0–35.0 µg/mL for TB, while the linearity range was 100.0–1000.0 µg/mL for method (C). Statistical comparison between the results obtained by these methods and the official method.

Keywords Trospium chloride, Stability Studies, Atomis Absorption Spectroscopy, Conductometric, Ion-pair complex, Ammonium reineckate, TB, BPB, BCP, Benzilic acid

1. Introduction

Trospium chloride (spiro [8-azoniabicyclo [3,2,1] octane-8,1-pyrrolidinium]-3 [(hydroxydiphenyl- acetyl)-oxy]chloride(1α, 3β, 5α) is an antimuscarinic agent indicated for the treatment of overactive bladder with symptoms of urge urinary incontinence, urgency, and urinary frequency [1].

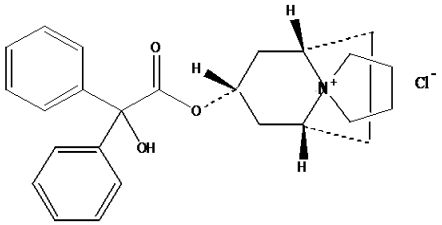


Figure 1: Structure of Trospium chloride,

Determination of Trospium chloride is described in European pharmacopoeia by titrimetric method [2]; using 0.1 M silver nitrate as titrant, then the end point was determined potentiometrically. Only two methods were reported for the determination of Trospium chloride, the first one was a fluorimetric method after derivatization with benoxaprofenchloride [3] while the second one was LC–MS method [4]. However, there is only one reported stability-indicating methods for the determination of the drug [5]. This paper presented the study of the acid and alkaline degradation of Trospium chloride, followed by the development of three stability-indicating methods for the determination of the drug in its pure powder form and in pharmaceutical dosage form.

2. Experimental

2.1. Instruments

A Thermo Elemental Atomic Absorption Flame Spectrophotometer,(Cambridge - UK) serial no.JE710572 computed with Solar data station software version 9.03. Chromium was measured at wavelength 357.9 nm, Band pass 0.5 nm, relative noise 1.0 nm, detection limit 0.01μg/mL, lamp current 10 mA, integration time 4 second. Deuterium lamp was used for background correction. The flame used was the acetylene-air mixture. For Conductometric method, a conductivity meter was used, model: HI 255 Combined Meter, HANNA Instruments, (Smithfield - USA). A dip type conductivity probe with a cell constant (K = 1) was used, while we used a Shimadzu UV-2400 PC Series Spectrophotometer with two matched 1cm quartz cell for the third method. For identification of the degradation product, IR Bruker Vector 22 8201 PCspectrometer (Bruker Instruments Ltd, Rheinstetten/ Karlsruhe, Germany) andMass Spectrophotometer, Hewlett Packard Model 5988A GC/MS (Agilent Technologies, Wilmington, DE) were used.

2.2. Materials and Reagents

Trospium chloride-Pure sample was kindly supplied by Hekma Pharma, Egypt, B.N. 21787. Its purity was found to be 99.95±0.644 % according to the official method. Trospikan tablet was supplied by HikmaPharma, (Cairo, Egypt), B.N.003. Each tablet is claimed to contain 20 mg of Trospium chloride. Benzilic acid was kindly supplied by HekmaPharma (Cairo, Egypt), its purity was certified to be 99.91±0.429 %.

Acetone was purchased from ADWIC [Cairo, Egypt] while ammonium reineckate, Bromo-phenol blue (BPB), Bromo-cresol purple (BCP) and Thymol-blue (TB) were purchased from Sigma Chemical Co. [St. Louis, USA]. Double distilled water.

2.3 Preparation of the degradation products

Trospium chloride (100 mg) was refluxed with 50 ml 1M NaOH solution into a100-ml round-bottom flask for 2 hours and tested for complete degradation by TLC using acetonitrile/glacial acetic acid (5:5 v/v) as the mobile phase. Two spots were observed not corresponding to Trospium chloride. One spot was visualized under UV lamp at 254 nm, while the other spot was visualized after spraying with potassium iodobismuthatereagent. The degraded solution was then cooled at room temperature, neutralized with 1M HCl solution respectively till pH was approximately 7. The solution was nearly evaporated to dryness, cooled and transferred quantitatively with methanol to a volumetric flask 50-ml then the volume was completed to the mark to prepare solution of concentration (equivalent to 2.0 mg/ml of intactTrospium chloride) in methanol and finally was filtered.The degraded solution and the reference standard degradation product solution (benzilic acid) were spotted on TLC plate. The plate was developed with the previously mentioned mobile phase. The spots were visualized under UV lamp at 254 nm, the R_f value of the resultant degradation product 1 was compared with that standard degradation product (benzilic acid), the results agreed with the published data.

