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Spectrophotometric and conductometric determination of tramadol hydrochloride

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ABSTRACT

Six simple and sensitive spectroscopic and conductometric procedures (A-F) were developed for the determination of tramadol hydrochloride. Method A, B and C are based on the reaction of cobalt (II) thiocyanate with tramadol to form stable ternary complex, which could be measured by spectrophotometric (method A), atomic absorption (method B) or conductometric (method C) procedures. Method (D and E) depended on the reaction of molybdenum thiocyanate with tramadol to form stable ternary complex, measured by spectrophotometric means (method D), or by atomic absorption procedures (method E), while method F depends on the formation of an ion pair complex between the studied drug and bromothymol blue which is extractable into methylene chloride, Tramadol hydrochloride could be assayed in the range of 80-560 μg ml^{-1}, 40-220 μg ml^{-1}, 1-15 mg, 2.5-22.5 μg ml^{-1}, 1.25-11.25 μg ml^{-1} and 5-22 μg ml^{-1} using methods A, B, C, D, E and F respectively. Various experimental conditions were studied. The results obtained showed good recoveries. The proposed procedures were applied successfully to the analysis of tramadol in its pharmaceutical preparations and the results were favorably comparable with the official method.

KEYWORDS

Tramadol;
Cobalt thiocyanate;
Molybdenum thiocyanate;
Bromothymol blue;
Spectrophotometry;
Conductometry;
Atomic absorption.

INTRODUCTION

Tramadol [(±) trans-2-(dimethylaminomethyl)-1-(3-methoxy- phenyl)-cyclohexanol hydrochloride] is an opoid analgesic used for moderate to severe pain[1]. Different methods for the analysis of the selected drug have been reviewed. The BP[2a] specifies non-aqueous titration technique detecting the end point potentiometrically for its determination. The literature reveals few methods for the determination of the mentioned drug in biological fluids and in pharmaceutical preparations. Among these methods are spectrophotometry[3,4], HPLC[5,6], GC[7], capillary electrophoresis[8], voltammetry[9] and potentiometry[10].

An inspection of the previous methods for the determination of the cited drug revealed that only few spectrophotometric ones have been reported. Although atomic absorption spectrometry AAS is rapid technique and has a low detection limit, it has not been yet applied to the determination of tramadol, so is the same case with the conductometric procedures which proved to be simple, sensitive, reliable and of a very conve-

nient and simple procedures.

Cobalt (II) thiocyanate reacts with tramadol to form a stable ternary complex extractable with methylene chloride, the complex was determined either spectrophotometrically (method A) by measuring the greenish blue extractable color at λ625 or by atomic absorption

spectrometry (method B) indirectly using the aqueous acidic extract of the combined cobalt (II) in the ternary complex or by measuring the change in the conductance (method C) following the titration of tramadol with Cobalt (II) thiocyanate, similar procedures using molybdenum thiocyanate were applied for determining tramadol either by spectrophotometric means (method D) or by atomic absorption spectrometry (method E), while method F depends on the reaction of bromothymol blue with tramadol at pH 3.7 to form a stable ion association complex extractable with methylene chloride, the complex has maximum absorbance at 411 nm.

EXPERIMENTAL

Apparatus

Shimadzu UV-260 double beam recording spectrometer with a 1 cm cell holder

Shimadzu Atomic Absorption flame spectrophotometer model AA-640-13

Conductometer model CM-1K, Tokyo TOA electronics ltd Japan.

Chemocadet pH meter.

Materials and reagents

All materials and reagent used were of analytical grade, solvents were of spectroscopic grade and bidistilled water was used.

1) Tramadol hydrochloride pure drug and Tramal capsules (labelled to contain 50 mg tramadol hydrochloride per capsule) were obtained from Minapharm, Egypt under licence of Grunenthal, Germany.
2) Tetrathiocyanato cobalt (II) solution (1), prepared by dissolving 56.25g of NH_4SCN and 13.80 g of $CoCl_2.6H_2O$ in water to give 100 ml of the solution[11].
3) 2.5×10^{-2} M tetrathiocyanato cobalt (II) solution (2) prepared by dissolving 0.025 mol of cobalt, as CoCl2·6H2O and the required amount of ammonium thiocyanate (0.1 mol), in 100 ml bidistilled water.
4) Sodium Molybdate (Fluka AG, Switzerland), 10^{-2} M solution prepared by dissolving 0.2419 gm in 100 ml distilled water.
5) Ascorbic Acid (El-Nasr pharm. Chem. Co., Egypt) prepared as 10% w/v solution in distilled water.
6) Ammonium thiocyanate (Belami Fine Chem., India), prepared as 10% w/v solution in distilled water.
7) Bromothymol blue (BDH Chemicals Ltd., Poole, England) was prepared as 0.05% solution in bidistilled water.
8) Acetate buffer pH 3.7: Dissolve 10 g of anhydrous sodium acetate in 300 ml water, adjust to pH 3.7 with glacial acetic acid and dilute to 1000 ml with water. If necessary, readjust to pH 3.7 with glacial acetic acid or anhydrous sodium acetate as required, before use[2b].

Standard drug solutions

Aqueous solution of 0.1 and 4 mg ml^{-1} tramadol hydrochloride, prepared by dissolving 10 and 400 mg of the pure drug in 100 ml distilled water respectively. Working solutions of lower concentrations were prepared by appropriate dilution of the standard solutions.

Construction of calibration curves

Spectrophotometric procedures (method A)

Into 125 ml separating funnels, transfer aliquots containing 0.8 -5.6 mg of tramadol drug solution, add 3 ml of cobalt thiocyanate (solution 1), mix then extract the aqueous solution with an equal volume of methylene chloride and shake for 45 seconds, allow the mixture to separate into two phases. Collect the organic layer and dry with anhydrous sodium sulfate, complete to 10 ml with methylene chloride, measure the absorbance of the extracts at 625 nm, against a reagent blank prepared according to the same treatment (Figure 1).

