Spectrophotometric Determination of Trace Quantities of Pure Atropine and Pharmaceutical Preparations with SbI_4^{2-} Ion

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Abstract: *This study aims to estimate a simple, rapid and sensitive method for a trace amount of atropine (ATR) in medicinal compounds. Two approaches were followed to accomplish this aim, i.e., spectrophotometric determination of pure ATR and pharmaceutical preparations using SbI_4^{2-} ion as a new reagent. The procedure involves the implementation of an ion-association complex with this alkaloid. The resulting complex was extracted and detected spectrophotometrically at 492 nm. Appropriate parameters were investigated, including the ion SbI_4^{2-} concentration and the pH value of the complex formation. Using chloroform to extract the complex, taking into consideration extraction time and volume of solvent used. The calibration graph is linear in the ranges of 0.5–5.0 × 10^{-3} M. Precision, accuracy, detection limit, and RSD %, as well as relative standard deviation (n = 5), were calculated. The test sensitivity was 0.013 μg cm^{-2}. Several interference additives were studied by investigating the effect of equal and duplicate quantities of some common excipients on selectivity, such as starch, glucose, lactose, glycerin, and talc. The molar ratio of the SbI_4^{2-}–ATR was determined. The amount of ATR in the pharmaceutical tablets and eye drop preparation was calculated using E_{rel} at ratios of 2.24 and 2.75%, respectively.*

Keywords: *atropine; spectrophotometer; SbI_4^{2-}-atropine complex; solvent extraction; pharmaceutical compounds*

■ INTRODUCTION

Numerous effects of atropine (ATR), such as treating slow heart rate or lowering secretions or intraocular pressure, are irreplaceable without this substance at specific concentrations when urgently needed and for a variety of objectives. For this reason, a number of research groups and scientists, including chemists and pharmacists, are working together to establish a valid procedure to estimate a trace amount of ATR, which leads to learning more about ATR, as well as the numerous analytical methods for determining trace amounts, following simple and rapid steps, and understanding its features. As a result, the concept of research was born [1-5].

Many analytical techniques are competing in research and focus on this important medicinal substance, its locations in the parts of plants and their concentration sites. We outline here some of the important results and key findings in this field. Optimization of parameters for conventional heated solid-liquid extraction of ATR from *Datura stramonium* seeds was obtained by the particle size, temperature, and ethanol concentration [6].

Electrochemiluminescence sensors are used for forensic analysis to detect and quantify ATR with linearity across a concentration range of 0.75 to 100 μM [7]. To measure ATR in belladonna leaves, an HPLC method was designed and validated. Analysis of the analytical curves of atropine revealed linearity 50–200 μg mL^{-1} with R^2 = 0.9996, LOD and LOQ of 3.75 and 11.4 μg mL^{-1}, respectively. The approach was precise, repeatable, and accurate, with a recovery rate of 103% [8].

Two methods have been applied for the spectrophotometric determination of ATR in bulk sample and in dosage form. The first with bromphenolblue (BPB). The calibration graph is linear

in the ranges of 0.5–40 μg mL^{-1}. The second method with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) with a linearity range of 2.5–50.0 μg mL^{-1} [9]. A planar electrochemical cell was used for the first time for the voltammetric determination of ATR with a LOD reaching 0.08 μM [10].

Methods for determining pharmaceuticals using extractive, sensitive spectrophotometers, and spectrofluorometric preparations such as tablets and suppositories have been carried out. Meralazine and 2,6-dihydroxybenzoic acid were found to yield colored products where oxidative coupling reaction with other reagents shows the highest absorption, Beer's rule was consistent at concentrations ranging from 1.25–30 and 0.5–12.5 μg mL^{-1} when measured at 640 and 515 nm alternately [11-14]. The utilization of ion pair production allows multiple analytical techniques, like as extraction, spectrophotometry, and their combination [15-16].

In this regard, the new research falls within the scope of competition for the speed and simplicity of the innovative scientific method, as well as minor quantities in the concentration found. Using the SbI_4^{2-} ion reagent, a new procedure for determining a pure alkaloid in pharmaceutical preparations was described. The goal of the method involves producing a simple, rapid, and sensitive, involve extracting an ion pair complex between the organic base ATR and the inorganic ion and measuring the intensity of the color complex in the organic phase using spectrophotometric analysis at 492 nm. In this procedure, the ideal experimental settings were investigated, including SbI_4^{2-} concentration, pH value, types of extraction solvents, volume and shaking time, phase ratio, and the number of extractions. In addition, to study the expected interactions of such compounds, we conducted stoichiometric studies on the molar ratio and compared the results with previous studies.