2.4. Standard solutions

2.4.1. For AASMethod: Trospium chloride standard solution: (0.2 mg/mL) in double distilled water.

Benzilic acid standard solution (degradation product 1): (0.2 mg/mL) in double distilled water.

2.4.2. For Colorimetric Method: Trospium chloride standardsolution: (0.25 mg/mL) in double distilled water**.**

Benzilic acid standard solution (degradation product 1): (0.25 mg/mL) in double distilled water.

2.4.3. For Conductometric Method,Trospium chloride stock standard solution: (2.5 mg/mL) in double distilled water. Benzilic acid standard solution (degradation product 1): (2.5 mg/mL) in double distilled water.

2.5. Laboratory prepared mixtures containing different ratios of Trospium chloride and its degradation product

2.5.1. For AAS method, Aliquots (1.5 – 14.25 mL) of Trospium chloride were accurately transferred from its stock standard solution (0.2 mg/mL) equivalent to (300.0 – 2850.0 µg/mL) into a series of 25-mL volumetric flasks. Aliquots (13.5 – 0.75 mL) of standard degradation product solution (0.2 mg/mL) equivalent to (2700.0 – 150.0 µg) were added. Then 2.0 mL of 1×10^{-2} M ammonium reineckate was added to each volumetric flask, the volume was completed to the mark with double distilled water to prepare mixtures containing 10–95 % of the degradation product

2.5.2. For Colorimetric Method

2.5.2.1. Using BCP: Aliquots (1.8–0.4 mL) of Trospium chloride were accurately transferred from its stock standard solution (0.25 mg/mL) equivalent to (450.0–100.0 µg) into a series of 100-mL separating funnels. Aliquots (0.2–1.6 mL) of standard degradation product solution (0.25 mg/mL) equivalent to (50.0–400.0 µg) were added, 1.0 mL of BCP solution ($1x10^{-3}$ M) was added, the complex was extracted two times with 10.0 mL chloroform, each. The solution was shaken for 2 min each time and the chloroform layer was passed through anhydrous sodium sulphate into a 25-mL volumetric flask, the volume was completed to the mark with chloroform to prepare mixtures containing 10–80 % of the degradation product.

2.5.2.2. Using BPB: Aliquots (2.25–0.25 mL) of Trospium chloride were accurately transferred from its stock standard solution (0.25 mg/mL) equivalent to (562.5–62.5 µg) into a series of 100-mL separating funnels. Aliquots (0.25–2.25 mL) of standard degradation product stock solution (0.25 mg/mL) equivalent to (62.5–562.5 µg) were added, 2.0 mL of BPB solution ($1x10^{-3}$ M) was added, the complex was extracted two times with 10.0 mL chloroform, each. The solution was shaken for 2 min each time and the chloroform layer was passed through anhydrous sodium sulphate into a 25-mL volumetric flask, the volume was completed to the mark with chloroform to prepare mixtures containing 10 – 90 % of the degradation product.

2.5.2.3. Using TB: Aliquots (3.15–0.7 mL) of Trospium chloride were accurately transferred from its stock standard solution (0.25 mg/mL) equivalent to (787.5–175.0 µg) into a series of 100-mL separating funnels. Aliquots (0.35–2.8 mL) of standard degradation product stock solution (0.25 mg/mL) equivalent to (87.5–700.0 µg) were added, 1.0 mL of TB solution ($1x10^{-3}$ M) was added, and the complex was extracted two times with 10.0 mL chloroform, each. The solution was shaken for 2 min each time and the chloroform layer was passed through anhydrous sodium sulphate into a 25-mL volumetric flask, the volume was completed to the mark with chloroform to prepare mixtures containing 10 – 80 % of the degradation product.

2.5.3. Conductometric method: Aliquots (18.0–2.0 mL) of Trospium chloride were accurately transferred from its stock standard solution (2.5 mg/mL) equivalent to (45.0–5.0 mg) into a series of 50-mL volumetric flasks. Aliquots (2.0–18.0 mL) of standard degradation product stock solution (2.5 mg/mL) equivalent to (5.0–45.0 mg) were added, the volume made up to the mark with double distilled water to prepare mixtures containing 10–90 % of the degradation product.

