CI&CEQ

Available on line at
Association of the Chemical Engineers of Serbia *AChE*
www.ache.org.rs/CICEQ

Chem. Ind. Chem. Eng. Q. 17 (3) 269–282 (2011) CI&CEQ

SARA M. ANIS
MERVAT M. HOSNY
HISHAM E. ABDELLATEF
MOHAMED N. EL-BALKINY

Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

SCIENTIFIC PAPER

UDC 661'8038:546:543.422.3

DOI 10.2298/CICEQ101206012A

SPECTROPHOTOMETRIC, ATOMIC ABSORPTION AND CONDUCTOMETRIC ANALYSIS OF TRAMADOL HYDROCHLORIDE

Six simple and sensitive spectroscopic and conductometric procedures (A-F) were developed for the determination of tramadol hydrochloride. Methods A, B and C are based on the reaction of cobalt (II) thiocyanate with tramadol to form a stable ternary complex, which could be measured by spectrophotometric (method A), atomic absorption (method B) or conductometric (method C) procedures. Methods D and E depend on the reaction of molybdenum thiocyanate with tramadol to form a 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 and 40--220 μg ml^{-1}, 1-15 mg ml^{-1} and 2.5-22.5, 1.25-11.25 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.

Key words: cobalt thiocyanate; molybdenum thiocyanate; bromothymol blue; spectrophotometry; conductometry.

Tramadol ((±)-*trans*-2-(dimethylaminomethyl)-1--(3-methoxyphenyl)-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 [2] specifies non--aqueous titration technique detecting the end point potentiometrically for its determination. The literature reveals several methods for the determination of the mentioned drug in biological fluids and in pharmaceutical preparations. Among these methods are spectrophotometry [3-5], HPLC [6-8], GC [9], LC-MS/MS [10], capillary electrophoresis [11], voltammetry [12] and potentiometry [13-18].

An inspection of the previous methods for the determination of the cited drug revealed that only a few spectrophotometric ones have been reported. Although atomic absorption spectrometry (AAS) is a rapid technique and has a low detection limit, it has not been yet applied to the determination of tramadol, the same case being with the conductometric procedures which proved to be simple, sensitive, reliable, very convenient 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 625 nm 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.

Correspondening author: H.E. Abdellatef, Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.
E-mail: ezzat_hisham@yahoo.com
Paper received: 6 December, 2010
Paper revised: 16 February, 2011
Paper accepted: 23 March, 2011

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 bi-distilled water was used. 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. Tetrathiocyanato cobalt(II) solution (**1**), prepared by dissolving 56.25 g of NH_4SCN and 13.80 g of $CoCl_2{\cdot}6H_2O$ in water to give 100 ml of the solution [19]. 2.5×10^{-2} M tetrathiocyanato cobalt(II) solution (**2**) prepared by dissolving 0.025 mol of cobalt, as $CoCl_2{\cdot}6H_2O$ and the required amount of ammonium thiocyanate (0.1 mol), in 100 ml bidistilled water. Sodium molybdate (Fluka AG, Switzerland), 10^{-2} M solution, prepared by dissolving 0.2419 gm in 100 ml bi-distilled water. Ascorbic acid (El-Nasr pharm. Chem. Co., Egypt) prepared as 10% w/v solution in bi-distilled water. Ammonium thiocyanate (Belami Fine Chem., India), prepared as 10% w/v solution in bi-distilled water. Bromothymol blue (BDH Chemicals Ltd., Poole, England) was prepared as 0.05% solution in bidistilled water. Acetate buffer, pH 3.7 was prepared by dissolving 10 g of anhydrous sodium acetate in 300 ml water, adjusting pH to 3.7 with glacial acetic acid and diluting to 1000 ml with water. If necessary, pH was readjusted to the value of 3.7 with glacial acetic acid or anhydrous sodium acetate as required before use [2].

Standard drug solutions

Aqueous solution of 0.1 and 4 mg ml^{-1} tramadol hydrochloride was 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, extract the aqueous solution with an equal volume of methylene chloride and shake for 45 s, then 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 performed the same as for the spectrophotometric method as far as “complete to 10 ml with methylene chloride”. The organic layer was

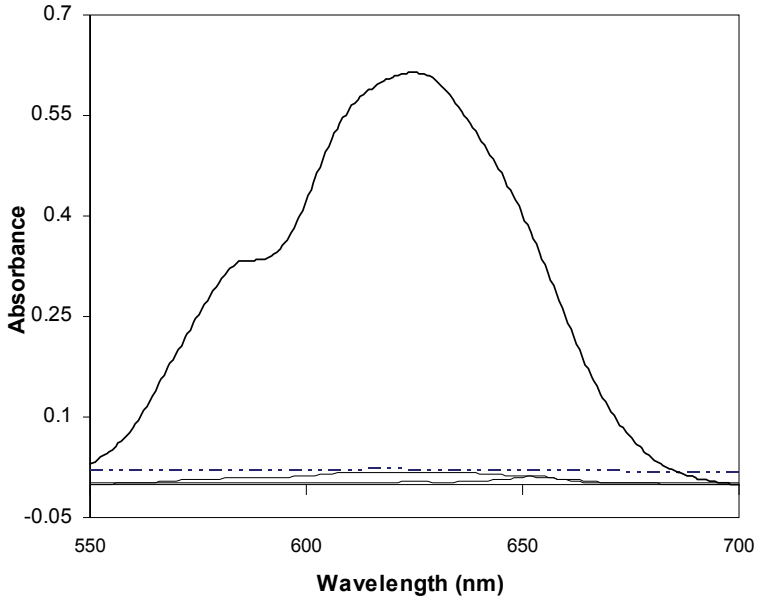

Figure 1. Absorption Spectra of the ternary complex formed through reaction of: 400 µg ml^{-1} tramadol HCl with cobalt thiocyante (—), 400 µg ml^{-1} tramadol HCl with cobalt chloride (--), 400 µg ml^{-1} tramadol HCl with ammonium thiocyanate (·····) and blank solution (-·-).

