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Kinetic spectrophotometric determination of tramadol hydrochloride in pharmaceutical formulation

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Abstract

Two simple and sensitive kinetic methods for the determination of tramadol hydrochloride are described. The first method is based upon a kinetic investigation of the oxidation reaction of the drug with alkaline potassium permanganate at room temperature for a fixed time at 20 min. The absorbance of the colored manganate ions was measured at 610 nm. The second method is based on the reaction of tramadol hydrochloride with 4-chloro-7-nitrobenzofurazan (NBD–Cl) in presence of 0.1 M sodium bicarbonate. The spectrophotometric measurements were recorded by measuring the absorbance at 467 nm, at fixed time at 25 min on thermostated water bath at 90 ± 1 °C. All variables affecting the development of the colour have been investigated and the conditions were optimised. The absorbance concentration plots in both methods were rectilinear over the range 5–25 and 50–250 μg ml^{-1}, for the first and second methods, respectively. The two methods have been applied successfully to commercial capsule and ampoule dosage form. The results obtained are compared statistically with those given by the reference spectrophotometric method. The determination of tramadol hydrochloride by the fixed concentration and rate constant methods is feasible with the calibration equations obtained, but the fixed time method proves to be more applicable.

Keywords: Kinetic determination; Tramadol hydrochloride; Potassium permanganate; 4-Chloro-7-nitrobenzofurazan (NBD-Cl)

1. Introduction

Tramadol [(±) trans-2-(dimethylaminomethyl-1-(3-methoxy-phenyl)-cyclohexanol hydrochloride] is a centrally acting analgesic-anodyne agent of high oral bioavailability. As a relatively new drug it is not yet included in internationally recognised pharmacopoeias through various mass—produced dosage forms containing tramadol are available on the market. The tramadol contains a weakly absorbing chromophore in its molecule and it was determined by HPLC with UV detection [1–3] or fluorescence detection [4] in pharmaceutical [1], urine [2] or blood plasma [3,4]. Gas chromatography with nitrogen—selective detector [5] liquid chromatography—mass spectrometry [6] capillary electrophoresis [7–9] or UV-spectrophotometry [10] were also used for determining tramadol. To my best knowledge, no

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attempts have yet been made to determine tramadol by any colorimetric method.

The literature are still poor in analytical procedures based on kinetic. Furthermore, some specific advantages in the application of kinetic methods can be expected [11]:

(a) Selectivity due to the measurement of the evolution of the absorbance with the time of reaction instead of the measure of a concrete absorbance value;
(b) Possibility of no interference of the coloured and/or turbidity background of the samples, and…
(c) Possibility of no interference of the other active compounds present in the commercial product if they are resisting the chemical reaction conditions established for the proposed kinetic method.

In the present work, kinetically based methods are proposed for the determination of tramadol hydrochloride by measuring the absorbance at 610 nm after oxidation reaction with alkaline $KMnO_4$ or at 467 nm after addition of NBD-Cl. Although, the poor selectivity of the proposed methods especially the oxidation with $KMnO_4$, they are simpler than the time consuming HPLC methods and are more sensitive than the UV-spectrophotometric method [10]. The aim of the present work was the development of simple and sensitive analytical methods for the determination of tramadol hydrochloride in pharmaceutical capsules and ampoules.

2. Experimental

2.1. Apparatus

Shimadzu 1601 UV recording spectrophotometric with 1 cm matched glass cells.

2.2. Materials and reagents

The following reagents were used;

1. Potassium permanganate (Merck, Germany), 5×10^{-3} M aqueous solution;
2. Sodium hydroxide (El-Nasr Chemical Co. Cairo, Egypt), 0.5 M aqueous solution.
3. Sodium bicarbonate (El-Nasr Chemical Co.), 0.1 M aqueous solution;
4. 4-chloro-7-nitrobenzofurazan (NBD-Cl) (Aldrich), a fresh solution (0.1% w/v) in acetone was prepared daily,
5. Tramadol hydrochloride (determined by the UV-spectrophotometric method [10] and was found to be 98.62%) was kindly supplied by Minapharm Co., Cairo, Egypt. Stock solutions containing 5 mg of tramadol hydrochloride in 50 ml distilled water for method A (solution A) and 25 mg in 25 ml acetone for method B (solution B). Capsules and ampoules containing tramadol hydrochloride were obtained from commercial sources.