2.6. Construction of Calibration Curves

2.6.1. For AAS Method

Aliquots (1.0, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mL) of Trospium chloride were accurately transferred from its stock standard solution (0.2 mg/mL) equivalent to (0.2, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg) into a series of 25-mL volumetric flasks; 2.0 mL of 1×10^{-2} M ammonium reineckate was added, the volume was completed to the mark with double distilled water. The solution was shaken well and left to stand for 30 minutes and filtered using Whatmann filter paper no. 44 (12.5 cm diameter). The obtained precipitate was washed two times with 10.0 mL double distilled water.

1. **For Direct method:** Each one of the obtained precipitates were dissolved in least amount of acetone and transferred quantitatively into a series of 25-mL volumetric flasks; the volume was completed to the mark with double distilled water.
2. **For Indirect method:** The filtrates and washings were collected and transferred into a series of 100-mL volumetric flasks; the volume was completed to the mark with double distilled water.

The contents of chromium (III) were determined either directly in the precipitate or indirectly in the filtrate by aspiration of the prepared solutions directly in the atomic absorption spectrometer. The absorbance obtained was plotted against the corresponding concentration in µg/mL directly and indirectly. The calibration curves were constructed and the regression equations were then computed.

2.6.2. For ColorimetricMethod

2.6.2.1. Using BCP: Aliquots (0.3, 0.4, 0.8, 1.2, 1.6 and 2.0 mL) of Trospium chloride were accurately transferred from its stock standard solution (0.25 mg/mL) equivalent to (75.0, 100.0, 200.0, 300.0, 400.0 and 500.0 µg) into a series of 100-mL separating funnels, 1.0 mL of BCP solution ($1x10^{-3}$ M) was added, the complex was extracted two times with 10.0 mL chloroform, each. The solution was shaken for 2 min each time and the chloroform layer was passed through anhydrous sodium sulphate into a 25-mL volumetric flask, the volume was completed to the mark with chloroform. The absorbance was measured at 412.0±2.0 nm against blank constructed the same as the experiment omitting the addition of the drug. Linear calibration curve was constructed relating the absorbance to the corresponding concentration of Trospium chloride and the corresponding regression equation was computed.

2.6.2.2. Using BPB: Aliquots (0.2, 0.3, 0.5, 1.0, 1.5, 2.0 and 2.5 mL) of Trospium chloride were accurately transferred from its stock standard solution (0.25 mg/mL) equivalent to (50.0, 75.0, 125.0, 250.0, 375.0, 500.0 and 625.0 µg) into a series of 100-mL separating funnel, 2.0 mL of BPB solution ($1x10^{-3}$ M) was added, the complex was extracted two times with 10.0 mL chloroform, each. The solution was shaken for 2 min each time and the chloroform layer was passed through anhydrous sodium sulphate into a 25-mL volumetric flask, the volume was completed to the mark with chloroform. The absorbance was measured at 412.0±2.0 nm against blank constructed the same as the experiment omitting the addition of the drug. Linear calibration curve was constructed relating the absorbance to the corresponding concentration of Trospium chloride and the corresponding regression equation was computed.

2.6.2.3. Using TB: Aliquots (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mL) of Trospium chloride were accurately transferred from its stock standard solution (0.25 mg/mL) equivalent to (125.0, 250.0, 375.0, 500.0, 625.0, 750.0 and 875.0 µg) into a series of 100-mL separating funnel, 1.0 mL of TB solution ($1x10^{-3}$ M) was added, the complex was extracted two times with 10.0 mL chloroform, each. The solution was shaken for 2 min each time and the chloroform layer was passed through anhydrous sodium sulphate into a 25-mL volumetric flask, the volume was completed to the mark with chloroform. The absorbance was measured at 412.0±2.0 nm against blank constructed the same as the experiment omitting the addition of the drug. Linear calibration curve was constructed relating the absorbance to the corresponding concentration of Trospium chloride and the corresponding regression equation was computed.

2.6.2.4. For Conductometric method

Aliquots (2.0, 4.0, 8.0, 12.0, 16.0 and 20.0 mL) of Trospium chloride were accurately transferred from its stock standard solution (2.5 mg/mL) equivalent to (5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg) into a series of 50-mL volumetric flasks. The volume made up to the mark with double distilled water. The content of the volumetric flask was transferred carefully to the titration cell, and then $5x10^{-3}$M ammonium reineckate was titrated from a 10.0 µL automatic micropipette and the conductance was measured subsequently after each addition of the reagent solution with thorough stirring of the solution. The conductance reading was taken (1 - 2 minutes) after each addition, corrected for the dilution bymeans of the following equation:

$$\Omega \text{ corrected} = \Omega \text{ observed } [(V1 + V2) /V1] \quad [6]$$

Ω corrected and Ω observed was the corrected and the observed electrolytic conductivities, respectively,V1: was the initial volume,V2: was the volume added of the reagent (titrant).A graph of the corrected conductivity values versus

the volume of the added titrant was constructed and the end point was determined, (Figure 2). The drug-titrant ratio was then determined from the intersection of the two linear lines of the graph. The concentration of the drug was calculated according to the equation [6],

Concentration of the drug = V.M.R / N

Where, V is volume of titrant, M is molecular weight of drug, R is molar concentration of titrant and N is no of moles of titrant consumed by one mole of drug.