■ EXPERIMENTAL SECTION

Materials

The materials used in this study were eye-drop atropine sulfate 1% cooper (S.A Pharmaceuticals) $(C_{17}H_{23}NO_3)_2{\cdot}H_2SO_4{\cdot}H_2O$). L-Ascorbic acid (Alpha chemika, Batch No.LA503). Different organic solvents were used, such as dichloromethane, chloroform, 1,2-dichloroethane, xylene and toluene. Sb(II) stock standard solution (BDH) and ATR sulfate analar from Fluka (Mr. 694.63) were purchased. The drugs used in this work were taken from the local market.

Instrumentation

The instrumentations used in this study were a Shimadzu UV-1800 UV/visible scanning spectrophotometer; 115 VAC, was used to measure the absorbance of all samples in this work. Operational spectrum was 190 to 1100 nm. The instrument was equipped with a quartz cell. The pH of samples was measured using Eutech Instruments/pH 700/pH/mV °C/°F meter.

Procedure

In this study, each 1 mL of eye-drop ATR sulfate contains 10 mg ATR sulfate. Alkaloid stock standard solutions were prepared. Lower concentrations of the stock solutions were prepared by dilution of 0.0600 g of ATR sulfate in a 50 mL volumetric flask. ATR 1000 μg mL^{-1} was prepared by dissolving 0.0600 g of atropine sulfate in a 50 mL volumetric flask. The working solution of SbI_4^{2-} was prepared by adding 1.0 mL of antimony stock solution to 50 mL volumetric flask with 10 mL of 1:1 H_2SO_4, 5 mL of 2% (wt/v) ascorbic acid, and 10 mL of 40% (wt/v) KI, stand for 10 min and proceed with deionized water (DIW) to the mark and shake for homogeneity and reading the maximum absorbance against H_2O as a blank [17]. A 1,000 μg mL^{-1} of Sb(II) stock standard solution was used. Intermediate standard solution concentrations were freshly generated by dilution ten times. Pharmaceutical grade ATR tablets SDI, Entro-stop, ATR sulfate (0.025 mg) were used in this work. All other chemicals and reagents were of analytical grade. DIW was used throughout this work.

Optimization of experimental parameters

The influence of varying parameters on the color intensity was studied to attain maximal sensitivity. A number of preparatory experiments were established for the fast and quantitative synthesis of colored complexes,

such as concentration of SbI_4^{2-}, pH-value, reaction time, kinds of extracting solvents, number of extractions, and shaking time.

Effect concentration of SbI_4^{2-}. The effect of SbI_4^{2-} concentration was studied by using a fixed quantity each time of ATR with different amounts of prepared SbI_4^{2-} (from 0.5 to 3.5 mL) of 2.0×10^{-4} M and extracting the colored complex with 4 mL of chloroform and measure the absorbance, at 492 nm (Table 2). It was concluded that 2.0 mL of the prepared concentration of SbI_4^{2-} is required to obtain maximum absorbance and remains constant by increasing the volume of the reagent, and it has no effect on the determination of the alkaloid. The influence of pH on the development of the SbI_4^{2-}-ATR complex has been investigated also. This is performed by changing the pH by 0.1 M of HCl or NaOH from pH 1 to 8 and measuring the absorbance. This is performed after fixing all the other conditions. Then the complex formed was extracted with 4 mL chloroform and the absorbance at 492 nm was measured against the blank; the highest absorbance was obtained at pH 2.0–3.0 (Table 1).

Influence of the reaction time. The reaction was carried out at different times (2–20 min) while keeping other conditions constant. It was estimated that 5 min is sufficient for a complete reaction to reach maximum absorbance.

Quantity of extractions, types of extraction solvents, and shaking duration. Different organic solvents were used to get better efficiency of extraction, such as dichloromethane, chloroform, 1,2-dichloroethane, xylene, and toluene. Extraction time and the number of extractions were studied to reach the maximum absorbance. Chloroform was found to be a sufficient solvent, and 1 min with one batch extraction is enough to complete extraction.

A spectrum of the SbI_4^{2-}-ATR

The maximum absorption of the complex was studied by scanning the spectrum of the extracted colored ion-association complex of SbI_4^{2-}-ATR from 200–600 nm, and it indicates that the maximum absorbance is at 492 nm.