2.3. General procedures

2.3.1. Method A (oxidation with $KMnO_4$)

Accurately measured aliquots (solution A) equal to 50–250 μg tramadol hydrochloride were transferred into separate 10 ml volumetric flasks, after that 1 ml of 0.5 M NaOH was added followed by 2 ml of 5×10^{-3} M $KMnO_4$, the mixture was shaken well and then the evolution of the absorbance at 610 nm with time was scanned during 20 min at ambient temperature (25 °C). The tramadol concentration was determined by measuring the rate of the reaction as the tangent to kinetic curve during the first 20 min of reaction and using the appropriate graphs. Log reaction rate versus log concentration of tramadol hydrochloride was plotted to get order of the reaction after 20 min. To get the standard calibration graph, the above procedure was carried out and the reaction mixture was allowed to stand for 20 min. volume with water was added. Measure the absorbance of the resulting solution at 610 nm against a blank solution prepared simultaneously. Plot the values of the absorbance against the final concentration in μg ml^{-1}. Alternatively, the regression equation was derived.

2.3.2. Method B (coupling reaction with NBD-Cl)

Accurately measured aliquots (solution B) equal to 5–2.5 mg tramadol were transferred into separate 10 ml volumetric flasks; after that 1 ml of 0.1 M sodium bicarbonate was added followed by

1 ml of 0.1% w/v NBD-Cl, the mixture was shaken, and diluted to mark with distilled water. The flask with its contents was shaken gently and then thermostated at 90 ± 1 °C in a water bath with a stop-watch turned on. At a fixed time of 25 min, the reaction was quenched by cooling under tap water for 3 min and the absorbance was measured directly at 467 nm. the tramadol concentration was then calculated from the corresponding equation for the calibration graph for the fixed time method.

2.3.3. Procedures for pharmaceutical formulations

2.3.3.1. For capsules. The content of five capsules was emptied out as completely as possible. An accurately weighed amount of the powder equivalent to 10 or 50 mg of the drug was dissolved in 50 ml of water or acetone, for method A or B, respectively. The procedure was continued as described under general procedures.

2.3.3.2. For ampoules. The content of two ampoules was mixed and diluted to 100 ml with water or acetone, for method A or B, respectively. An accurately amount equivalent to 5 or 25 mg of the drug was further diluted with the same solvent in 25 ml volumetric flask. The procedure was continued as described under general procedures.

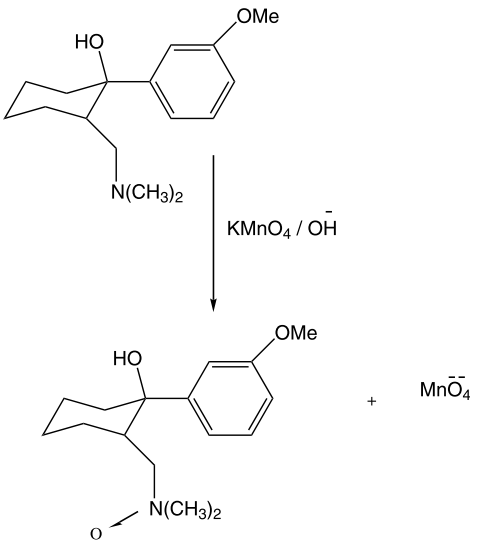
3. Results and discussion

3.1. Optimisation of the reactions conditions

3.1.1. Method A: (oxidation with $KMnO_4$)

The reaction between tramadol and $KMnO_4$ in alkaline solution yields a green colour as a result of manganate species (Scheme 1), which absorbs at 610 nm. As the intensity of colour increases with time, it was deemed useful to elaborate a kinetically based method for the determination of tramadol hydrochloride. In order to come to this conclusion, the reaction was investigated under various conditions of reagent concentration and alkalinity.

At room temperature, the reaction increased substantially with time, as revealed by the intensification of the developed colour and subsequent increase in the slop of the calibration graph (Table 1), indicating high analytical sensitivity. Therefore, room temperature was selected as the optimum temperature.



Scheme 1. Proposed reaction between tramadol and potassium permanganate in alkaline medium.

