Spectrophotometric Determination of Isopropamide Iodide based on Ion-pair Complex Formation with Thymol Blue

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Abstract

A simple, accurate, affordable, and rapid spectrophotometric approach has been established for the quantification of the medicinal compound of Isopropamide iodide (ISO) in pharmaceutical preparations. An ion-pair complex which absorbs at 404nm has formed the reaction of the drug and the coloring reagent of Thymol blue (TB) at an acidic medium of pH=4. The calibration curve showed excellent linearity over a quantification range of (1-40 µg/ml) with an excellent coefficient of correlation (= 0.9993). The examined limit of detection (LOD) was (0.3 µg/ml) for repeatability of (n= 3). The approach witness no interference of the commonly used excipients in such pharmaceutical formulation that contains Isopropamide iodide. Validation of the method was accomplished by calculating the relative standard deviation (RSD %) and found to be in the range of (1.1938-0.5954). While recovery percentage for (n=3) was (97.4 – 101.4%).

The analytical parameters of the method were optimized and were efficaciously applied to the Isopropamide iodide determination in pure form and in the mixture of a tablet form. The approach can be functional for determining other active pharmaceuticals.

Keywords: *Isopropamide iodide, spectrophotometry, ion-pair complex, Thymol blue, pharmaceuticals.*

Introduction

Isopropamide iodide (ISO), in IUPAC "(4-amino-4-oxo-3,3-diphenylbutyl)-methyl-di(propan-2-yl) azanium;iodide", an off-white odorless bitter powder with a molar mass of 480.434 g/mol [1,2] as it shown in (Figure 1). Its an anticholinergic medication having long effect duration[3]. ISO is a wide spectrum pharmaceutic drug used in the treatment of several disease symptoms, not limited to; Assist in gastrointestinal (GI) spasm relief[4], the remedy of peptic ulcer, thyroid disorder[5], rhinitis redaction, some types of diarrhea, duodenitis spasm, chronic cholelithiasis, biliary dyskinesia, curing of gastritis, rest of spastic colon, disturbance of the urinary tract[6] those correlated to the spasm of smooth muscle, and some other disorders[7–10].

The Iraqi "State Company For Drugs Industry And Medical Appliances (SDI – Samarra)" produces tablets for the treatment of "irretable bowel syndrome" called Salabid which composed of ISO and trifluperazine hydrochloride11.

A literature survey revealed that ISO has been determined in the presence of other active drugs by derivative spectrometry[12,13]. Also, it has been extracted from physiological fluids via solid-phase extraction cartridges[14] (SPE) followed by the chromatographic analysis of HPLC at 220 nm[15,16]. Likewise, platforms of electrochemical sensing were used for a simultaneous determination of ISO in a bulk pharmaceutical mixture[17].

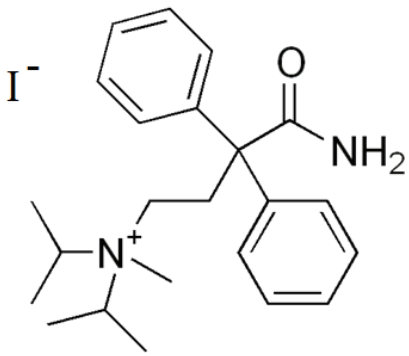

Fig. 1. The chemical structure of the pharmaceutical molecule of Isopropamide iodide[15]

A literature survey comes out with different approaches for finding the concentration of ISO in pharmaceutical mixtures. Many spectrophotometric approaches have been described for the quantification of ISO and measurement were accomplished within the ultraviolet (UV) region[18]**sensitive, rapid, accurate, precise and economical spectrophotometric method based on simultaneous equation method for the simultaneous estimation of isopropamide and trifluoperazine in combined tablet dosage form. Materials and Methods: The method is based on the simultaneous equation and first-order derivative method for analysis of both the drugs using methanol:water in the ratio of 7:3 (v/v**. Simultaneous spectrophotometric estimation of ISO in the existence of trifluoperazine as a tranquilizer and its oxidative degradation was reported as well[19]. Frequently, trifluoperazine comes in a dualistic mixture with ISO for their anti-vomiting and anti-convulsive influence[20]. Similarly, derivative spectrometries were reported also since they have the privilege of simultaneous analysis of multi-component systems. Usually, as a subject to second derivative measurement within the UV region in a methanolic medium or in a slightly basic medium of 0.1 N sodium hydroxide followed an extraction step with chloroform. The procedure provides a linear quantification range for the determination of ISO within 20-80 μg/ml. However, complexation with methyl orange or sodium taurodeoxycholate[21] as an ion-pair, or with iodine[22] as a charge-transfer complex were common too[23].