Interferences sample preparation

Stock solutions of 1,000 ppm of the common excipients such as starch, glucose, lactose, glycerin, and talk were prepared by dissolving 0.025 g each with suitable solvents and continued to 25 mL of the solvent.

M/L ratio

An aliquot of the ATR standard solution (0.5 to 4.0 mL) was transferred to 10 mL test tubes, each containing 1.0 mL of SbI_4^{2-} standard solution at the greatest wavelength.

Analytical procedure

Calibration graph. The calibration graph was managed using standard solutions at the optimum conditions of the experiment. A series of 10 mL graduated cylinders are added aliquots of stock solution of 2.0×10^{-5} M ATR concentration. A stock solution of 2.0 mL of SbI_4^{2-} was then added to the mixtures. After shaking the mixture, it was left to stand for 5 min. The volume was subsequently increased to 5.0 mL with DIW and extracted with 4.0 mL chloroform. The absorbance of the purple-colored ion-pair complex was measured at 492 nm using a 1 cm path cell against a blank constructed in the same way but without the addition of medication.

To calculate the phase ratio, the volume of the aqueous layer was fixed by increasing the organic layer volume from 4.0 to 8.0 mL after stabilizing other optimum conditions. The volume of the organic layer was then fixed at 4.0 mL, with an increase in the aqueous layer from 4.0 to 8.0 mL. The complex was extracted, and the absorbance was measured. To determine the adequacy of the extraction, this factor was determined after completely removing the organic layer for the first time. This is followed by withdrawing the aqueous layer to another separating funnel. Finally, an equal volume of the organic solvent used in the first time was added. This process was repeated again to find the second absorbance. After observing the adequacy of the solvents used in extracting the formed complex and determining the appropriate ones, it was used to experiment on a blank solution containing the same concentration of SbI_4^{2-} ion solution with the application of optimal conditions and absorbance measurement.

Analytical characteristics. Standard solutions were prepared and analyzed in triplicates to study the linearity, Sandell sensitivity, slope (b), correlation coefficient (R^2), and LOD values.

Stoichiometry between ATR and SbI_4^{2-}. This was investigated using the mole ratio method, equimolar from the reagent and the ATR 2.0×10^{-4} M, to a series of 10 mL graduated cylinder, volume from a stock solution of the ligand ATR with exceeding volumes of the reagent and applied the optimum conditions and read the absorbance at λ_{max} against the mole ratio, and plot the curve [18].

Interferences. The selectivity of the present method was tested by examining the effect of equal and duplicate quantities of some common excipients (starch, glucose, lactose, glycerin, and talk) on selectivity. Stock solution for these excipients was prepared by dissolving 0.0250 g in a 25 mL volumetric flask, from each one in an appropriate solvent and continue to the mark to get 1,000 µg mL^{-1}. The results indicated that excipients do not affect or interfere with the determination of ATR in pharmaceutical preparations or dosage forms.

Analytical applications. The standard addition method procedure was used to determine the content of ATR in tablets and eye drop preparations; the contents of 10 tablets (entro-stop) were grounded and mixed well. A 0.05 g of this powder was dissolved in ethanol:water solvent and then filtered and poured into a 10 mL volumetric flask and continue with solvent to the mark. Then, we took 500 µL to several test tubes containing a series increasing amount of ATR and continued with all optimum conditions and extraction with 4 mL chloroform, and established the standard calibration curve. The same procedure for eye drops was used after preparing the suitable sample by taking 1 mL from the content and diluting it to 25 mL in a volumetric flask.

■ RESULTS AND DISCUSSION

The ion-pair complex's absorbance was measured in the region of 200–600 nm in comparison to a blank solution. Fig. 1 illustrates the maximum absorbance peak which was found to λ_{max} equal to 492 nm. Fig. 2 depicts the influence of SbI_4^{2-} ion concentration on the formation of the [ATR-SbI_4^{2-}] complex. There was an increase in absorbance as we added the reagent. It was anticipated that the reaction would be concluded with the addition of 2 mL of the reagent. Fig. 3 depicts the influence of pH values on the absorbance of the alkaloid complex in question. It was discovered that a pH range of 2–3 was the best setting for the reaction; alkaloids do not ionize at pH above 7, therefore, no ion-association complex formation is expected [19]. In an acidic media, the alkaloid's nitrogen atom is protonated.

$$\text{Alkaloid}^+ + \text{ML}_{n^-} \rightarrow \text{Alkaloid} - \text{ML}_n$$

ML_n^- symbolizes the SbI_4^{2-} and $Alkaloid^+$ is the protonated ATR [17]. The interaction of two oppositely