Atomic absorption spectrometric procedures (method B)

Procedures were proceeding as under spectrophotometric method as far as "complete to 10 ml with methylene chloride ". The organic layer was evaporated to dryness, the residue was dissolved in 1 ml 1N HCl and the volume was completed to 10 ml with bidistilled water (TABLE 1).

TABLE 1 : Aspirate in a suitable atomic absorption spectrometer under the following conditions

Analysis line wavelength	2407A^o
Lamp current	9 m A
Slit width	1.9A^o
Burner height	6 mm
Burner slot / flame	10 cm (air-C_2H_2)
Support gas flow	10 I min^{-1}
Fuel gas flow	2.5 I min^{-1}
Absorption sensitivity	0.16ppm

TABLE 2 : Aspirate in a suitable atomic absorption spectrometer under the following conditions

Analysis line wavelength	313.3 nm
Lamp current	9 m A
Slit width	3.8A^o
Burner height	5 mm
Burner slot / flame	5 cm (O-C_2H_2)
Support gas flow	10 L min^{-1}
Fuel gas flow	4.8 L min^{-1}
Absorption sensitivity	0.77ppm

The concentration of the consumed cobalt was calculated from calibration graph of standard cobalt chloride solution.

Conductometric procedures (method C)

Transfer suitable aliquot of sample solution containing 1-15 mg of drug to a 50 mL calibrated flask and make up to the mark with distilled water. Transfer the contents of the calibrated flask to a beaker and immerse the conductivity cell. Titrate using 2.5×10^{-2} M cobalt thiocyanate (solution 2). Measure the conductance subsequent to each addition of reagent solution and after thorough stirring for two minutes, correct it for dilution effect[12] by means of the following equation, assuming that conductivity is a linear function of dilution.

$$\Omega^{-1}_{correct} = \Omega^{-1}_{obs} [v_1+v_2/v_1]$$

where Ω^{-1}_{obs} is the observed electrolytic conductivity, $v1$ is the initial volume and v_2 is the volume of reagent added.

Construct a graph of corrected conductivity *versus* the volume of added titrant and determine the endpoint (Figure 2).

Spectrophotometric procedures (method D)

Into 125 ml separating funnels mix 2 ml of 10^{-2} M sodium molybdate solution, 1 ml of 5 M HCl, 0.5 ml of 10% NH_4SCN and 0.5 ml of 10% ascorbic acid, and allow the mixture to stand for10 minutes at room temperature (20±5°C). Add aliquots containing 0.025 - 0.225 mg of tramadol drug solution to the mixture, and stand for another 5 minutes, extract the formed complex with an equal volume of methylene chloride and shake for 75 seconds, allow the mixture to separate into two phases.

Collect the organic layer and dry with anhydrous sodium sulfate, complete to 10 ml with methylene chloride. Measure the absorbance of the extracts at 467 nm, against a reagent blank prepared according to the same treatment (Figure 3).

Atomic absorption spectrometric procedures (method E)

Procedures were proceed as under spectrophotometric method as far as "complete to 10 ml with methylene chloride ". The organic layer was evaporated to dryness, the residue was dissolved in 1 ml 1N HCl and the volume was completed to 10 ml with bidistilled water (TABLE 2).

The concentration of the consumed molybdenum was calculated from calibration graph of standard sodium molybdate solution.

Ion pair procedures using bromothymol blue (method F)

Into 125 ml separating funnels, transfer aliquots containing 0.05 – 0.22 mg of tramadol drug solution, treat with about 2 ml of acetate buffer pH 3.7, add 1.5 ml of 0.05 % bromothymol blue mix then extract the aqueous solution with an equal volume of methylene chloride and shake for 30 seconds, allow the mixture to separate into two phases. Collect the organic layer and dry with anhydrous sodium sulfate, complete to 10 ml with methylene chloride, measure the absorbance of the extracts at 411 nm, against a reagent blank prepared according to the same treatment (Figure 4).

Procedure for the assay of the pharmaceutical formulations

The contents of ten capsules were emptied, pulverized. An accurately weighed amount equivalent to 10 and 400 mg tramadol hydrochloride was extracted

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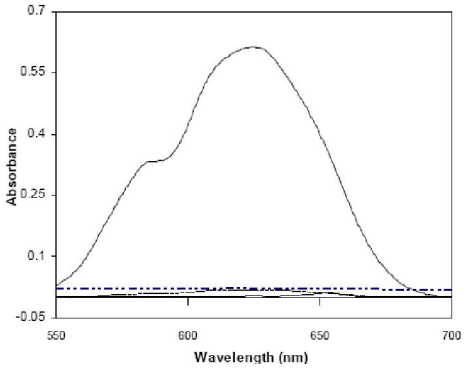

Figure 1 : Absorption Spectra of the ternary complex formed through reaction of: 400 µg ml^{-1} tramadol HCl with Cobalt thiocyante (—), 400 µg ml^{-1} tramadol HCl with Cobalt chloride (— — —), 400 µg ml^{-1} tramadol HCl with ammonium thiocyanate (. . . . .), Blank solution (- - - -)

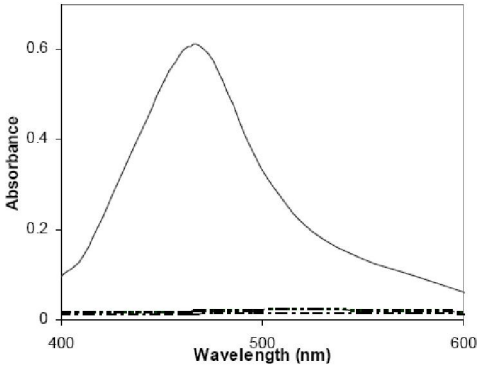

Figure 3 : Absorption Spectra of the complex formed through reaction of: 20µg ml^{-1} tramadol HCl with molybdenum thiocyante (——), 20 µg ml^{-1} tramadol HCl with sodium molybdate (— — —), 20 µg ml^{-1} tramadol HCl with ammonium thiocyanate (. . . . .), Blank solution (- - —)

by shaking with 50 ml distilled water, filtered, transferred to a 100 ml volumetric flask, 10 completed to the mark using distilled water. Aliquots from this solution were used for the application of proposed methods applying standard addition technique or applying the direct procedures.