ISO can be formally determined ion-exchange liquid chromatography, or by direct titration with perchloric acid in an organic medium[20]

The proposed method was built on the reaction of Isopropamide iodide with Thymol blue in an acidic medium at 404 nm. The method proved to be effective for the determination of Isopropamide iodide in pure form and its pharmacological formulation.

Materials and Method

Instrumentation

- Spectrophotometric measurements were made by a UV-Visible Spectrophotometer (T80+ PG Instruments Ltd.).

- Analytical Electronic balance (Sartorius, with four decimals).

- The acidity of the prepared solutions and buffers were adjusted using a digital pH meter (pH/mV Bench Meter, Hanna Instruments) equipped with a combined glass pH electrode.

Material and Reagents

- ISO has been provided freely by "The State Drug Industries and Medical Appliances Company" (SDI), Samarrah, Iraq.

- Solvents and chemicals were of analytical grade.

- Thymol blue (TB), 0.1% (w/v) stock solution: a 0.1 g of the reagent TB was dissolved in 5 ml of methyl alcohol and then diluted with distilled water to 100 ml. While the standard working solutions of TB were prepared freshly by consequent dilutions.

- Hydrochloric acid (0.1M): A 0.83 ml of a concentrated HCl (Sp.gr. 1.18 g/ml, 37% w/w) was diluted into 25 ml of deionized distilled water then adjusted to a 100 ml.

- NaOH (0.1 M): the solution was prepared by dissolving 0.40 g of sodium hydroxide in 100 ml of distilled water.

- Preparation of copper sulfate solution was accomplished by dissolving a 0.25 g of $CuSO_4$ and 4.5 g of CH_3COONH_4 with the solution of 0.1M acetic acid in a 100ml volumetric flask. Adjustment of acidity was fulfilled using the dilute solutions of HCl and NaOH previously prepared.

- Preparation of ISO stock solution (100 μg/ml): 0.01g of ISO was dissolved in 100ml of distilled water.

Assay procedure of pure ISO

One milliliter of the ISO standard solution (20μg/ml) was moved into a set of 50 ml separatory funnel with a 0.5 ml of pH 4 buffer solution as well as a 0.5 ml of the reagent TB (). A half milliliter of chloroform was added and the separatory funnel was shaken for one minute. The funnel was kept aside to allow a clear separation of the binary phase. Spectrometric measurement of the yellow organic phase was accomplished at a maximum wavelength of 404 nm against a blank. The calibration curve was built by graphing the absorbance intensity of the organic phase as a function to the medicine concentration.

Analysis of pharmaceutical formulation

Ten tablets of (Salabid tablets, a product of SDI) was ground and weighed accurately. Aliquot of 5mg of the fine powder was transferred quantitatively and dissolved into a 100ml of distilled water. The resulting solution was filtered to avert the presence of any suspended particles. Whereas, the standard working solutions were made by successive dilution.

Results and Discussion

In general, spectrometric procedures are well known as sensitive approaches for the determination of medicinal compositions. Spectrometric ion-pair complexation is commonly used in the determination of the concentration of various pharmaceutical composites.

A series of experimental investigations in buffer mediums have revealed the possibility of ISO compound to react with TB. The resulting yellow ion-pair derivative has been extracted from the aqueous medium by chloroform. The complex was formed in an acidic buffer medium and found to exhibit an absorption peak with a maximum at the wavelength of 404nm as its obvious in (Figure 2).