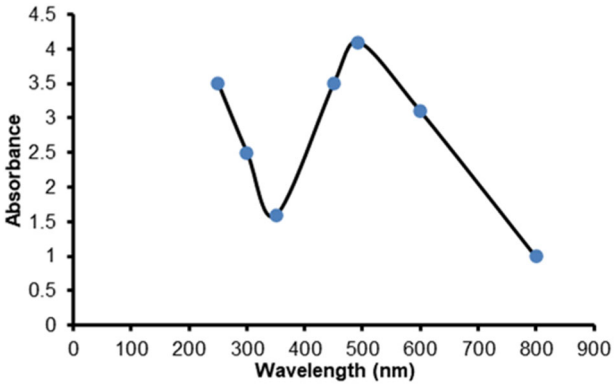


Fig 1. Absorption spectra of the complex SbI_4^{2-}-ATR formed against reagent blank

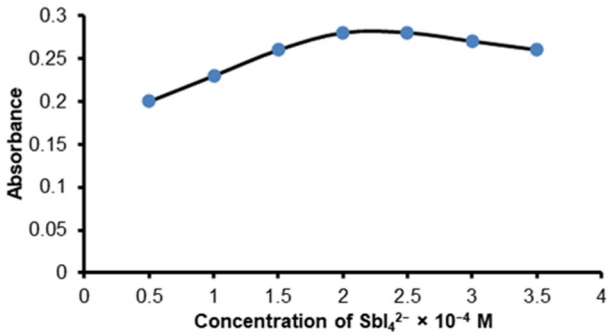


Fig 2. The absorbance of the complex produced is influenced by the concentration of SbI_4^{2-}

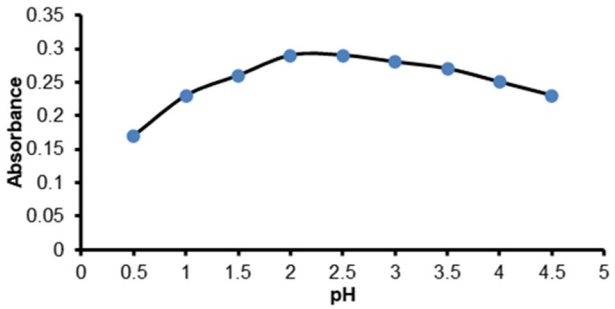


Fig 3. The effect of pH on the development of the SbI_4^{2-}-ATR complex

charged species under optimal conditions results in the production of an ion-pair complex in an aqueous medium when ATR combines with the SbI_4^{2-} ion in an acidic medium. The outcomes demonstrated that an ion pair complex was produced in a 1:1 ratio (Scheme 1) via electrostatic attraction between the positive protonated ATR and the SbI_4^{2-} reagent's anion.

The interaction of two oppositely charged species under suitable conditions results in the production of an ion-pair complex in an aqueous medium when ATR combines with the SbI_4^{2-} ion in an acidic medium. The outcomes demonstrated that an ion pair complex was produced in a 1:1 ratio via electrostatic attraction between the positive protonated ATR and the SbI_4^{2-} reagent's anion.

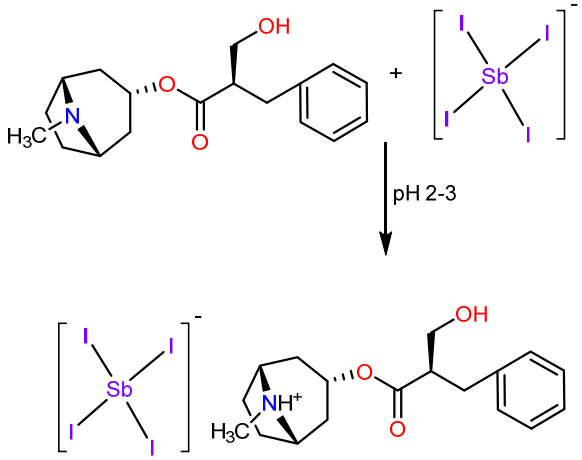
Experiments have demonstrated that 4 mL of the organic phase is sufficient for the complete depletion of the organic-SbI_4^{2-} complex in the aqueous phase as well as reading the absorbance. The extraction of the ATR complex required only 1 min of shaking time.