Determination of the stoichiometry of the reaction

Job's method of continuous variation

In order to ascertain the stoichiometry of reaction Job's method of continuous variation[13] was carried out.

RESULTS AND DISCUSSION

Spectrophotometric procedures using cobalt thiocyanate (method A)

Ternary complexes have been widely used in

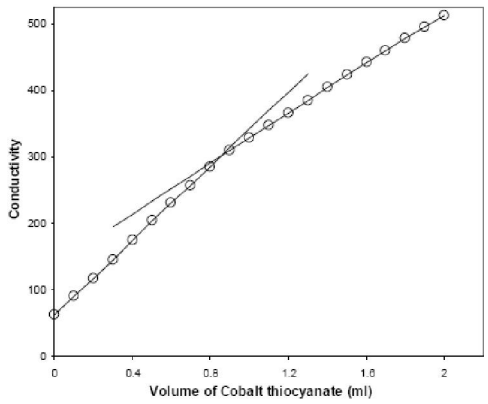

Figure 2 : Conductometric titration curve of 13 mg tramadol *vs* (2.5×10^{-2}) M cobalt thiocyanate

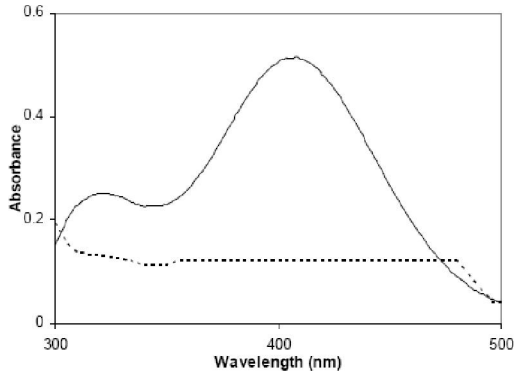

Figure 4 : Absorption spectra of the ion pair formed through reaction of : 14 µg ml^{-1} tramadol HCl with 0.05 % bromothymol blue (——), Blank solution (……)

spectrophotometric analysis of many pharmaceutical compounds[14-16]. In this paper, the formed ternary complex consists of the studied drug tramadol hydrochloride as main ligand, thiocyanate as second ligand and the metal ions, cobalt (II). This triple complex is extractable with methylene chloride with absorption maximum at 625 nm, whereas the binary systems (metal: drug), (metal: thiocyanate) and (drug: thiocyanate) have no absorbance in the visible region (Figure 1). The effects of the reagent concentrations, pH, extraction time, organic solvent type and aqueous to organic phase ratio with respect to maximum sensitivity, adherence to Beer's law and stability, have been studied through control experiments. The optimum conditions were established by varying one variable at a time and observing its effect on the absorbance of colored species:

Effect of cobalt thiocyanate concentration

A high concentration of $[Co(SCN)_4]^{-2}$ was necessary for quantitative complex formation, 3 ml of cobalt

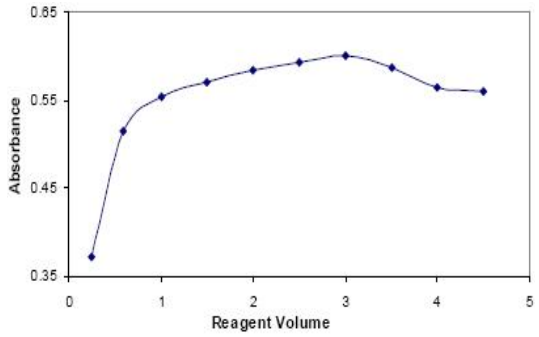

Figure 5 : Effect of volume of cobalt thiocyanate (solution 1) on the reaction of cobalt thiocyanate with 400 µg ml^{-1} tramadol

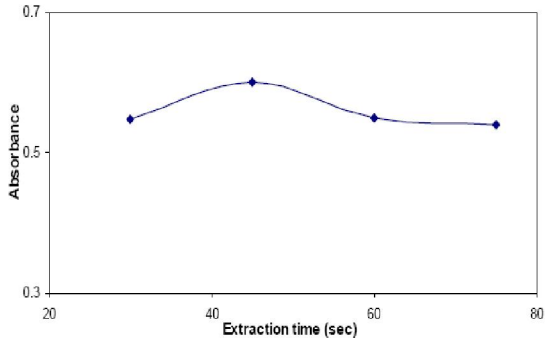

Figure 6 : effect of extraction time on the reaction of cobalt thiocyanate with 400 µg ml^{-1} tramadol