The reaction rate and maximum absorbance increased with time and with increasing $KMnO_4$

Table 1
Calibration equations at different fixed times for tramadol in the ranges 5–25 and 50–250 μg ml^{-1} applying methods A and B, respectively

Time (min)	Calibration equation	Correlation coefficient
Method A: (oxidation with $KMnO_4$)		
5	$A = 0.1088 + 0.02024C$	0.9891
10	$A = 0.1482 + 0.02608C$	0.9926
15	$A = 0.1422 + 0.02844C$	0.9991
20	$A = 0.1466 + 0.02964C$	0.9996
25	$A = 0.1650 + 0.02940C$	0.9984
Method B: (reaction with NBD-Cl)		
10	$A = -0.0101 + 0.00090C$	0.8942
15	$A = 0.0365 + 0.00157C$	0.9921
20	$A = 0.0663 + 0.00169C$	0.9988
25	$A = 0.0664 + 0.00168C$	0.9991
30	$A = 0.0712 + 0.00163C$	0.9981

C, concentration in μg ml^{-1} of the final measured solution.

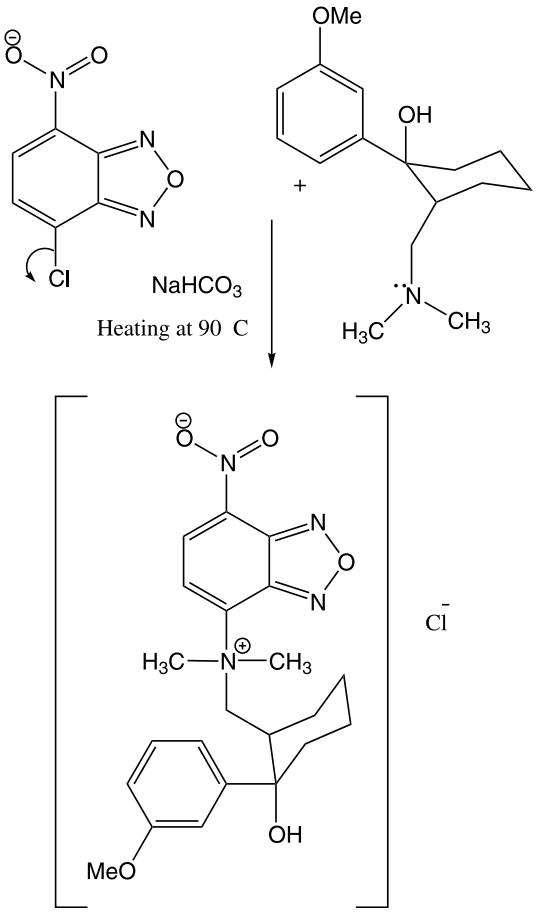
concentration. It was found that 2 ± 0.2 ml of 5×10^{-3} M $KMnO_4$ was adequate for the maximum absorbance. The influence of NaOH concentration on the reaction rate was studied between 0.1 and 1.0 M, it was found that increasing NaOH concentration increases the reaction rate with maximum absorbance being reached in shorter time. It was also observed that there was no significant difference in the absorbance of reactant solutions at NaOH concentration above 0.5 M, while decreasing NaOH concentration resulted in lower absorbance values. Therefore, 0.5 M NaOH was chosen as the most suitable concentration.

3.1.2. Method B: (coupling reaction with NBD-Cl)

The possibility of the reaction of tramadol with NBD-Cl was investigated under various conditions. It was found that the reaction proceeds only in alkaline media and at elevate temperatures. Like many alkylamine [12–15] tramadol reacts with NBD-Cl to form a yellow colored product (Scheme 2), that absorbs at 467 nm. The extent of formation of this species depends on the concentration of both reactants, alkalinity and temperature, and, therefore, the effects of these variables were studied.

The reaction rate was found to increase with increasing temperature with a subsequent increase in the slope of calibration graph (Table 1), indicating higher analytical sensitivity. Above 90 °C unwanted chemical changes might occur, so 90 °C was chose as an adequate temperature.

A volume of 1 ml of 0.1% w/v NBD-Cl solution in acetone was found to be satisfactory and was used throughout this investigation. Owing to the presence of labile chloride a daily fresh solution is recommended. To generate the nucleophile from tramadol, different bases were tried such as disodium hydrogen phosphate, sodium bicarbonate, sodium acetate and borax, all prepared as aqueous solution. Best results were obtained in case of 1 ml of 0.1 M sodium bicarbonate, where with other bases either precipitation of white colloid occurred upon addition of NBD-Cl or non reproducible results and weak sensitivity were observed.



Scheme 2. Proposed reaction between tramadol and NBD-Cl in presence of sodium bicarbonate.