Calculation: each 0.1-mL of $5x10^{-3}$ M ammonium reineckate was theoretically equivalent to 0.214 mg of Trospiumchloride. Intersection of the two linear lines of the resultant plot were plotted against the corresponding concentration in µg/mL. The calibration curve was constructed; and the regression equation was then computed.

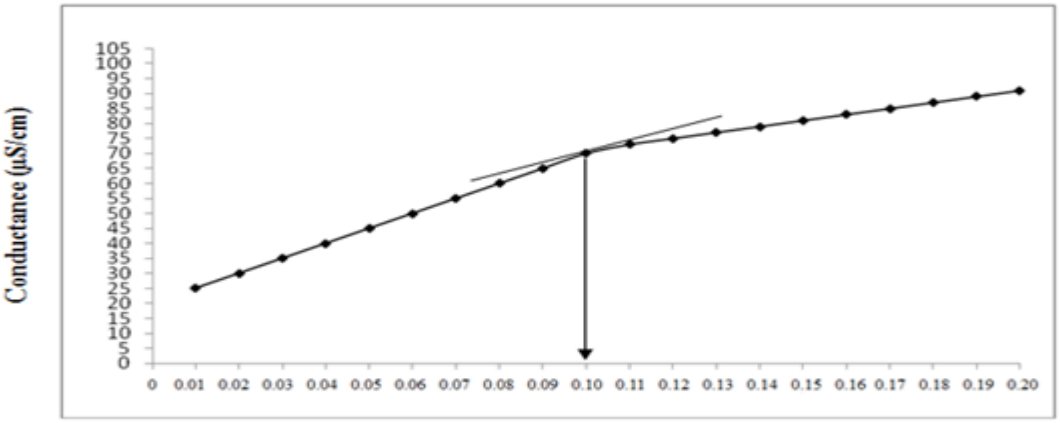


Figure 2: Conductometric titration curve of Trospium chloride (200 µg/ml) vs mL of ammonium reineckate solution

2.7. Application of the proposed methods for the analysis of laboratory prepared mixtures of Trospium hloride and its degradation product

2.7.1.For AAS: The laboratory prepared mixtures containing different percentages of Trospium chloride and its degradation product (2.5.1.) were gotten, then the procedure was completed as described under subsection (2.6.1.) of Linearity. The concentrations of Trospium chloride were calculated using the regression equations.

2.7.2. For Colorimetric Method: The absorption spectra of laboratory prepared mixtures containing different percentages of Trospium chloride and its degradation product as prepared in subsection (2.5.2) were recorded, then the concentrations of Trospium chloride were calculated from the corresponding regression equation.

2.7.3. For Conductometric method: The procedure under linearity (2.6.3) was done using the previous laboratory prepared mixtures containing different percentages of Trospium chloride and its degradation product (2.5.3). Then the concentrations of Trospium chloride were calculated using the regression equation.

2.8. Application of the proposed methods for the analysis of Trospium chloride in pharmaceutical preparation

2.8.1. For AAS: Five tablets of Trospikan were weighed accurately and finely powdered in a small dish. An amount of powder equivalent to 20.0 mg Trospium chloride was accurately transferred into a 100-mL volumetric flask, 50.0 mL of double distilled water was added. The flask was sonicated for 30 minutes, then the volume was completed to the mark with the same solvent and finally was filtered to prepare solution of concentration equivalent to (0.2 mg/mL). Aliquot of 2.5 mL of the prepared solution was transferred into a 25-mL volumetric flask. Then the procedure was completed as described under subsection (2.6.1.) of Linearity. The concentration of Trospium chloride was calculated from the regression equations.

2.8.2. For Colorimetric: Five tablets of Trospikan were weighed accurately and finely powdered in a small dish. An amount of powder equivalent to 25.0 mg Trospium chloride was accurately transferred into a 100-mL volumetric flask, 50.0 mL of the double distilled water was added. The flask was sonicated for 30 minutes, the volume was then completed to the mark with the same solvent and finally was filtered to obtain solution of final concentration equivalent to (0.25 mg/mL). Aliquot of 0.5 mL was transferred into100-mL separating funnel. Then the procedure was completed as described under subsection (2.6.2.) of Linearity. The concentration of Trospium chloride was calculated from the corresponding regression equation.