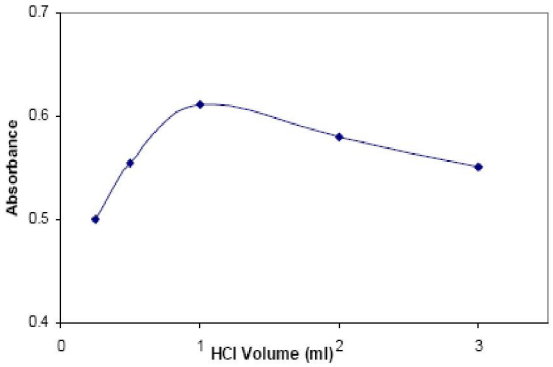

Figure 7 : Effect of volume of 5 M HCl on the reaction of molybdenum thiocyanate with 20 µg ml^{-1} tramadol

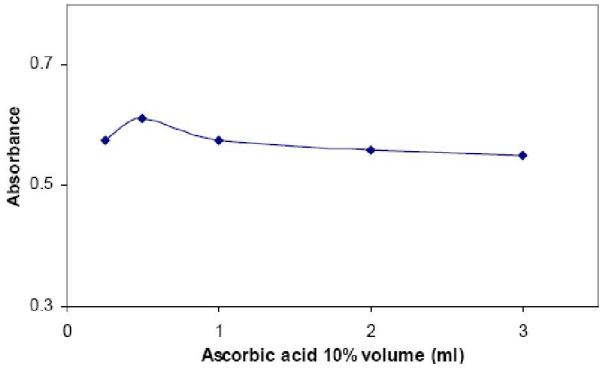

Figure 8 : Effect of volume of 10% ascorbic acid on the reaction of molybdenum thiocyanate with 20 µg ml^{-1} tramadol

thiocyanate (solution 1), was found sufficient for tramadol, more than this optimal concentration would decrease the absorbance of the ternary complex (Figure 5).

Effect of buffer pH

Variation of the pH of the aqueous phase in the range from (2-11), had no effect on the intensity of the absorbance of the complex.

Effect of extraction time and times of extractions

It was found that a single extraction of the ternary complex for 45 seconds was sufficient for quantitative extraction (Figure 6).

Effect of organic solvent type

Chloroform, methylene chloride, diethyl ether and benzene were all tried.

Methylene chloride was found to be most convenient for the studied drug.

Effect of aqueous to organic phase ratio

Varying the ratio from (2:1) to (1:2) didn't cause any reasonable change in the results so a (1:1) ratio was rather used.

Atomic absorption procedures using cobalt thiocyanate (method B)

It was not practical to aspirate the organic solvent of the ternary complex in the atomic absorption spectrometer, the high chlorine/carbon ratio would lead to the formation of a large quantity of HCl in the flame, which would damage the instrument[17,18]. It was better to extract the ternary complex with organic solvent (methylene chloride), evaporate, and then dissolve the ternary complex residue with HCl, which could be aspirated directly in the atomic absorption spectrometer. The effects of the reagent concentrations (cobalt thiocyanate solution 1) pH, extraction time, solvents with respect to maximum sensitivity, minimum blank, adherence to Beer's law and , have been studied through control experiments. The optimum conditions were established by varying one variable and observing its effect on the absorbance of metal ion. It was found that the optimum experimental conditions are the same as in the extractive spectrophotometric procedures and incorporated into the general procedures.

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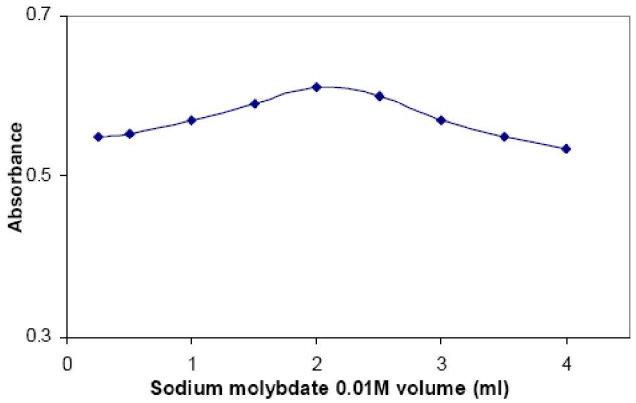
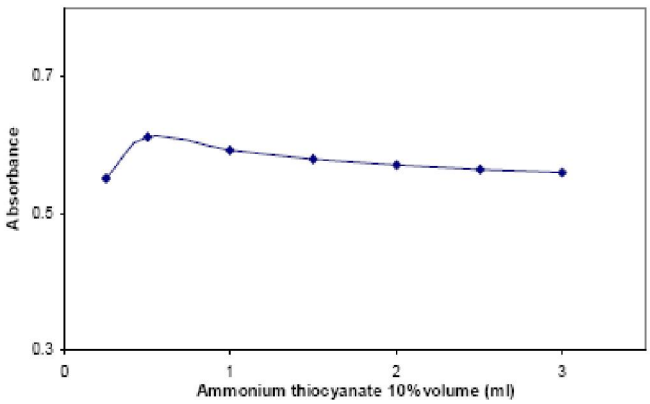


Figure 9 : Effect of volume of 0.01 M sodium molybdate on the reaction of molybdenum thiocyanate with 20 µg ml^{-1} tramadol



Figuer 10 : Effect of volume of 10% ammonium thiocyanate on the reaction of molybdenum thiocyanate with 20 µg ml^{-1} tramadol

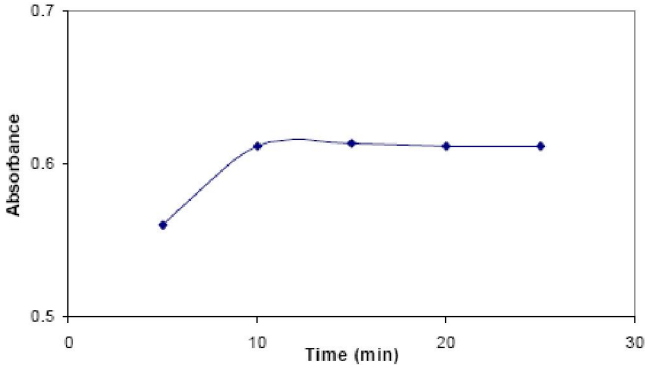


Figure 11 : Effect of standing time before addition of the drug to Mo (V) thiocyanate complex on the reaction of molybdenum thiocyanate with 20 µg ml^{-1} tramadol