evaporated to dryness, the residue was dissolved in 1 ml 1N HCl and the volume was completed to 10 ml with bidistilled water. Aspirate in a suitable atomic absorption spectrometer under the following conditions: analysis line wavelength: 2407 Å, lamp current: 9 mA, slit width: 1.9 Å, burner height: 6 mm, burner slot/ /flame: 10 cm (air-C_2H_2), support gas flow: 10 l min^{-1}, fuel gas flow: 2.5 l min^{-1} and absorption sensitivity: 0.16 ppm.

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 bi-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 effects [20] 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, v_1 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 end-point (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 for 10 min 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 min, extract the formed complex with an equal volume of methylene chloride and shake for 75 s, 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 performed the same as for the 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. Aspirate in a suitable atomic absorption spectrometer under the following conditions: analysis line wavelength: 313.3 nm, lamp current: 9 mA, slit width: 3.8 Å, 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} and absorption sensitivity: 0.77 ppm.

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

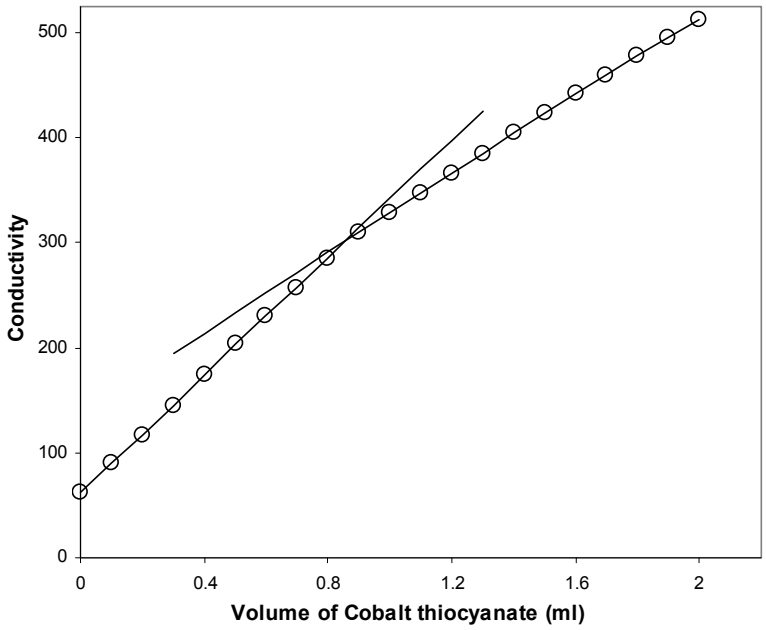

Figure 2. Conductometric titration curve of 13 mg tramadol vs. 2.5×10^{-2} M cobalt thiocyanate.

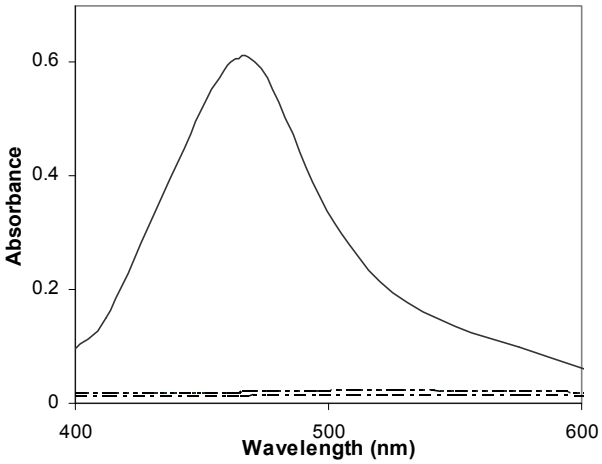

Figure 3. Absorption spectra of the complex formed through reaction of: 20 μg ml^{-1} tramadol HCl with molybdenum thiocyante (—), 20 μg ml^{-1} tramadol HCl with sodium molybdate (--), 20 μg ml^{-1} tramadol HCl with ammonium thiocyanate(·····) and blank solution (-··-).

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 s, 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 by shaking with 50 ml bi-distilled water, filtered, transferred to a 100 ml volumetric flask, completed to the mark using bi-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 was carried out [21].

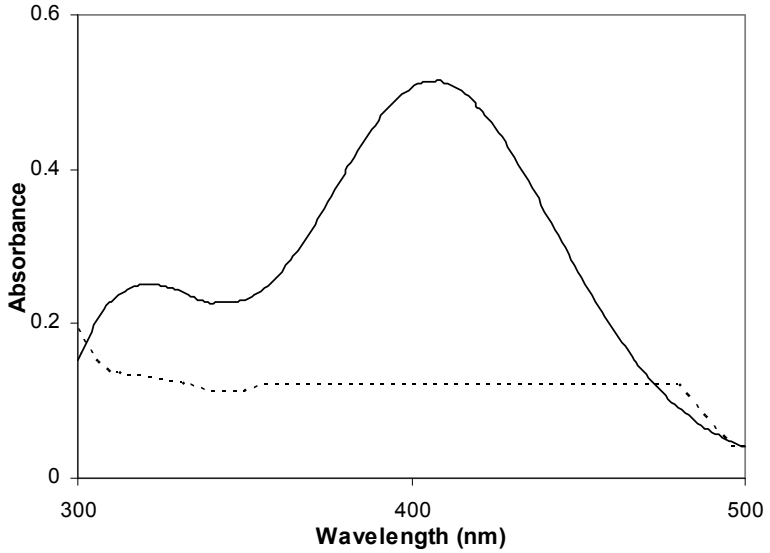

Figure 4. Absorption spectra of the ion pair formed through reaction of: 14 μg ml^{-1} tramadol HCl with 0.05 % bromothymol blue (—) and blank solution (·····).