2.8.3. For Conductometric: Twenty tablets of Trospikan were weighed accurately and finely powdered in a small dish. An amount of powder equivalent to 250.0 mg Trospium chloride was accurately transferred into a 100-mL volumetric flask, 50.0 mL of the double distilled water was added. The flask was sonicated for 30 minutes, then the volume was completed to the mark with the same solvent and finally was filtered to prepare solution of concentration equivalent to (2.5 mg/mL). Further Dilution with double distilled water was done to obtain solution of final concentration of (200.0 µg/mL). Then the procedure was completed as described in subsection (2.6.3.) of Linearity. The concentrations of Trospium chloride were calculated by substitution in the corresponding regression equations.

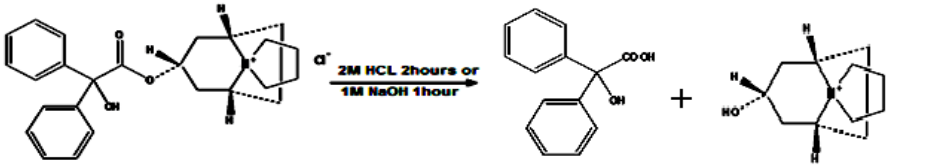
3. Results and Discussion

The stability of Trospium chloride was studied according to ICH guidelinesQ2 (R1) [7] for:

(a) Stress Acid and Alkaline: 1M NaOH/1M HCl for 2 hours, 2M HCl/2M NaOH for 2 hours.
(b) Oxidative Condition: 3% H_2O_2 for 2, 4, 6 and for 10 hours.
(c) Thermal Degradation: at 100°C in an oven for 2, 4 and for 6 hours.

The degradation process under the previously mentioned conditions was followed using TLC and the compound was found to be liable to acid and alkaline degradations. There were two components which confirmed by TLC as indicated by the appearance of two spots with the same R_f values, at the acid and alkaline degradation conditions after complete degradation. In this work, we concerned with the alkaline degradation of Trospium chloride as it was completely degraded under very mild conditions. Furthermore, standard solution of benzilic acid showed similar R_f(0.71), UV, IR and MS spectra as the isolated laboratory prepared degradation product 1. Trails were carried out to isolate and identify the degradation product 2 R_f (0.25) which was invisible on TLC plates unless after successive spraying with potassium iodobismuthate reagent to give a very faint orange colour. Unsuccessful results were obtained; therefore degradation product 1 only be considered as the drug degradation product.

This finding suggested the degradation pathway and indicates that degradation product of Trospium chloride has the following structure, (scheme 1):



Scheme 1: The degradation pathway of Trospium chloride

3.1. For AAS: this method was based on the formation of stable ion-pair complex between Trospium chloride and ammonium reineckate. The formed precipitate is water insoluble red metal complex [8], while the degradation product of Trospium chloride did not give any precipitate when reacted ammonium reineckate, therefore the method can be used for the determination of the drug in presence of its degradation product.

The optimization of the method was carefully studied to achieve complete reaction formation, highest sensitivity and maximum absorbance. The stoichiometry of the reaction on studying the molar ratio of the interaction between Trospium chloride with chromium ions using Job's method [9] for continuous variation was found to occur at a ratio of (1:1). Considering the influence of metal ion concentration on precipitation, it was found that, 2 mL of 1×10^{-2} M of ammonium reineckate solution was optimum for complete precipitation as shown in (Figure 3). The time required for complete precipitation was determined and it was found that, 30 minutes were sufficient for completion of precipitation.

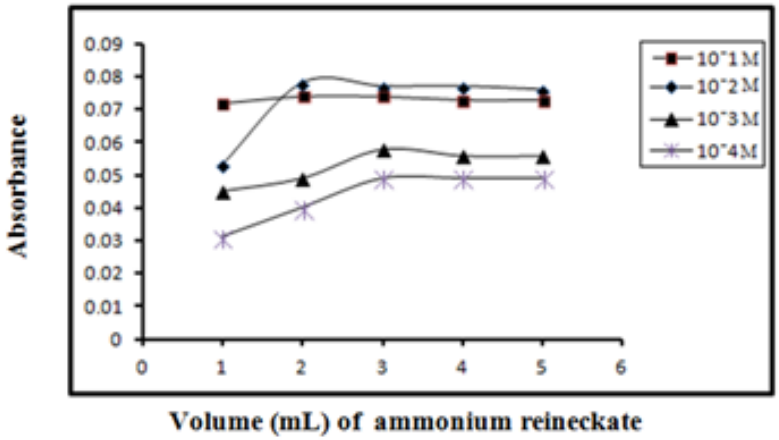


Figure 3: Effect of volume and concentration of ammonium reineckate solution on the absorbance of (80.0 µg/mL) of Cr-Trospium complexat 357.9 nm