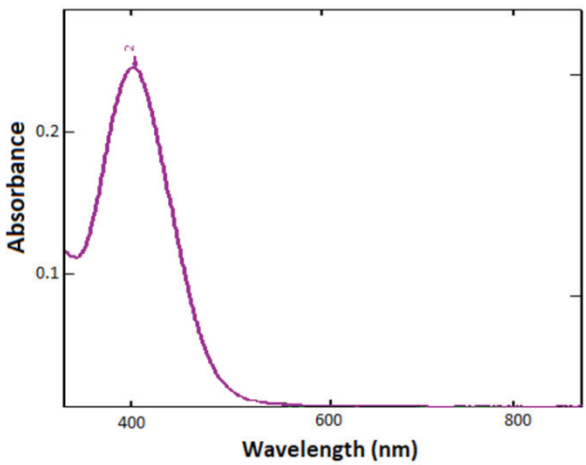

Fig. 2. The absorption spectrum of the ISO-TB yellow complex

Optimization of experimental variables

Univariable method

The experimental variables affecting the stability and the formation yield of the produced colored complex. They have been specified by the application of different sets of preliminary experiments. These variables include pH, reaction time, shaking time, reagent concentration, the order of addition, and type of the extraction solvent. Thus, the univariable approach was accomplished by modifying one variable at a time while keeping the other variables at constant values. Once the optimum value of the variable gets specified, then another parameter will be chosen for modification while maintaining all the others constant.

Effect of acidity

For the purpose of finding the optimum acidity range, ISO solutions were mixed separately with the coloring reagent of TB in mediums of different pH(s). The acidity of the reaction mediums was adjusted to a value between (2 - 5) via the addition of a few drops of either (0.1M) NaOH or (0.1M) HCl. Maximum absorbance was obtained at pH 4.0 (Figure 3). Therefore, an acidic medium with a pH of 4 was employed in all the subsequent preparations.

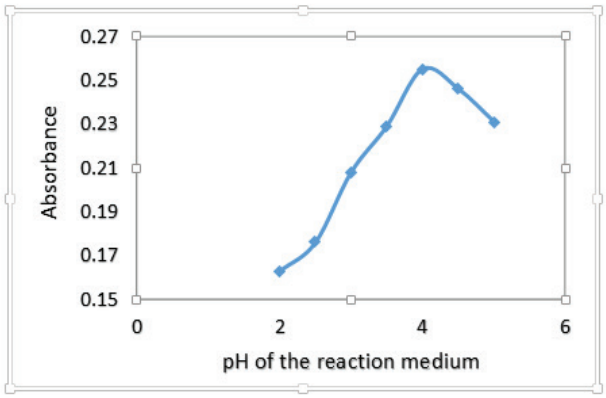

Fig. 3. The absorbance intensity for the formation of the ion-pair complex as a function of the pH of the reaction medium

Effect of reaction time

The optimization of the reaction time was accomplished by examining the development of the yellow color of the formed complex at ambient temperature (25 ± 2 °C). The reaction was instant as the colored product reached its maximum intensity with stable absorbance directly after the blending of ISO with the reagent of TB. The color intensity remains stable for the next 24hr (Table 1).

Tab. 1. The reaction time for the formation of the ion-pair complex as a function of absorbance intensity

Time reaction (min)	Absorbance
1	0.256
2	0.251
3	0.252
4	0.251
5	0.250

Effect of reagent mole ratio

Absorbances were measured when different volumes of the TB solution in the concentration of () were added to 0.5ml of ISO standard solution (20µg/ml). An increment in TB concentration causes a reduction in the absorbance which may be due to the formation of new species (Figure 4).

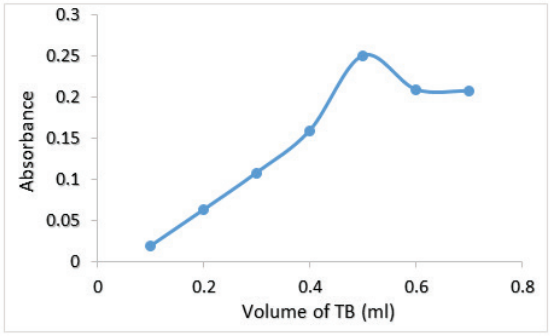

Fig. 4. The absorbance intensity for the formation of the ion-pair complex as a function for the addition of TB

Optimization of the extraction shaking duration

Optimization of the shaking time required to achieve a maximum extract recovery was studied over a time range of 1-5 minutes at ambient room temperature. No significant influence was observed for this parameter (Figure 5). However, a one minute found to be enough as an optimum shaking time for all the upcoming extractions.