RESULTS AND DISCUSSION

Spectrophotometric procedures using cobalt thiocyanate (method A)

Ternary complexes have been widely used in spectrophotometric analysis of many pharmaceutical compounds [22-24]. In this work, the formed ternary complex consists of the studied drug tramadol hydrochloride as the main ligand, thiocyanate as the 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 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 s 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 solvent as it gave the best results and the results were stable.

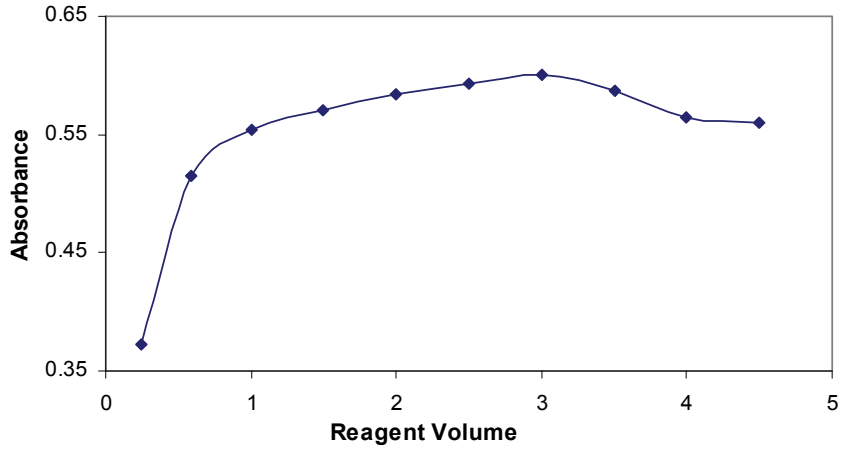

Figure 5. Effect of volume of cobalt thiocyanate (solution 1) on the reaction of cobalt thiocyanate with 400 μg ml^{-1} tramadol.

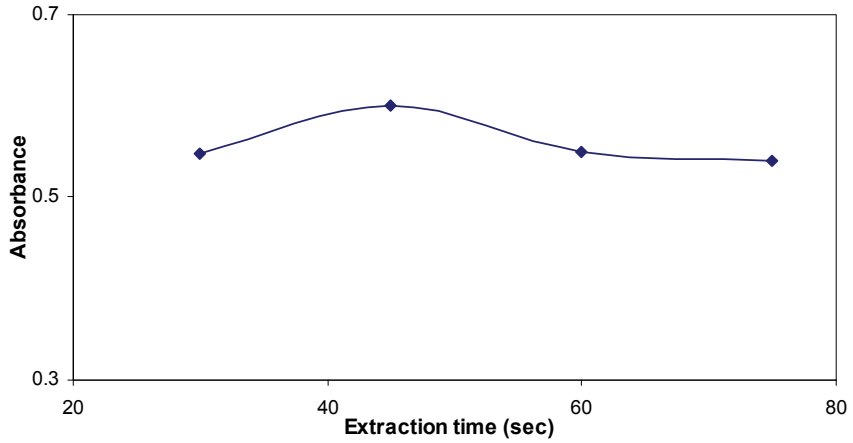

Figure 6. Effect of extraction time on the reaction of cobalt thiocyanate with 400 μg ml^{-1} tramadol.

Effect of aqueous to organic phase ratio

Varying the ratio from 2:1 to 1:2 did not cause any reasonable change in the results so an 1:1 ratio was 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, because the high chlorine/carbon ratio would lead to the formation of a large quantity of HCl in the flame, which would damage the instrument [25--26]. It was better to extract the ternary complex with an 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 and adherence to Beer's law 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 were the same as in the extractive spectrophotometric procedures and incorporated into the general procedures.

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: *i*) aqueous drug solution with aqueous reagent solution, *ii*) ethanol drug solution with ethanol reagent solution, *iii*) drug solution with reagent solution, both in ethanol--water (50%, v/v) mixture, *iv*) methanol drug solution with methanol reagent solution, *v*) drug solution with reagent solution, both in methanol-water (50%, v/v) mixture, *vi*) acetone drug solution with acetone reagent solution and *vii*) 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 a turbid solution was formed which, causing some errors.

Reagent's concentration. Different concentrations of cobalt thiocyanate solution were tried ranging from 2×10^{-1} to 5×10^{-3} M 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 lower than these limits led to unstable readings and more time was needed to obtain constant conductance values.

The 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. This increase of the conductance is due to the mobility of Cl^-, 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 Cl^-, 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 equation:

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

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 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:

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

Upon addition of the investigated drug solution, an orange red complex is formed and extractable with methylene chloride, and had an absorption maximum at 467 nm against a reagent blank.

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.

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. Through this study 0.5 ml of 10% ascorbic acid was found to be the most convenient concentration.

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 7). The data shows that 2 ml of 0.01 M sodium molybdate was required for maximum absorbance.