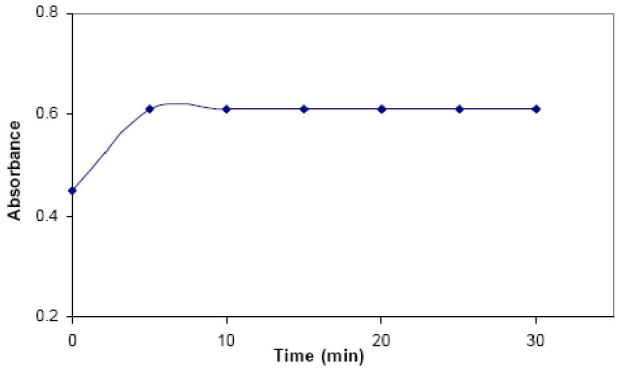


Figure 12 : Effect of standing time after addition of the drug to Mo (V) thiocyanate complex on the reaction of molybdenum thiocyanate with 20 µg ml^{-1} tramadol

Conductometric procedures using cobalt thiocyanate (method C)

Conductometric analysis can be used in many titration procedures when ionic solutions are involved. As the conductance of a solution is related to the total ionic content, it can be applied to follow reactions that result in a change in this quantity. Conductance measurements are used successfully in quantitative titration of systems in which the conductance of the solution varies before and after the equivalence point. In these cases, the titration curve can be represented by two lines intersecting at the end point.

Investigations were carried out to establish the most favorable conditions for the ion associates formation of tramadol with cobalt thiocyanate to achieve sharp end point. The influence of some variables on the reaction has been tested as follow:

The optimum conditions for performing the titration in a quantitative manner were elucidated as described below.

Titrations in different media were attempted to obtain the best results

Preliminary experiments in:

1. Aqueous drug solution with aqueous reagent solution
2. Ethanol drug solution with ethanol reagent solution
3. Drug solution with reagent solution, both in ethanol–water (50%, v/v) mixture
4. Methanol drug solution with methanol reagent solution
5. Drug solution with reagent solution, both in methanol—water (50 v/v) mixture
6. Acetone drug solution with acetone reagent solution and
7. Drug solution with reagent solution, both in acetone–water (50% v/v) mixture

Preliminary experiments showed that procedure in aqueous media was the most suitable for successful results, because in other procedures turbid solution was formed which caused some errors.

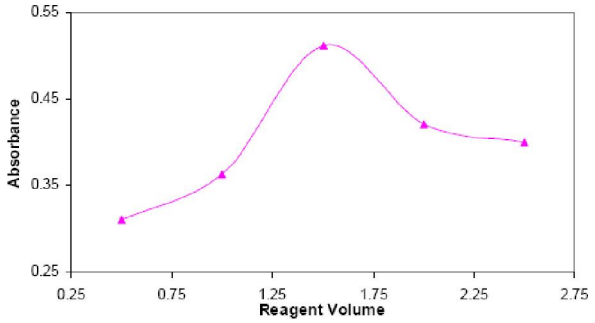


Figure 13 : Effect of volume of bromothymol blue on the reaction of bromothymol blue with 14 µg ml^{-1} tramadol

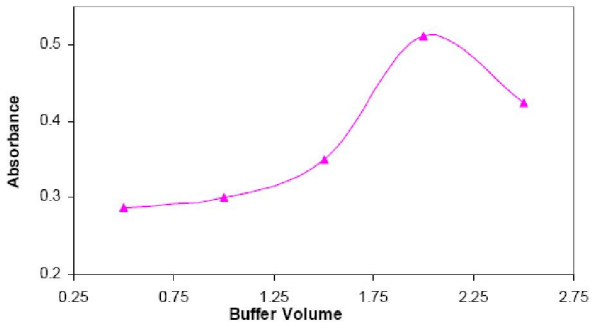


Figure 14 : Effect of volume of acetate buffer pH 3.7 on the reaction of bromothymol blue with 14 µg ml^{-1} tramadol

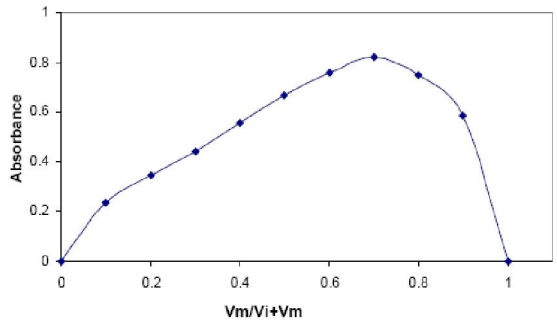


Figure 15 : Determination of the stoichiometry of the reaction of: Tramadol (2.5 × 10^{-2}M) and $CoCl_2$ (2.5 × 10^{-2} M) in presence of NH_4SCN (3M)

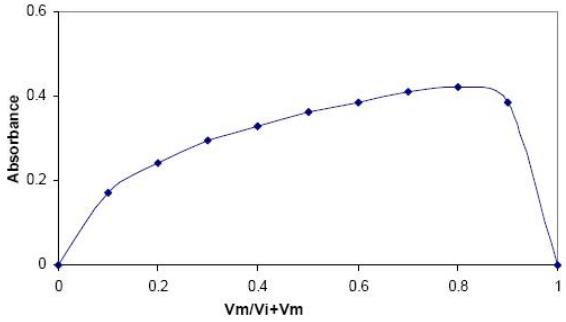


Figure 16 : Determination of the stoichiometry of the reaction of: Tramadol (1×10^{-3}M) and sodium molybdate (1 × 10^{-3} M) in presenece of 10% ammonium thiocyanate

Reagent's concentration

Different concentrations of cobalt thiocyanate solution were tried ranging from 2×10^{-1} to 5×10^{-3} Molar solution The optimum concentration of the reagent was 2.5×10^{-2} M in titration of the studied drug to achieve a constant and highly stable conductance reading within 1-2 min of mixing. Concentrations less than these limits led to unstable readings and more time was needed to obtain constant conductance values.