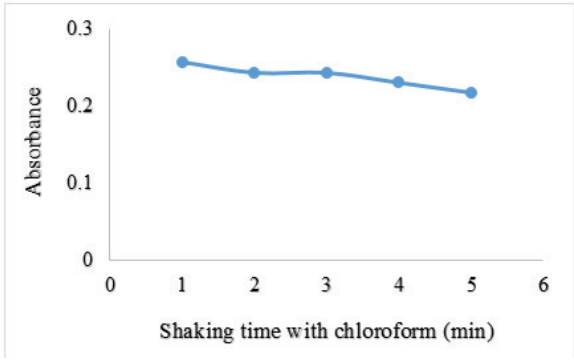

Fig. 5. The absorbance intensity for the formation of the ion-pair complex as a function for the shaking time of extraction

Effect of the extracting solvent

Various organic solvents; Benzene, carbon tetrachloride, dichloromethane, cyclohexane, and chloroform, were inspected for their extractability ISO colored complex. Chloroform observed to have the most efficient extraction ability (Figure 6). Furthermore, experiments exhibited that a single extraction of a 5ml batch of chloroform was very satisfactory in terms of recovery.

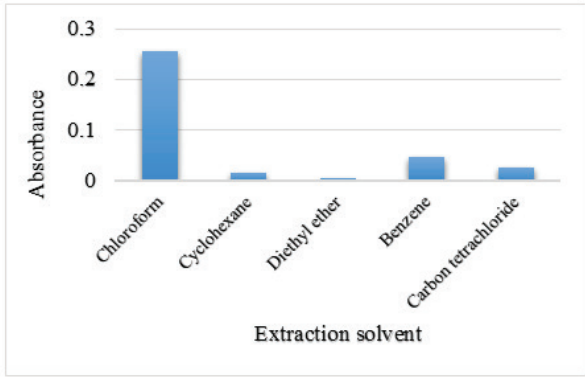

Fig. 6. The absorbance of ISO/TB ion-pair colored complex at the wavelength of 404 nm in different solvents.

Influence of the order of addition

The sequence of addition of the reactants is important and has been tested. The optimum order was by dissolving the ISO drug into the buffer then adding TB reagent to it. Starting with the ISO in the buffer gave a higher absorption (0.257 au) at the wavelength of 404nm than by starting with TB in the buffer (0.141 au).

Stoichiometry of the complex

The mole ratio of ISO to TB was established via Job's method of continuous variation. Two standard solutions of ISO and TB both have the same concentration of were prepared. The reagent TB was added gradually to ISO buffer solution by a 0.1 ml portion so that the total volume of the mixture maintains to 1ml and the

absorption at the wavelength of 404nm is measured with each addition. The resulted graph has exhibited that the ion-pair complex is formed with a mole ratio of 1:1 to ISO:TB (Figure 7). As one positive protonated ISO molecule requires one anion of TB to form an ion-pair complex6,24.

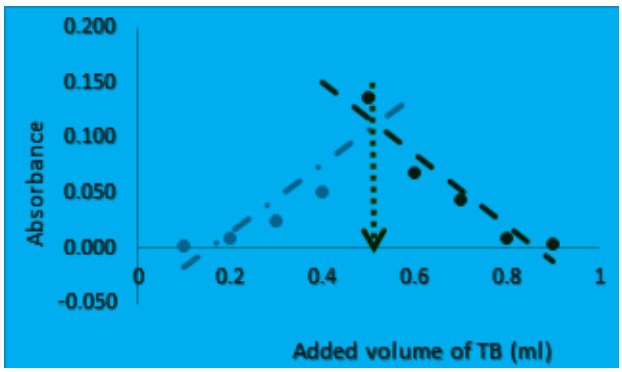

Fig. 7. Determination of the mole ratio of ISO:TB via Job's method of continuous variation

Calibration curve

A linear calibration curve was built for the colorimetric determination of ISO in a pharmaceutical composition under optimum conditions for the formation of the ion-pair complex of ISO with TB. The resultant calibration curve excellent agreement to beer's law on the quantification range of 1-40µg/ml with linearity of 0.9993 (Figure 8). Nevertheless, more analytical parameters are listed in (Table 2) below.