Effect of ammonium thiocyanate concentration. 0.5 ml of 10% ammonium thiocyanate solution was required to obtain maximum absorbance for the formed complex (Figure 8).

Effect of standing time. Mo (V) thiocyanate binary complex was stable after 10 min, while Mo (V) thiocyanate drug ternary complex needed another 5 min for its complete formation (Figures 9 and 10).

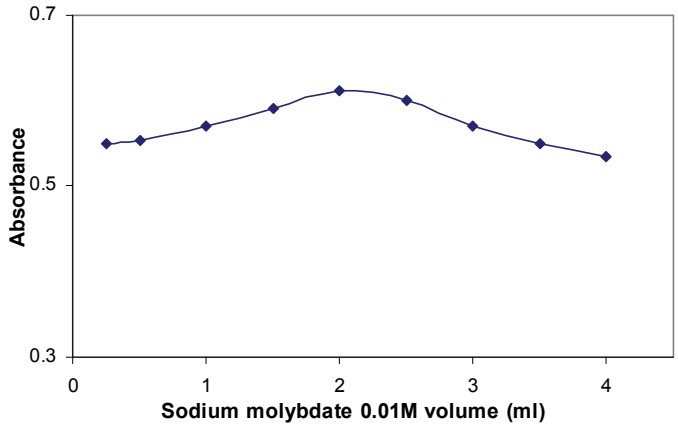

Figure 7. Effect of volume of 0.01 M sodium molybdate on the reaction of molybdenum thiocyanate with 20 μg ml^{-1} tramadol.

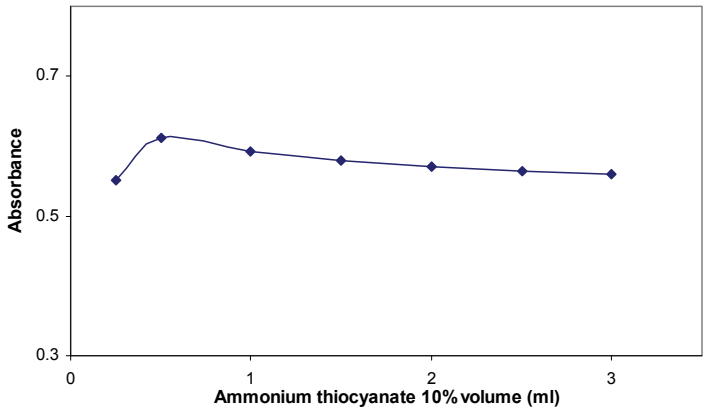

Figure 8. Effect of volume of 10% ammonium thiocyanate on the reaction of molybdenum thiocyanate with 20 μg ml^{-1} tramadol.

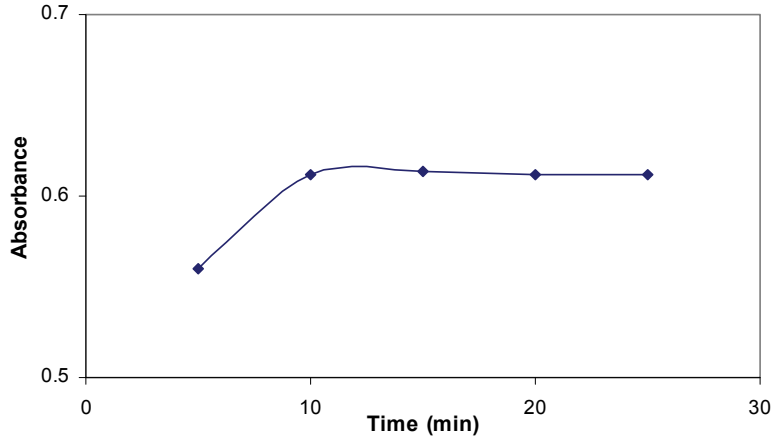

Figure 9. Effect of standing time before addition of the drug to Mo(V) thiocyanate complex on the reaction of molybdenum thiocyanate with 20 μg ml^{-1} tramadol.

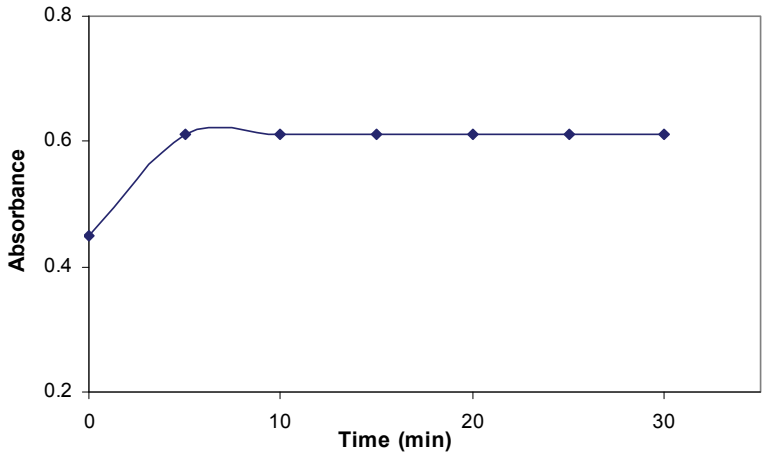

Figure 10. Effect of standing time after addition of the drug to Mo(V) thiocyanate complex on the reaction of molybdenum thiocyanate with 20 μg ml^{-1} tramadol.

Effect of extraction time and times of extractions. It was found that a single extraction of the ternary complex for 75 s 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 did not cause any reasonable change in the results so an 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 was investigated here. The spectra of the reaction products show characteristic λ_{max} 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 11).
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.
Effect of extraction time and times of extractions. It was found that a single extraction of the ternary complex for 30 s was sufficient for quantitative extraction.