3.2. Kinetics study of the reactions

The rate of the reactions was also found to be [tramadol] dependent. The rates were followed

(a) At room temperature with various concentrations of tramadol in the range of 5–25 μg ml^{-1}, keeping $KMnO_4$ and NaOH constant at high concentration as above, applying method A
(b) At 90 °C with various concentrations of tramadol in the range 50–250 μg ml^{-1}, keeping the other reactants, base and NBD-Cl constant at high concentration above, applying method B.

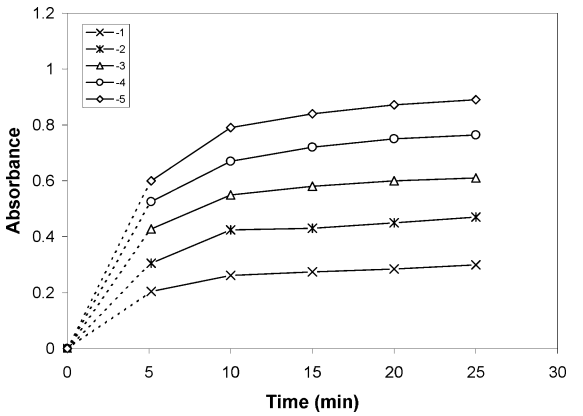


Fig. 1. Absorbance vs. time graphs for the reaction of tramadol and alkaline $KMnO_4$ (Method A) showing the dependence of the reaction on tramadol concentration. Concentration of tramadol, (1) 1.9×10^{-5}; (2) 3.8×10^{-5}; (3) 5.7×10^{-5}; (4) 7.6×10^{-5}; (5) 9.5×10^{-5}.

The graphs shown in Figs. 1 and 2, applying methods A and B, respectively, were obtained, from which it is clear that the rate increases as the tramadol concentration increases, indicating that the reactions rates obeys the following equation:

Table 2
Logarithms of the rates for different concentrations of tramadol applying methods A and B

Log (rate), $\log \Delta A/\Delta t$	Log [tramadol] (M)
Method A (oxidation with $KMnO_4$)	
−4.110	−4.72
−3.942	−4.42
−3.821	−4.24
−3.701	−4.12
−3.620	−4.02
Method B (reaction with NBD-Cl)	
−4.240	−3.72
−4.110	−3.42
−4.050	−3.24
−3.950	−3.12
−3.900	−3.02

$$\text{Rate} = K'[\text{tramadol}]^n \quad (1)$$

where K' is the pseudo-order constant of the reaction and n is the order of the reaction. The rate of the reaction may be estimated by the variable-time method measured [16] as $\Delta A/\Delta t$, where A is the absorbance and t is the time in