Under the optimized experimental conditions, linear calibration curves between the absorbance at 357.9 nm (λ of chromium) and its corresponding concentration of Trospium chloride was obtained in the range of (8.0–120.0 µg/mL) and (10.0–120.0 µg/mL) for the direct and indirect procedures, respectively. The characteristic parameters for regression equations were computed and found to be:

Direct method: A = 0.0005 C + 0.0382 (r = 0.9996), **Indirect method:** A= - 0.00009 C + 0.1916 (r = 0.9995)

Where, A: chromium ion absorbance at λ = 357.9 nm, C: the concentration of Trospium chloride in µg/mL,

r: correlation coefficient.

The proposed method was successfully applied for the determination of the drug in pure powdered form with mean percentage recoveries of 100.06±1.096 % and 100.21± 0.629% for the direct and indirect methods, respectively Table (1).

3.2. For Colorimetric, ion pair formation has been exploited for separation and analysis of a large numbers of pharmaceuticals such as alkaloids and amines. The extractive spectrophotometric method is based on the reaction of basic nitrogenous compound with acidic dye to yield ion pair salts [10] which have intense absorption band in the visible region spectra that are extracted into organic solvent.

The method was based on that the drug reacts with BCP, BPB and TB to form yellow chloroform-extractable complex, while the degradation product of Trospium chloride did not give any yellow color when reacted the three dyes, therefore the method can be used for the determination of the drug in presence of its degradation product. The absorption spectra of the extracted complex were recorded over the range 300-600 nm. The complex showed a maximum absorbance at 412 nm, which could be used as the wavelength for determination. The reaction conditions were optimized, it was found that 1ml of 1×10^{-3} M of BCP and TB reagents and 2 ml of $1x10^{-3}$M of BPB reagent were optimum. Maximum color intensity was observed upon using chloroform as solvent. The absorbance of the ion pair complexes remains stable for at least 60 min.

Table 1: Results of validation parameters of the responses and the regression equations obtained by the proposed methods

Parameter	Atomic absorption spectroscopy		Colorimetric method			Conductometric method
	Direct Method	Indirect Method	BCP	BPB	TB	
Validation of regression equation:						
Slope	0.0005	-0.00009	0.0537	0.0384	0.0241	0.3034
S.E. of slope	0.000006	0.000001	0.000343	0.000458	0.00023	0.002574
Intercept	0.0382	0.1916	0.0316	0.1145	0.0771	7.5719
S.E. of intercept	0.000433	0.000086	0.004025	0.006447	0.005151	1.4464
Correlation coefficient	0.9996	0.9995	0.9999	0.9996	0.9998	0.9997
Validation of response:						
Concentration range (µg/ml)	8.0 -120.0	8.0 -120.0	3.0 – 20.0	2.0 – 25.0	5.0 - 35.0	25.0 – 1000.0
Average accuracy %	100.06	100.21	100.39	100.20	100.18	100.12
S.D.	1.097	0.630	0.558	0.836	0.782	0.842
R.S.D. %	1.096	0.629	0.556	0.834	0.781	0.841
Specificity ± R.S.D. %	100.26±0.957	100.49±1.236	99.77±0.747	99.68±0.804	99.38±0.624	99.76±0.943
Repeatability[*a] %	100.51±0.736	99.81±0.848	99.60±0.949	100.69±0.650	99.69±0.444	100.25±0.846
Intermediate precision[*b] %	100.38±0.458	100.49±0.694	99.99±0.812	100.45±0.720	99.68±0.495	100.19±0.105

[*a] 3x3 [*b] 3x3

The stoichiometry of reaction of Trospium chloride and the three dyes was proved by applying jobs methodusing equimolar concentrations of the drug and the dyes .It was found that the reaction proceeds in a molar ratio of 1:1 in the three dyes, (Figure 4).