Representative titration curve is shown in (Figure 2). Two straight lines are obtained, intersecting at the end-point, the first branch ascending the second one.

The conductance measured before the addition of the titrant (volume of $NH_4[Co(SCN)_4]$ equals zero) is related to the slight dissociation of the drug cations and chloride ions. Up to the equivalence point, the titration involves the gradual dissociation of the protonated cation drug as a result of the formation of ion-pair in the solution releasing Cl^- into the medium þ. This increase of the conductance is due to the mobility of the Cl^- ionsþ, causing an increase in the slope of the conductometric curve (first branch of the curve). After the equivalence point, the measured conductance is mainly due to NH_4^+ present in the solution. As the mobility of those ions is smaller than that of the Cl^- ions, there is a decrease in the slope of the second section of the titration curve. The equivalence point is defined as the point of intersection of the two straight segments.

The shape of the titration curve depends on all the species present during the titration process and other factors such as viscosity, dielectric constant, solvation, ion-pair association and proton transfer.

The conductometric titrations of different volumes of 2.5×10^{-2} M cobalt thiocyanate solution was performed. The results show an obvious maximum in the conductance curve at drug-reagent molar ratio of (2:1). The reactions may be represented by the equations:

2 TRM HCl + $(NH_4)_2[Co(SCN)_4] \rightarrow (TRM)_2[Co(SCN)_4]$ + $2NH_4^+ + 2Cl^-$

The optimum concentration ranges for determination of tramadol was in the range of 1-15 mg. At such range, distinct inflections (Figure 2) and stable conductance reading were obtained.

Spectrophotometric procedures using molybdenum thiocyanate (method D)

Molybdenum(V) formed by the reduction of

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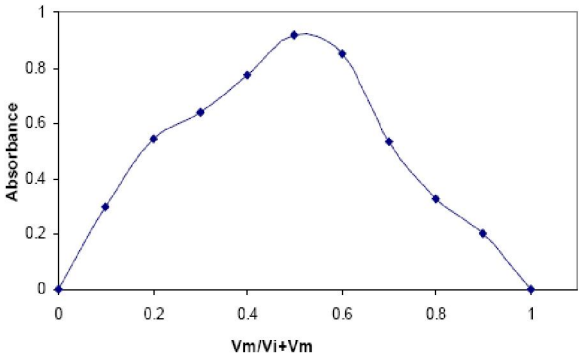

Figure 17 : Determination of the stoichiometry of the reaction of: Tramadol (2.5×10^{-4}M) and bromothymol blue (2.5×10^{-4} M)

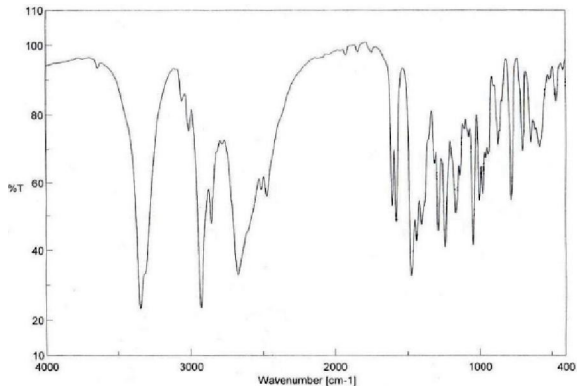

Figure 18 : IR of tramadol Hydrochloride

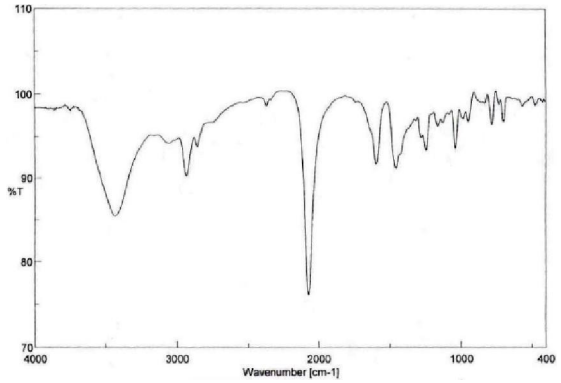

Figure 19 : IR of tramadol Co (II) thiocyanate complex

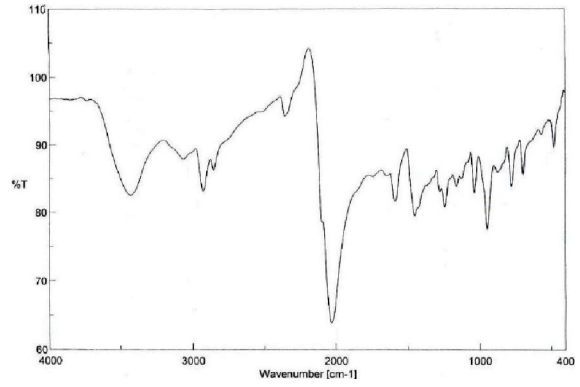

Figure 20 : IR of tramadol Mo (V) thiocyanate complex

molybdenum(VI) by ascorbic acid or by thiocyanate in presence of 5 M hydrochloric acid, it then combines with ammonium– thiocyanate to form a red binary molybdenum(V)–thiocyanate complex, the complex is non-extractable with methylene chloride. On adding of the investigated drug solution, an orange red complex is formed and extractable with methylene chloride and had an absorption maximum at 467 against a reagent blank.