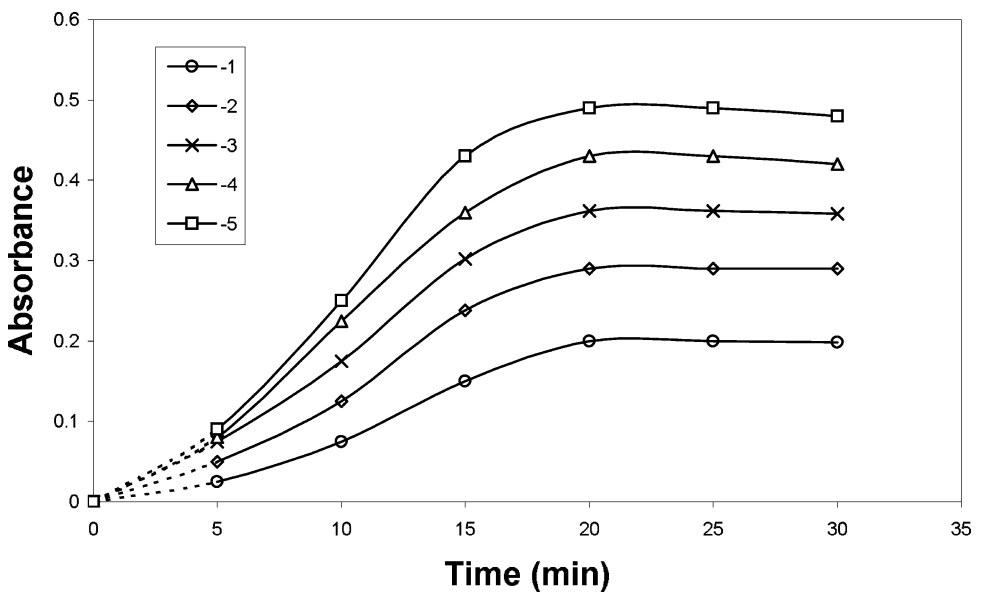


Fig. 2. Absorbance vs. time graphs for the reaction of tramadol and NBD-Cl in alkaline medium (Method B) showing the dependence of the reaction on tramadol concentration. Concentration of tramadol, (1) 1.9×10^{-4}; (2) 3.8×10^{-4}; (3) 5.7×10^{-4}; (4) 7.6×10^{-4}; (5) 9.5×10^{-4}.

seconds. Taking logarithms of rates and concentration (Table 2) (Eq. (1)) is transformed into:

$$\log(\text{rate}) = \log \frac{\Delta A}{\Delta t} = \log K' + n \log[\text{tramadol}] \quad (2)$$

Regression of log (rate) versus log (tramadol) gave the regression equation:

$$\log(\text{rate}) = -0.814 + 0.7027 \log C \quad (r = 0.9992)$$

Method A

$$\log(\text{rate}) = -2.45 + 0.4816 \log C \quad (r = 0.9974)$$

Method B

hence $K' = 0.153$ or 3.55×10^{-3} S^{-1}, applying methods A or B, respectively, and the reactions are first order with respect to tramadol concentration.

3.3. Evaluation of the kinetic methods

The quantitative of tramadol under the optimised experimental conditions outlined above would result in a pseudo-first order reaction with respect to their concentration where

(a) $KMnO_4$ concentration was at least ten times of the concentration of tramadol and NaOH concentration was at least 400 times the initial concentration of tramadol, applying method A, and
(b) NBD-Cl concentration was at least 2.5 times of the concentration of tramadol and sodium bicarbonate concentration was at least 100 times the initial concentration of tramadol, applying method B.

However, the rates will be directly proportional to tramadol concentration in a pseudo-first order rate equation as follows:

$$\text{Rate} = K'[\text{tramadol}] \quad (3)$$

where K' is the pseudo-first order constant.

Eq. (3) was the basis for several experiments, which were run to obtain tramadol concentration using the rate data. Rate constant, constant concentration and fixed-time methods [17,18] were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the correlation coefficient (r) and the intercept.

3.3.1. Rate-constant method

Graphs of log (absorbance) versus time for tramadol concentration in the range 1.9×10^{-5}–9.5×10^{-5} and 1.9×10^{-4}–9.5×10^{-4} M applying methods A or B, respectively, were plotted and all appeared to be rectilinear.

Pseudo-first order rate constants corresponding to different tramadol concentrations (C) were calculated from the slopes multiplied by -2.303 and are presented in Table 3.

Regression of (C) versus K' gave the equation:

$$K' = 2.866 \times 10^{-4} - 0.0209C \quad (r = 0.8362)$$

Method A

$$K' = -2.8 \times 10^{-4} - 1.1263C \quad (r = 0.6372)$$

Method B

The value (r) indicates poor linearity, which is probably due to inconsistency of K' as a result of slight changes due to the elevated temperature of the reaction.

3.3.2. Fixed-concentration method

Reaction rates were determined for different tramadol concentrations in the range 5.7×10^{-5}–9.5×10^{-5} and 5.7×10^{-4}–9.5×10^{-4} M applying methods A or B, respectively. A pre-selected value of the absorbance was fixed and the time was measured in seconds. The reciprocal of time

Table 3
Values of K' calculated from slops of log A vs. t graphs multiplied by -2.303 for different concentration of tramadol applying methods A and B

K' (S^{-1})	[Tramadol] (M)
Method A (oxidation with $KMnO_4$)	
-2.833×10^{-4}	1.9×10^{-5}
-3.081×10^{-4}	3.8×10^{-5}
-2.652×10^{-4}	5.7×10^{-5}
-2.877×10^{-4}	7.6×10^{-5}
-2.952×10^{-4}	9.5×10^{-5}
Method B (reaction with NBD-Cl)	
-1.294×10^{-3}	1.9×10^{-4}
-1.094×10^{-3}	3.8×10^{-4}
-9.693×10^{-4}	5.7×10^{-4}
-9.917×10^{-4}	7.6×10^{-4}
-2.716×10^{-4}	9.5×10^{-4}