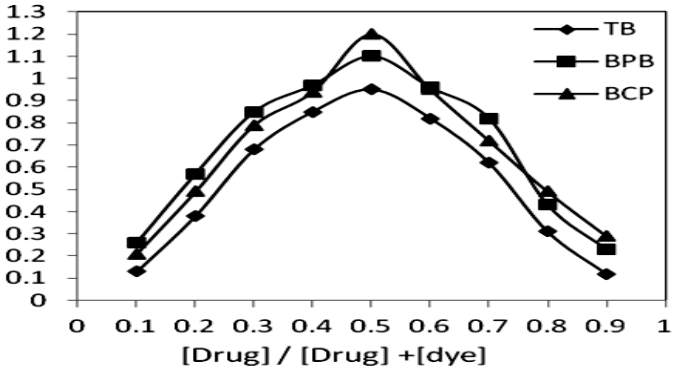


Figure 4: Job's plot for stoichiometric ratio between Trospium chloride and BCP (1×10^{-3} M), Trospium chloride and BPB (1×10^{-3} M) and Trospium chloride and TB (1×10^{-3} M).

Linear relationship was obtained between the absorbance and the corresponding concentration of Trospium chloride in the range of (3-20 µg/ml) by using BCP, (5-35 µg/ml) by using TB and (5-25 µg/ml) by using BPB. The regression equations were computed and found to be:

$A_1 = 0.0537C + 0.0316$ r = 0.9999 using BCP

$A_2 = 0.0384C + 0.1145$ r = 0.9996 using BPB

$A_3 = 0.0241C + 0.0771$ r = 0.9998 using TB

Where A_1 is the absorbance of Trospium chloride-BCP ion-pair complex, A_2 is the absorbance of Trospium chloride-BPB ion-pair complex, A_3 is the absorbance of Trospium chloride-TB ion-pair complex, C is the concentration µg/ml and r is the correlation coefficient.

The proposed method was successfully applied for the determination of the drug in pure powdered form with mean percentage recoveries of 100.39±0.556%, 100.20±0.834% and100.18±0.781% for BCP, BPB and TB respectively, (Table 1).

3.3. For Conductometric

In this section, a highly simple, sensitive, inexpensive and time saving conductometric titration method was developed for determination of Trospium chloride in raw material and in pharmaceutical formulation.

Table 2: Results of analysis of Trospium chloride in laboratory prepared mixtures containing different ratios of Trospium chloride and its degradation product in pure powder form by the proposed methods

a. AAS Method

Degradation %	Concentration ((µg/mL)		Recovery %*	
	Trospium Chloride	Degradation product	Direct method	Indirect method
10	108.0	12.0	100.86	101.32
30	84.0	36.0	101.19	101.65
50	60.0	60.0	98.78	100.41
70	36.0	84.0	99.35	99.89
90	12.0	108.0	100.64	101.32
95	6.0	114.0	100.72	98.34
		Mean	100.26	100.49
		S.D.	0.959	1.242
		R.S.D. %	0.957	1.236

b. Colorimetric Method

Degradation %	BCP			TB			Degradation %	BPB		
	Conc (µg/mL)		Recovery %	Conc (µg/mL)		Recovery %		Conc (µg/mL)		Recovery %
	Trospium Chloride	Degradation product		Trospium Chloride	Degradation product			Trospium Chloride	Degradation product	
10	18.0	2.0	99.81	31.5	3.5	100.10	10	22.5	2.5	99.34
20	16.0	4.0	99.75	28.0	7.0	99.32	20	20.0	5.0	99.14
30	14.0	6.0	100.81	24.5	10.5	99.47	40	15.0	10.0	98.68
50	10.0	10.0	100.53	17.5	17.5	99.61	50	12.5	12.5	100.47
60	8.0	12.0	98.57	14.0	21.0	99.07	70	7.5	17.5	101.0
70	6.0	14.0	99.31	10.5	24.5	99.89	80	5.0	20.0	99.71
80	4.0	16.0	99.63	7.0	28.0	98.21	90	2.5	22.5	99.40
		Mean	99.77			99.38				99.68
		S.D.	0.745			0.620				0.801
		R.S.D. %	0.747			0.624				0.804

c. Conductometric Method

Conc (µg/mL)		Recovery %
Trospium Chloride	Degradation product	
900.0	100.0	99.34
800.0	200.0	98.28
600.0	400.0	101.16
500.0	500.0	100.38
300.0	700.0	99.64
200.0	800.0	100.31
100.0	900.0	99.23
	Mean	99.76
	S.D.	0.941
	R.S.D. %	0.943