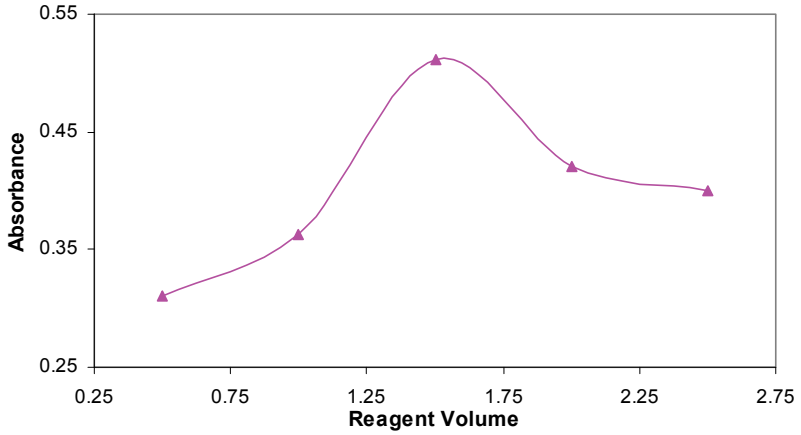

Figure 11. Effect of volume of bromothymol blue on the reaction of bromothymol blue with 14 μg ml^{-1} tramadol.

Effect of organic solvent type. Chloroform, methylene chloride, diethyl ether and benzene were all tried. Methylene chloride was found to be most convenient solvent for dissolving the formed precipitate other solvents are not suitable owing to the limited solubility of the precipitate in these solvents.

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 (Figures 12-14).

IR Charts

The ternary complexes formed from applying procedures 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 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 (Figures 15-17).

Methods validation

Under the experimental conditions described above the optical characteristics such as Beer's law limits, Sandell's sensitivity and molar absorptivity [27] were calculated for the proposed methods and the results are summarized in Table 1.

Regression equations, intercepts, slopes and correlation coefficients for the calibration data are presented also in the same table while standard deviation, relative standard deviation and standard error are summarized in Tables 2 and 3.

The percent recoveries of the pure drug using the proposed methods compared with that given by

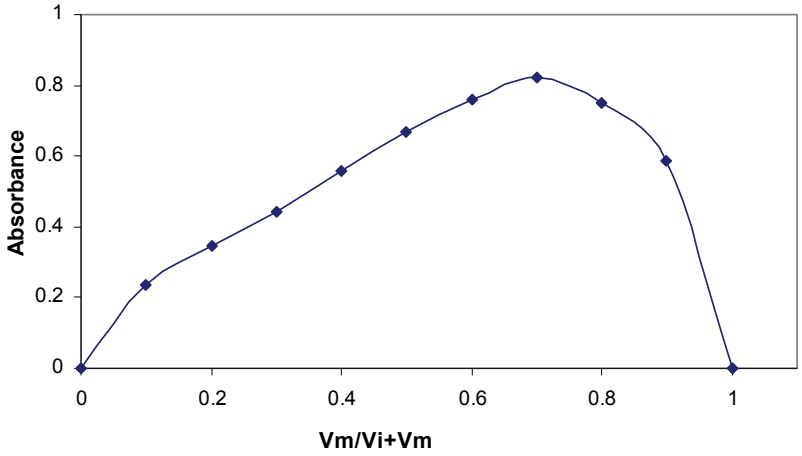

Figure 12. Determination of the stoichiometry of the reaction of: tramadol (2.5×10^{-2} M) and $CoCl_2$ (2.5×10^{-2} M) in presence of NH_4SCN (3 M).

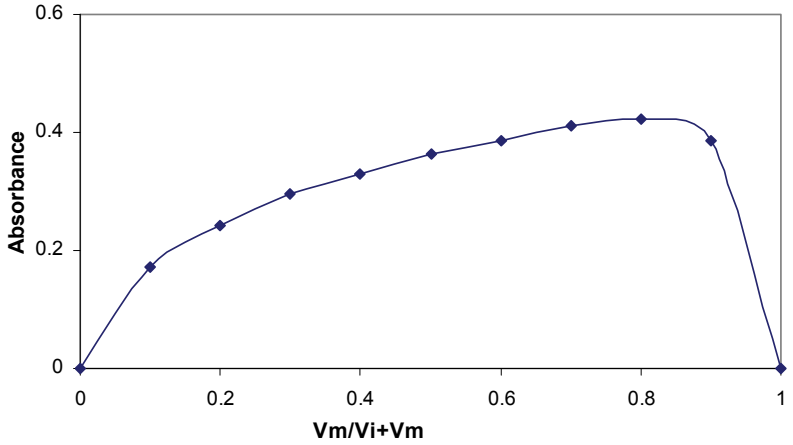

Figure 13. Determination of the stoichiometry of the reaction of: tramadol (1×10^{-3}M) and sodium molybdate (1×10^{-3} M) in presence of 10% ammonium thiocyanate.