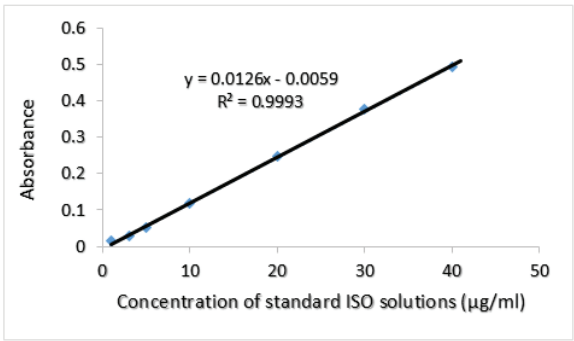

Fig. 8. Calibration curve for the quantification of (ISO) in optimum conditions.

Tab. 2. Some spectral parameters related to the regression equation for ISO determination via ion-pair formation.

Parameter	Value
λmax (nm)	404
color	Yellow
Range of linearity (µg/ml)	1 - 40
Molar absorptivity (L.mol .cm 1)	1000x408.434x0.0126
Regression equation	Y= 0.0126X0.0059
Correlation of Linearity (R2)	0.9993
Calibration Sensitivity	0.0126
Sandells Sensitivity (µg. cm 2)	0.0793
LOD (µg/ml)	0.714
LOQ (µg/ml)	2.380

Method validation

The accurateness of the suggested method was confirmed by three replicate analyses, i.e. n=3. Three different concentrations of ISO were used (10, 20, 30 µg/ml) to calculate the method recovery and the relative error percentage, as shown in (Table 3). The results were satisfactory as indicating the high accuracy of the method. The precision was expressed as a function of the "relative standard deviation" (RSD %) which found to be within the range of (1.1938-0.5954).

Tab. 3. Estimation of accuracy and precision of the suggested method.

Concentration of ISO (µg/mL)		Recovery percentage	Relative Error %	R.S.D. %
Taken	Found (n=3)			
10	10.140	101.4	1.4	1.1938
20	19.478	97.4	-2.61	1.1762
30	29.531	98.4	-1.56	0.5954

Interference studies

Under optimum experimental conditions, the influence of several foreign constituents which may be existing in medicinal products and may have an impact on the reaction of ISO with TB were examined. The tests indicated that common excipients like in the case of glucose, sucrose, lactose, and starch do not interfere with the colorimetric analysis of ISO even when they present in a high concentration of 1000 µg/ml (Table 4).

Tab. 4. The recovery percentage of 20µg/ml of ISO in the presence of 1000µg/ml of excipients.

Excipient present (1000µg/ml)	ISO initial Conc. (20 µg/ml)	
	ISO Conc. Found (µg/ml)	Recovery %
Lactose	19.769	98.849
Sucrose	19.928	99.642
Starch	20.325	101.626
Glucose	19.531	97.658

Analysis of dosage forms

The good validation of the method outcomes has made the suggested method suitable for quality control analysis of ISO in commercial tablets (Table 5).

Tab. 5. Determination of Isopropamide iodide in pharmaceutical formulations (n=3)

Pharmaceutical formulations	Label amount (mg)	Found by proposed method (mg)	Recovery (%)
Tablet	5mg/tab	5.00	100.1

Conclusion

A spectrometric methodology has been established and validated for the quantification of Isopropamide iodide (ISO) in pure form and as a medicine. The method has a number of privileges over other instrumental techniques of ISO analysis. It is affordable, especially when compared to chromatographic techniques, as well as rapid, and highly accurate. Moreover, common excipients did not interfere with the proposed procedure. For the prementioned, this method is highly recommended for routine analysis of ISO and may apply for other active pharmaceuticals with a similar functional group.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Chemistry Department and all experiments were carried out in accordance with approved guidelines.

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