The optimum conditions for the formed metal complex were carefully studied and the obtained observations were recorded as follows; The influence of ammonium reineckate ion concentration on conductance of Trospium chloride was investigated. The results showed that titrant solutions lower than $5x10^{-3}$ M was not suitable for conductometric titrations, as the conductance readings were unstable and the inflection at the end point wasn't detected. The stoichiometry of the reaction on studying the molar ratio of the interaction between Trospium chloride with ammonium reineckate using the Mole – Ratio method was found to occur at a ratio of (1:1). Different temperatures were tested (25, 30, 35 and 40 ºC) and it was found that the same results were obtained, so room temperature (25 ºC) was selected for the determination. Temperature couldn't be increased more than 40 ºC as conductometry electrode (conductivity cell) and conductivity of the ions could be affected by elevated temperature. Under the optimized experimental conditions, linear calibration curves between the values of the measured conductivities after dilution correction at each end point obtained from the intersection of the two linear lines of the resultant plots and their corresponding concentrations were obtained in the range of "100.0 – 1000.0 µg/mL'' for Trospium chloride.

The characteristic parameters for the regression equation were computed and found to be:

A = 0.3034C + 7.5719 r = 0.9997

Where, A: the measured conductivities at each end point,C: the concentration of Trospium chloride in µg/mL

r: correlation coefficient

The proposed method was successfully applied for the determination of the drug in pure powder form with mean percentage recovery of 100.12 ± 0.841 %, Table (1).

Table 3: Quantitative determination of Trospium chloride in pharmaceutical formulation by the proposed methods and results of application of standard addition technique

Pharmaceutical formulation Trospikan-Tablet 20.0mg Batch number 003	**AAS**		**Colorimetric Method**			**Conductometric Method**
	Direct Method	**Indirect Method**	**Bromocresol purple**	**Bromophenol blue**	**Thymol blue**	
Found %[a]	100.34±1.195%	100.30±0.983%	99.78±0.873%	99.40±1.524%	99.74±0.735%	100.17±1.041%
Recovery of standard added %[b]	99.91±1.304%	99.89±0.852%	99.95±0.465%	99.46±0.477%	99.42±0.413%	99.89±0.451%

[a] Average of six determinations

[b] Average of six determinations

Table 4: Statistical analysis between the results obtained for the determination of Trospium chloride in pure samples by the proposed methods and those obtained by the reported method

Item	**AAS**		**Colorimetric Method**			**Conductometric Method**	*** European pharmacopoeia's method**
	Direct Method	**Indirect Method**	**Bromocresol purple**	**Bromophenol blue**	**Thymol blue**		
Mean	100.06	100.21	100.39	100.20	100.18	100.12	99.95
S.D	1.097	0.630	0.558	0.836	0.782	0.842	0.644
R.S.D%	1.096	0.629	0.555	0.834	0.781	0.841	0.644
Variance	1.203	0.397	0.311	0.698	0.612	0.709	0.414
N	6	6	6	6	6	6	6
Student-t	0.212 (2.228)*	0.707 (2.228)*	1.265 (2.228)*	0.580 (2.228)*	0.556 (2.228)*	0.393 (2.228)*	
F test	2.899 (5.05)*	1.045 (5.05)*	1.331 (5.05)*	1.685 (5.05)*	1.475 (5.05)*	1.708 (5.05)*	

*The values between parenthesis are the theoretical values of t and F at (p = 0.05).

*Official method; is titration with 0.1 M silver nitrate then determination of the end point potentiometrically.

The specificity of the methods was proved by the analysis of laboratory prepared mixtures containing different percentages of the degradation product. Method (A) was found to be specific for Trospium chloride in presence of up to 95 % of its degradation product. While the specificity of (BCP and TB) in method (B) was achieved in presence of its degradation up to 80 % , while achieved to 90 % in case of BPB in method (B) and method (C), (Table 2).

The usefulness of the proposed methods for the analysis of Trospium chloride was studied by assaying Trospikan tablet, (Table 3). Standard addition technique was also applied to assess the validity of the proposed method, (Table 3).

Results obtained by the proposed method for the determination of pure samples of the drug were statistically compared [11] to those obtained by European pharmacopoeia's method and no significant differences were observed, (Table 4).

Validation of the proposed methods was made by measuring range, accuracy, precision, repeatabilities, interday precision, linearity and specificity. Results obtained are depicted in Table (1). This data render the applicability of the proposed methods for the quality control of the drug formulation.

4. Conclusion

The AAS, Colorimetric and Conductometric methods proposed are accurate, precise and reproducible. They are stability-indicating methods, so can be used for simple accelerated stability studies to predict the expiry dates of pharmaceuticals. All methods complied with the validation guidelines of the International Conference on Harmonization and could be used for purity testing, stability studies, quality control, and routine analysis of Trospium.

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