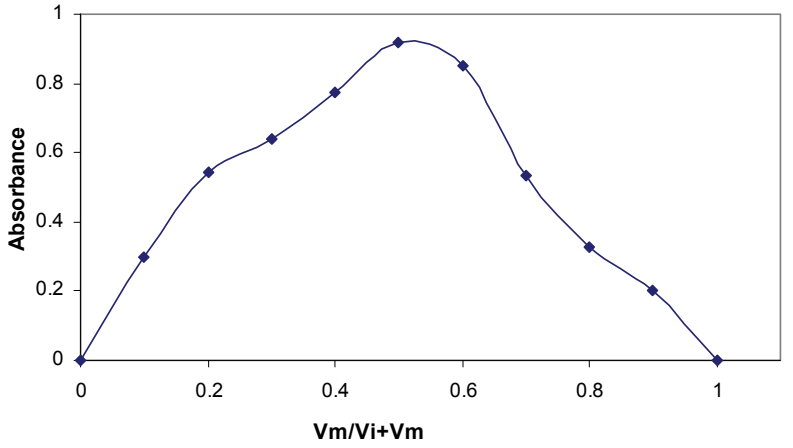

Figure 14. Determination of the stoichiometry of the reaction of: tramadol (2.5×10^{-4}M) and bromothymol blue (2.5×10^{-4} M).

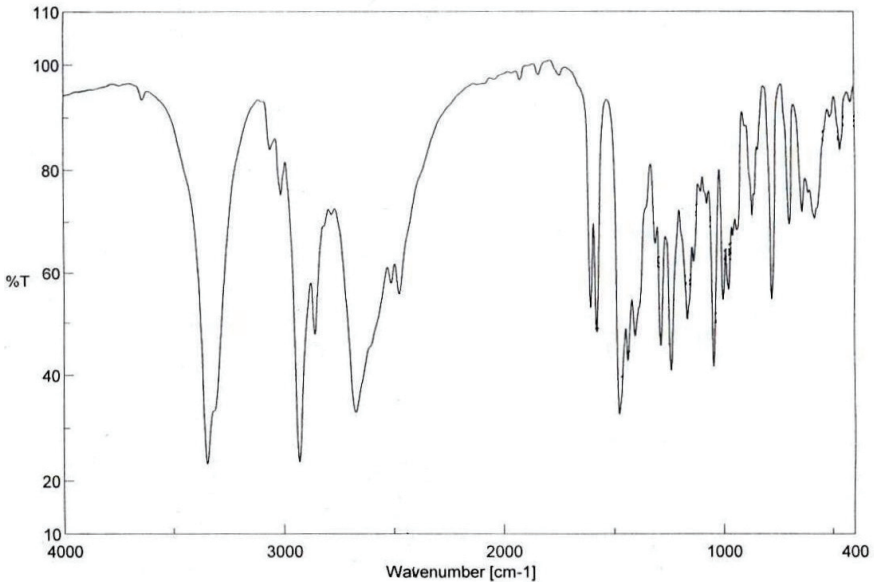

Figure 15. IR of tramadol hydrochloride.

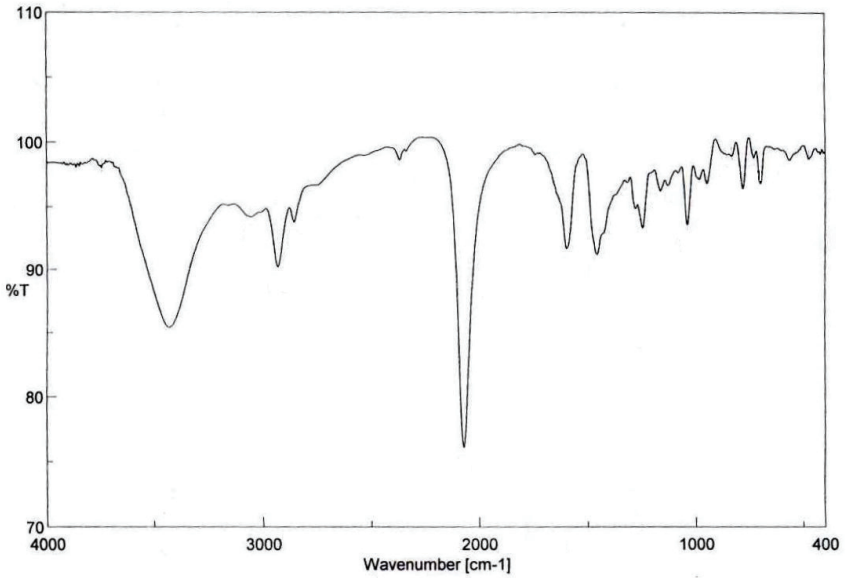

Figure 16. IR of tramadol Co (II) thiocyanate complex.

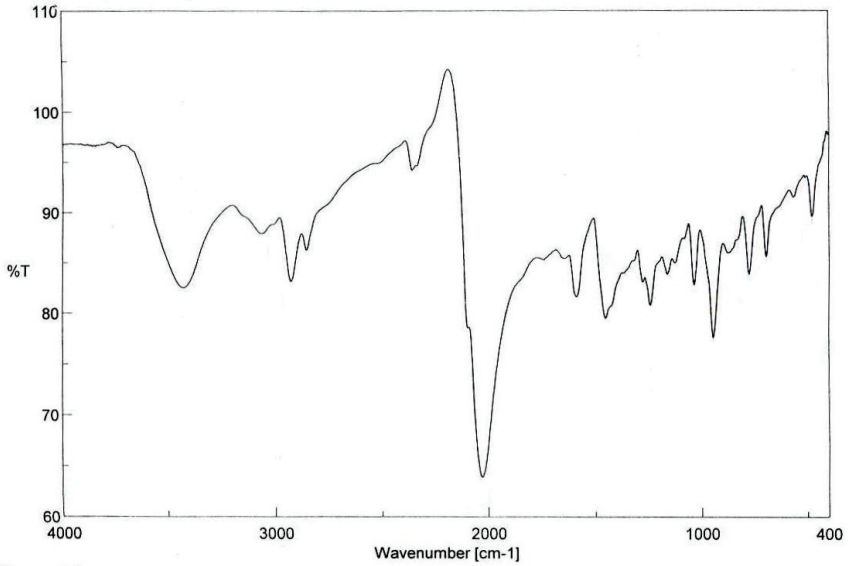

Figure 17. IR of tramadol Mo (V) thiocyanate complex

the official method 2 are illustrated in Table 4. The official method recomonded non-aqueous titration technique by detecting the end point potentiometrically.