$$\text{Mo(VI)} \xrightarrow[\text{5M HCl}]{\text{ascorbic}} \text{Mo(V)} \xrightarrow{6SCN^-} [Mo(SCN)_6]^-$$

Effect of acidity

The formation of a ternary complex was only achieved in acidic medium, the complex was not formed in acetic or perchloric acid medium, but it was formed in hydrochloric, sulphuric or nitric acids medium. 1 ml of 5 M HCl was sufficient for maximum absorbance and the formation of Mo(V)–thiocyanate-drug complex (Figure7).

Effect of ascorbic acid

It was found that the reduction probability of Mo(VI) to Mo(V) may occur by ascorbic acid or by SCN^- in acidic medium. The rapidity, sensitivity and stability of Mo (V)–thiocyanate binary complex is enhanced considerably by using ascorbic acid. Ascorbic acid gives reproducible values and masks many interfering ions. 0.5 ml 10% ascorbic acid was used through the study (Figure 8).

Effect of sodium molybdate concentration

The effect of varying sodium molybdate on the complex formation and its extraction in methylene chloride is shown in (Figure 9). The data shows that 2 ml of 0.01 M sodium molybdate was required for maximum absorbance.

Effect of ammonium thiocyanate concentration

ml of 10% ammonium thiocyanate solution was required to obtain maximum absorbance for the formed complex (Figure10).

Effect of standing time

Mo (V) thiocyanate binary complex was stable after 10 minutes, while Mo (V) thiocyanate drug ternary

TABLE 3 : Spectral data for determination of tramadol HCl using methods (A, B, D, E and F)

Items	Method A	Method B	Method D	Method E	Method F
Linearity range (μg ml^{-1})	80-560	40-220	2.5-22.5	1.25-11.25	5-22
Apparent molar absorptivity* (mol^{-1} cm^{-1})	4.37×10^{2}	7.72×10^{5}	1.09×10^{4}	1.29×10^{5}	9.96×10^{3}
Sandell's sensitivity (μg ml^{-1} per 0.001 A)	1.46×10^{-4}	2.57×10^{-1}	3.63×10^{-3}	4.33×10^{-2}	3.323×10^{-3}
Regression equation :	-3.5×10^{-2}	12.18			1.2×10^{-1}
Intercept (a)	1.6×10^{-3}	2.46	1.15×10^{-1}	1.198	4.5×10^{-2}
Slope (b)	0.9997	0.9997	2.5×10^{-2}	1.6×10^{-1}	0.9998
Correlation coefficient (r)	1.17	0.746	0.9999	0.9999	0.757
Variance			0.799	1.01	

***Calculated on the basis of the molecular weight of the drug**

complex needed another 5 mintues for its complete formation (Figure 11,12).

Effect of extraction time and times of extractions

It was found that a single extraction of the ternary complex for 75 seconds was sufficient for quantitative extraction.

Effect of organic solvent type

Chloroform, methylene chloride, diethyl ether and benzene were all tried.

Methylene chloride was found to be most convenient for the studied drug.

Effect of aqueous to organic phase ratio

Varying the ratio from (2:1) to (1:2) didn't cause any reasonable change in the results so a (1:1) ratio was rather used.

Atomic absorption procedures using molybdenum thiocyanate (method E)

The optimum conditions were established by varying one variable and observing its effect on the absorbance of metal ion. It was found that the optimum experimental conditions are the same as in the extractive spectrophotometric procedures.

It was found that: 0.1 μg ml-1 Mo (V) = 1.25 μg ml-1 tramadol hydrochloride.

Ion pair procedures using bromothymol blue (method F)

The utility of bromothymol blue as ion-pairing reagent in assay of tramadol is investigated here. The spectra of the reaction products show characteristic λmax_at 411 nm (figure 4). The experimental conditions were established by varying one variable and observing its effect on the absorbance of the colored species as discussed below:

Effect of bromothymol blue concentration

1.5 ml of 0.05 % bromothymol blue was found sufficient for tramadol, more than this optimal concentration would decrease the absorbance of the formed complex (Figure 13).

Effect of buffer pH and volume

Using different buffers of different pH in the range from (2-11), the intensity of the color of the formed complex increased when 2 ml of acetate buffer of pH 3.7 was used (Figure 14).

Effect of extraction time and times of extractions

It was found that a single extraction of the ternary complex for 30 seconds was sufficient for quantitative extraction.

Effect of organic solvent type

Chloroform, methylene chloride, diethyl ether and benzene were all tried.

Methylene chloride was found to be most convenient for the studied drug.

Effect of aqueous to organic phase ratio

Varying the ratio of aqueous phase to organic phase didn't cause any reasonable change in the results so a (1:1) ratio was rather used.

Stoichiometric relationship

Using Job's method of continuous variation, the molar ratio of tramadol to cobalt thiocyanate and molybdenum thiocyanate was found to be (2:1) and (4:1) respectively, while for bromothymol blue it was found to be (1:1) (Figure 15-17).