Table 4
Values of reciprocal of time taken at fixed absorbance for different rates of various concentrations of tramadol applying methods A and B

$1/t$ (S^{-1})	[Tramadol] (M)
Method A (oxidation with $KmnO_4$)	
8.333×10^{-4}	5.7×10^{-5}
2.083×10^{-3}	7.6×10^{-5}
3.333×10^{-3}	9.5×10^{-5}
Method B (reaction with NBD-Cl)	
9.803×10^{-4}	5.7×10^{-4}
1.111×10^{-3}	7.6×10^{-4}
1.389×10^{-3}	9.5×10^{-4}

(i.e. $1/t$) versus the initial concentration of tramadol (Table 4) was plotted. The following equation for calibration graph was worked out by linear regression:

$$\frac{1}{t} = -2.92 \times 10^{-3} + 65.78C \quad (r = 0.9991)$$

Method A

$$\frac{1}{t} = 1.10 \times 10^{-3} + 0.0786C \quad (r = 0.9873)$$

Method B

The range of tramadol concentration giving the most acceptable calibration graph with the above equations were very limited (15–25 and 150–250 μg ml^{-1}, applying methods A and B, respectively) which could be disadvantage.

3.3.3. Fixed time method

Reaction rates were determined for different concentration of tramadol. At a pre-selected fixed time, which was accurately determined, the absorbance was measured. Calibration graphs of the absorbance versus initial concentration of tramadol were obtained at fixed times of 5, 10, 15, 20 and 25 min applying method A and fixed times of 10, 15, 20, 25 and 30 min applying method B with the calibration equation shown in Table 1

It is clear that, both the slopes and intercepts increase with time. The correlation coefficient and more reaction products (indicated by higher absorbance readings as shown in Figs. 1 and 2) were obtained for a fixed time of 20 or 25 min applying methods A and B, respectively, which was, therefore, chosen as the most suitable time interval for measurements. The detection limit [19] was 7.6×10^{-6} M (2 μg ml^{-1}) and 3.8×10^{-5} M (10 μg ml^{-1}), while the quantification limit, was 1.9×10^{-5} M (5 μg ml^{-1}) and 1.9×10^{-4} M (50 μg ml^{-1}) applying methods A and B, respectively.

3.4. Application

The fixed time method was applied to the determination of tramadol in the supplied drug formulations in tablet and ampoule forms. The concentration of tramadol was calculated using the corresponding calibration equation in Table 1 at fixed time of 20 and 25 min. applying methods A and B, respectively. The result obtained for the analysis of tramadol in drug formulations employed was compared with those obtained with the reference spectrophotometric method [10], (Table 5). The Student t-test and F-test values of 95% confidence level did not exceed the theoretical values of 2.306 and 6.39 for t-and F-tests, respectively, indicating no significant difference between the accuracy and the precision of the two methods.

Table 5
Statistical comparison of the results obtained by the fixed-time method with those obtained by the spectrophotometric reference method [10]

Pharmaceutical formulation	Recover (%) [a] ± S.D. (%)	
	Kinetic method[b]	Spectrophotometric method [10]
Tramal[c] capsules	99.87 ± 1.12	101.45 ± 1.78
t[d]		1.672 (2.306)
F[d]		2.512 (6.39)
Tramal[c] ampoules	100.16 ± 0.65	101.05 ± 0.97
t[d]		0.837 (2.306)
F[d]		2.237 (6.39)

[a] Average of five determination.
[b] Fixed-time method.
[c] Tramal capsules and ampoules labelled to contain 50 or 100 mg tramadol hydrochloride per capsule or ampoule, respectively, were kindly supplied by Minapharm Co.
[d] Theoretical values of t and F at $P = 0.05$ are 2.306 and 6.39, respectively.

4. Conclusion

The proposed methods are advantageous when compared with the only exiting spectrophotometric procedure in the literature [10] in having higher sensitivity. The data given above reveal that the proposed methods are accurate and sensitive (method A, oxidation with $KMnO_4$ > method B, coupling reaction with NBD-Cl) with good precision and accuracy. With these methods, one can do the analysis with speed at law cost without losing accuracy. The most important limitation of the proposed methods is their poor selectivity, especially for compounds of similar structure, this shortcoming does not affect the usefulness in routine analysis and content uniformity determination of tramadol as it is singly prescribed. 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 depending upon the availability of chemicals and equipment.

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