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

Linear regression equations were obtained over the concentration range given in Table 1.

The validity of the proposed methods was evaluated by statistical analysis [28] between the results obtained and that of the official one. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Table 4), there is no significant difference between the proposed methods and the official one regarding accuracy and precision, The calculated standard deviation, relative standard deviation, standard error for the proposed methods showed that the precision was good.

Limit of detection (*LOD*) and limit of quantification (*LOQ*) indicate the sensitivity of the method. *LOD* is the lowest detectable concentration of the analyte by the method, while *LOQ* is the minimum quantifiable concentration. The results (Table 1) indicate that the proposed methods are highly sensitive. These methods were successfully applied to determine tramadol HCl in its commercial pharmaceutical preparations using standard addition technique (Table 3) without interference of commonly used excipients and additives.

Table 1. Spectral data for determination of tramadol HCl using methods A, B, D, E and F

Item	Method				
	A	B	D	E	F
Linearity range, μg ml^{-1}	80-560	40-220	2.5-22.5	1.25-11.25	5-22
Apparent molar absorptivity[a], 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				
Intercept (*a*)	-3.5×10^{-2}	12.18	1.15×10^{-1}	1.198	1.2×10^{-1}
Slope (*b*)	1.6×10^{-3}	2.46	2.5×10^{-2}	1.6×10^{-1}	4.5×10^{-2}
Correlation coefficient (*r*)	0.9997	0.9997	0.9999	0.9999	0.9998
Variance	1.17	0.746	0.799	1.01	0.757
LOD, μg ml^{-1}	0.938	0.979	0.895	1.14	0.787
LOQ, μg ml^{-1}	3.13	3.26	2.98	3.80	2.62

[a]Calculated on the basis of the molecular weight of the drug

Table 2. Determination of tramadol HCl using methods A-F

Method A		Method B		Method C			Method D		Method D		Method F	
Taken μg ml^{-1}	Recovery %	Taken μg ml^{-1}	Recovery %	Taken mg	Found mg	Recovery %	Taken μg ml^{-1}	Recovery %	Taken μg ml^{-1}	Recovery %	Taken μg 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[a], (p = 0.05): 100.07		99.867		100.107			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	

[a]Mean of three different experiments

Table 3. Determination of tramal capsule using methods A-F

Method A		Method B		Method C			Method D		Method E		Method F	
Taken/ /added μg ml^{-1}	Recovery %	Taken μg ml^{-1}	Recovery %	Taken mg	Found mg	Recovery %	Taken μg ml^{-1}	Recovery %	Taken μg ml^{-1}	Recovery %	Taken/ /added μg 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
80/80	98.44	60	100.83	4	4.04	101.00	5.00	100.00	2.50	101.75	5/6	100.37
80/160	98.05	100	99.11	6	5.92	98.67	7.50	98.67	3.75	99.50	5/8	101.94
80/200	99.38	120	99.53	8	8.02	100.25	10.00	101.20	5.00	100.25	5/9	100.25
80/240	101.30	140	98.67	10	9.89	98.90	12.50	99.20	6.25	101.80	5/10	101.11
80/400	98.59	200	100.98	12	11.99	99.92	20.00	98.80	7.50	101.75	5/11	99.79
80/480	98.83								10.00	99.81	5/14	101.90
80/560	98.44											

Table 3. Continued

Method A	Method B	Method C	Method D	Method E	Method F
Mean[a] (p = 0.05)					
99.00	99.76	99.71	99.51	100.84	100.89
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

[a]Mean of three different experiments

Table 4. Statistical data for the determination of tramadol HCl using methods 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^a	-	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^a	-	1.195 (3.98)	1.31 (5.14)	1.64 (5.14)	1.22 (4.46)	1.03 (5.14)	1.293 (4.10)

[a]Theoretical values of t and F at p = 0.05

When comparing the 6 methods it was found that methods (D and E), which depends on the reaction of molybdenum thiocyanate with tramadol, are the most sensitive methods in this work.

CONCLUSION

The data presented above reveal that the proposed methods introduce new techniques for the determination of tramadol. The proposed methods are 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.

REFERENCES

[1] S.C. Sweetman, Martindale, The Complete Drug Reference, 33rd ed., Pharmaceutical Press, London, 2002, p. 89

[2] British Pharmacopoeia, Stationary office, Her Majesty's Stationary Office, London (2003), p.1868 , p A136-A137

[3] H.E. Abdellatef, J. Pharm. Biomed. Anal. **32** (2002) 835-842

[4] K.K. Srinivasan, J. Alex, A.A. Shirwaikar, S. Jacob, M.R. Sunil Kumar, S.L. Prabu, Ind. J. Pharm. Sci. **69** (2007) 540-545

[5] H.E. Abdellatef, M.M. El-Henawee, H. M. El-Sayed, M. M. Ayad, Spectrochim. Acta A Mol. Biomol. Spectrosc. **65** (2006) 1087-1092

[6] R.S. Pedersen, K. Broesen, F. Nielsen, Chromatographia **57**(2003) 279-285

[7] S. H. Gan, R. Ismail, J Chromatogr. B Biomed. Sci. Appl. **759** (2001) 325-335