IR charts

The ternary complexes formed from applying procedure A and D were isolated and subjected to structural elucidation by means of infra red (IR). Tramadol gave principal peaks at 2672.86, 2858.95, 2928.38, 3011.3, 3059.51, 3344.93, when the complexes were

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TABLE 4 : Determination of tramadol HCl using methods (A-F)

Method A		Method B		Method C			Method D		Method D		Method F	
Taken ($\mu g\ ml^{-1}$)	Recovery %	Taken ($\mu g\ ml^{-1}$)	Recovery %	Taken (mg)	Found (mg)	Recovery %	Taken ($\mu g\ ml^{-1}$)	Recovery %	Taken ($\mu g\ ml^{-1}$)	Recovery %	Taken ($\mu g\ ml^{-1}$)	Recovery %
80	101.56	40	100.43	1	1.02	102	2.50	97.60	1.25	100.50	5	98.67
120	98.44	60	100.15	3	3.04	101.33	5.00	99.20	2.50	98.00	6	100.74
160	99.61	100	99.52	7	6.89	98.43	7.50	99.73	3.75	101.17	7	101.59
200	101.88	120	99.19	9	8.99	99.89	10.00	100.00	5.00	100.13	8	99.44
240	100.00	140	99.54	11	10.87	98.82	12.50	99.84	6.25	100.70	10	98.89
280	99.55	200	101.39	13	13.04	100.31	15.00	100.53	8.75	100.14	11	100.20
320	100.78	220	98.82	15	14.99	99.93	17.50	100.57	11.25	100.11	12	100.37
360	99.83						20.00	99.40			14	100.32
400	99.22						22.50	99.20			17	100.00
440	100.14										19	100.82
480	98.70										22	99.49
560	101.12											
Mean* 100.07 (p = 0.05)		99.86			100.10		99.56		100.11		100.05	
N 12		7			7		9		7		11	
S.D. 1.083		0.863			1.27		0.894		1.01		0.8699	
R.S.D. 1.082		0.864			1.26		0.0898		1.00		0.8695	
V 1.17		0.746			1.61		0.799		1.01		0.757	
S.E. 0.313		0.326			0.480		0.298		0.380		0.262	

***Mean of three different experiments**

TABLE 5 : Determination of tramal capsule using methods (A-F)

Method A		Method B		Method C			Method D		Method E		Method F	
Taken ($\mu g\ ml^{-1}$)	Recovery %	Taken ($\mu g\ ml^{-1}$)	Recovery %	Taken (mg)	Found (mg)	Recovery %	Taken ($\mu g\ ml^{-1}$)	Recovery %	Taken ($\mu g\ ml^{-1}$)	Recovery %	Take ($\mu g\ ml^{-1}$)	Recovery %
80 -	100.78	40	99.41	2	1.99	99.50	2.50	99.20	1.25	101.00	5 -	99.11
80	98.44	60	100.83	4	4.04	101.00	5.00	100.00	2.50	101.75	6	100.37
160	98.05	100	99.11	6	5.92	98.67	7.50	98.67	3.75	99.50	8	101.94
200	99.38	120	99.53	8	8.02	100.25	10.00	101.20	5.00	100.25	9	100.25
240	101.30	140	98.67	10	9.89	98.90	12.50	99.20	6.25	101.80	10	101.11
400	98.59	200	100.98	12	11.99	99.92	20.00	98.80	7.50	101.75	11	99.79
480	98.83								10.00	99.81	14	101.90
560	98.44											
Mean* 99.00 (p = 0.05)		99.76			99.71		99.51		100.84		100.89 ± 0.902	
N 7		6			6		6		7		6	
S.D. 1.09		0.938			0.870		0.949		0.983		0.902	
V 1.19		0.881			0.757		0.900		0.967		0.813	
S.E. 0.413		0.383			0.355		0.387		0.401		0.368	

***Mean of three different experiments**

isolated, they gave the same peaks but with low intensity, in addition to the appearance of peak at 2071.17 or 2036.46 due to the presence of C=N group in the products (Figure 18-20).

Quantification, accuracy and precision

The proposed methods were tested for linearity, accuracy and precision.

Linear regression equations were obtained over the

TABLE 6 : Statistical data for the determination of tramadol HCl using method (A-F) compared with official method[2]

Item	Official method	Method A	Method B	Method C	Method D	Method E	Method F
Mean ± S.D. (p= 0.05)	100.66 ± 0.989	100.07± 1.083	99.86 ± 0.863	100.10 ± 1.27	99.56 ± 0.894	100.11 ± 1.01	100.05 ± 0.8699
N	3	12	7	7	9	7	11
S.D.	0.989	1.083	0.863	1.27	0.894	1.01	0.8699
R.S.D	0.982	1.082	0.864	1.26	0.898	1.00	0.8695
V	0.979	1.17	0.746	1.61	0.799	1.01	0.757
t	-	0.856 2.160)*	1.29 (2.306)*	0.671 2.306)*	1.81 (2.228)*	0.796 (2.306)*	1.051 (2.179)*
F	-	1.195 (3.98)*	1.31 (5.14)*	1.64 (5.14)*	1.22 (4.46)*	1.03 (5.14)*	1.293 (4.10)*

***Theoretical values of t and F at p = 0.05**

concentration range given in (TABLE 3).

Precision and accuracy of the proposed methods was determined. The analytical results obtained are listed in (TABLE 4). The proposed methods were applied for determination of the selected drugs in their pharmaceutical formulations (TABLE 5). Results obtained were compared with the official[2a] method, the calculated t- and F- values did not exceed the theoretical values (95% confidence limit), and so we conclude that the proposed method doesn't differ significantly from the official one, (TABLE 6).

CONCLUSION

The data given above reveal that the proposed methods introduce new techniques for the determination of tramadol a rather simple, accurate and sensitive ones with good precision and accuracy. With these methods, one can do the analysis in a short time at low cost without losing accuracy. The proposed methods can be used as alternative methods to reported ones for the routine determination of tramadol in the pure form and in pharmaceutical formulations.

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