Table 1 shows the appropriate concentration of the SbI_4^{2-} reagent, as well as the optimal pH range and absorbance for one (A1) and two (A2) batch extractions, also with the comparison with the blank extraction (A). It can be concluded that the former is the most suitable. The complex formed is slightly soluble in aqueous media but freely soluble in organic solvent, and a 5 min interval is sufficient. One extraction was shown to be sufficient for achieving a quantitative recovery of the complex in the minimum amount of time.

The results indicate that satisfactory accuracy and precision could be attained by the current method (Table 2). The E_{rel} % value was 2.38, which appeared a high value of accuracy with the RSD% of 3.76. The maximum color intensity was attained almost instantly, and 5 min was enough to complete the formation of the ion-association complex between the reagent SbI_4^{2-} and ATR, and the color intensity was stable for more than 10 h.

Table 3 shows regression value, slope, and correlation coefficient of the procedure, and it appears interesting results. Linear regression was used to derive a linear equation for the standard curve. Fig. 4 illustrates the linearity from the range 0.5–5.0 × 10^{-5} M. After this concentration the line start to be little bit bending to appositive deviation.

The present method has been effective for the evaluation of ATR in pharmaceutical preparations. The obtained results are shown in Tables 4 and 5. It refers to the ATR content measured by the proposed method being in good agreement with those results by manual reference British pharmacopeia method [20], and these



Scheme 1. Proposed reaction pathway between atropine-SbI_4^{2-} ion pair complex under recommended procedure

Table 1. Effect of SbI_4^{2-} concentration and pH-values

Alkaloid conc. (× 10^{-5} M)	SbI_4^{2-} conc. (M)	pH	Absorbance		
			Extr. 1 A1	Extr. 2 A2	Extr. blank A
ATR (2.0)	2.0 × 10^{-4}	2–3	0.280	0.025	0.012

Table 2. Linearity, precision, accuracy, limit of detection (LOD), sensitivity, and confidence limit are all analytical parameters

Comp.	Linearity (× 10^{-5} M)	RSD% (n = 5)	E_{rel}%	D.L (μM)	Sensitivity (μg cm^{-2})	Confidence limit
ATR	0.5–5.0	3.76	2.38	1.13	0.013	0.420±0.018

Table 3. Regression value, slope, and correlation coefficient

Sample	Regression Y = BX + A	Slope	Corr. coeff. (R^2)
ATR	Y = 0.1001X + 0.0008	0.1001	0.9999

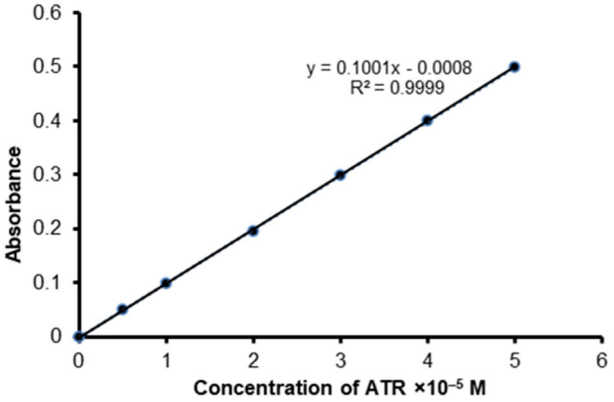


Fig 4. The linearity of the determination of the ATR

experiments are used easily and rapidly on pharmaceutical preparations.

Table 6 demonstrates that a comparison with an earlier technique for the determination of Qunidine (QND) using another reagent (PdI_4^{2-}) was made [17], and it was discovered that the treatment is competitive in these areas. This demonstrates that a more sensitive LOD allows the linearity to reach the lowest concentration.

Fig. 5 and 6 demonstrate the results obtained using the standard addition method to find the exact concentrations in the pharmaceutical preparations, and these results prove that the method of standard additions is appropriate and compatible with this method and can be used in a simple and accurate manner in this line of work.

Fig. 7 shows the molar ratio method used in estimating the rate of the ligand to the reagent used at 492 nm, and it is clear from the obtained figure that the

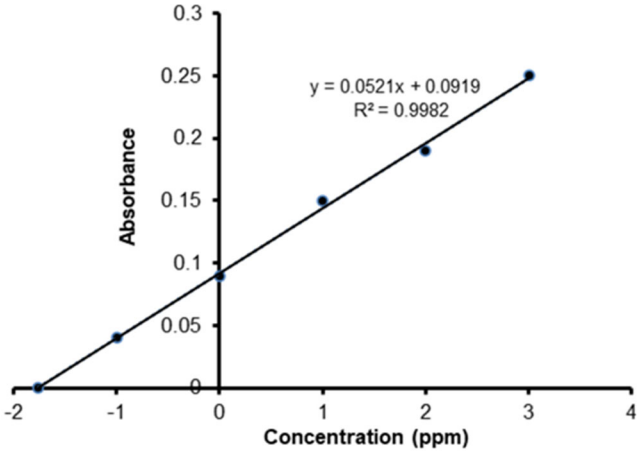


Fig 5. The standard addition method for the determination of ATR in the Entro-stop tablet pharmaceutical preparations