[8] A. Curticapean, D. Muntean, M. Curticapean ,M. Dogaru, C. Vari, J. Biochem. Biophys. Methods **70** (2008) 1304-1312

[9] A. Valli, A.Polettini, P. Papa, M. Montagna, Ther. Drug Monit. **23** (2001) 287-294

[10] B.N. Patel, N. Sharma, M. Sanyal, P.S. Shrivastav, J. Pharm. Biomed. Anal. **49** (2009) 354-366

[11] W.D. Cao, J.F. Liu, H.B. Qiu, X.R Yang, E.K. Wang, Electroanalysis **14** (2002) 1571-1576

[12] E.M.P.J. Garrido, J.M.P.J. Garrido, F. Borges, C. Delerue-Matos, J. Pharm. Biomed. Anal. **32** (2003) 975-981

[13] J.X. Feng, X.Q. Wang, L.P. Wang, Y.C. Zhang, Fenxi Huaxue **29** (2001) 1487-1491 (in Chinese)

[14] G.A. Zhong, Phys. Test. Chem. Anal., B **39** (2003) 59-60

[15] L.C. Huang, R. Xiu, Y.Z. Liu, C.Y. Li, J.C. Meng, Fenxi Kexue Xuebao **21** (2005) 48-50 (in Chinese)

[16] Y.J. Wu, J. Shi, H.J. Zhang, L.K. Zhao, P.Z. Zhang, Fenxi Shiyanshi **24** (2005) 49-51 (in Chinese)

[17] H.M. Abu-Shawish, N. Abu Ghalwa, F.R. Zaggout, S.M. Saadeh, A.R. Al-Dalou, A.A. Abou Assi, Biochem. Engin. J. **48** (2010) 237-245

[18] H.M. Abu-Shawish, S.M. Saadeh, A.R. Al-Dalou, N. Abu Ghalwa, A.A. Abou Assi, Mater. Sci. Eng. C **31** (2011) 300-306

[19] M.M. Ayad, H. M. Khalil, M. El-Henawee, M. Hosny, Sci. Pharm. **67** (1999) 241-252

[20] J. Lingane, Electroanalytical Chemistry, 2nd Ed., Interscience, New York (1958), p. 90

[21] J. Rose, Advanced Physio-Chemical Experiments, Pitman, London (1964), p. 54

[22] H.E. Abdellatef, Spectrochim. Acta, A **66** (2007) 1248-1254

[23] M.M. Ayad, A.A Shalaby, H.E. Abdellatef, M.M. Hosny, J. Pharm. Biomed. Anal. **28** (2002) 311-321

[24] A.M. El-Walily, S.F. Belal, R.S. Bakry, J. Pharm. Biomed. Anal. **14** (1996) 561-569

[25] N. Cristina, G. Agustin, Anal. Chem. **57** (1985) 34-38

[26] C. Nerin, J. Cacho, A. Garania, Anal. Lett. **18** (1985) 1887-1891

[27] H. Zavis, D. Ludvik, K. Milan, S. Ladislaw, V. Frantisck, Handbook of Organic Reagents in Inorganic Analysis. Translated by Stanislav, K, Dr. Chalmers (The Series and Translation Editor: University of Aberdem, Ellis Horwood Limited, Chichester), A Division of John Wiley & Sons IC, New York, London, Sydney, Toronto, 1976, p. 364

[28] R. Caulcut, R. Boddy, Statistics for Analytical Chemists, Chapmann and Hall, London, 1983.

SARA M. ANIS
MERVAT M. HOSNY
HISHAM E. ABDELLATEF
MOHAMED N. EL-BALKINY

Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

NAUČNI RAD

SPEKTROFOTOMETRIJSKA, ATOMSKO-APSORPCIONA I KONDUKTOMETRIJSKA ANALIZA TRAMADOL HIDROHLORIDA

Razvijeno je šest jednostavnih i osetljivih spektroskopskih i konduktometrijskih procedura (A-F) za određivanje tramadol-hidrohlorida. Metode A, B i C su zasnovane na reakciji kobalt(II)-tiocijanata sa tramadolom u kojoj nastaje stabilni ternarni kompleks koji se može odrediti spektrofotometrijom (metoda A), atomsko-apsorpcionom spektrofotometrijom (metoda B) i konduktometrijom (metoda C). Metode D i E zavise od reakcije molibden-tiocijanata sa tramadolom u kojoj nastaje stabilni ternarni kompleks, koji se određuje spektrofotometrijom (metoda D) i atomsko-apsorpcionom spektrofotometrijom (metoda E), dok metoda F zavisi od formiranja kompleksa jonskog para između proučavanog leka i bromtimol plavog koji se ekstrahuje metilen-hloridom. Tramadol-hidrohlorid se može analizirati u sledećim opsezima koncentracije: 80-560, 40-220 μg ml^{-1}, 1-15 mg ml^{-1}, 2.5-22,5, 1,25-11,25 i 5-22 μg ml^{-1} koristeći metode A, B, C, D, E i F, respektivno. Ispitivanja su izvršena u različitim uslovima, a dobijeni rezultati ukazuju na dobre vrednosti % prinosa. Sve metode su uspešno primenjene za analizu tramadola u farmaceutskim preparatima, a dobijeni rezultati se dobro slažu sa standardnom oficijelnom metodom.

Ključne reči: kobalt(II)-tiocijanat, molibden-tiocijanat, bromtimol plavo, spektrofotometrija, konduktometrija.