Table 4. Comparison between the concentrations found against the stated one (Entro-stop) using the standard addition method

Alkaloid	Preparation sample	Manufacture	Stated concentration (mg)	Concentration found (mg)	$\%E_{rel}$
ATR tablet	Entro-stop	SDI/IRAQ	0.0250	0.0255	2.24

Table 5. Comparison between the concentrations found against the stated ATR using the standard addition method

Alkaloid	Preparation sample	Manufacture	Stated concentration (mg mL^{-1})	Concentration found (mg mL^{-1})	$\%E_{rel}$
ATR eye-drop	Atropine sulfate/cooper	Cooper pharmaceutical	1.0	1.027	2.75

Table 6. A comparison of the novel method to the prior one that used the SbI_4^{2-} reagent

Alkaloid	Inorganic complex	Linearity ($\times 10^{-5}$ M)	D.L (μm)	RSD%
ATR	SbI_4^{2-}	0.5–5.0	1.13	3.76
QND	PdI_4^{2-}	2.0–6.0	1.50	2.84

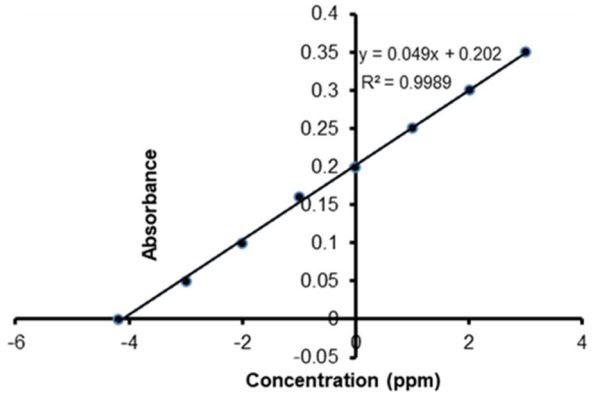


Fig 6. The standard addition method for the determination of ATR in the eye-drop pharmaceutical preparation

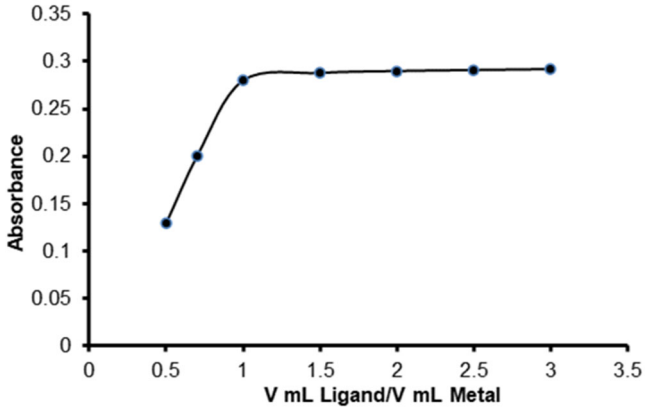


Fig 7. Molar ratio method for SbI_4^{2-}-ATR complex

results are proven after reaching the result 1:1, the curve begins to take the form of a straight line parallel to the x-axis, which indicates the sufficiency of the interaction.

■ CONCLUSION

The proposed method was new, simple, and proved to be sensitive for the spectrophotometric determination of Atropine (ATR) drugs in pure and pharmaceutical preparations by the formation of the ion association complex using a new reagent SbI_4^{2-}. It is evident from the data presented in this work that we can use the SbI_4^{2-} ion in analytical technique as a suitable one when we need to analyze trace or ultra-trace quantities of ATR in pharmaceutical samples. When compared to previous methods that used different techniques and metals, the detection limits for the three alkaloids detected were found to be between 0.006–0.011 ppm, Precision RSD = 0.73–2.69%, and Accuracy E_{rel} 0.45–1.11%, for three alkaloids detected. The proposed method was a simple one and did not contain any difficult reaction conditions and can be used as a new method for determining the presence of ATR in pharmaceutical tablets and eye drop preparations. For future research, we recommend combining different hybridization techniques with other techniques to improve sensitivity.

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