University of Benghazi
Faculty of Education Al marj
Global Libyan Journal

بنغازي جامعة
كلية التربية المـــرج
المجلة الليبية العالمية

العدد العشرون – 30/ مايو 2017

Spectrophotometric determination of some selected analgesic drugs based on complex formation reactions

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Abstract.

Accurate and precise spectrophotometric methods for the determination of four analgesic drugs namely, tramadol (TRM), morphine (MRF), nalbuphine (NLB) and naltrexone (NLT) in pharmaceutical formulations and biological fluids were developed and optimized. The proposed methods involve the addition of a measured excess of bromate-bromide mixture in acid medium and subsequent estimation of the residual bromine by reacting with a measured excess of iron (II), the remaining iron (II) is complexed with 1, 10-phenanthroline (method A) or with 2, 2' bipyridyl (method B) and measuring the increase in absorbance at 510 and 522 nm, respectively. In both methods, the amount of bromine reacted corresponds to the amount of drugs. The calibration graphs are found to be linear over 2.4 – 14.4 µg ml^{-1} and 1.6 – 12.8 µg ml^{-1} for method A and method B, respectively. Under the optimum conditions, Beer's law limit, molar absorptivities and Sandell's sensitivity are calculated. The limits of detection and quantification are also reported for both methods. Statistical evaluation of the methods was examined by determining intra-day and inter-day precisions. The methods were successfully applied to the assay of drugs in their pharmaceutical formulations and biological fluids. No interference was observed from common additives and the validity of the methods was tested.

Keywords: Analgesic drugs; 1, 10-phenanthroline; 2, 2' bipyridyl; bromate-bromide mixture.

Introduction

Tramadol hydrochloride is a centrally acting analgesic, used to treat moderate to moderately severe pain and most types of neuralgia, including trigeminal neuralgia. Chemically it is [2-(dimethylaminomethyl)-1-(3-methoxyphenyl) cyclohexanol)], (Scheme 1a). It is the BP [1], specifies non-aqueous titration technique detecting the end point potentiometrically for determination of tramadol. Because of its wide use, several techniques have been reported for its assay in biological and pharmaceutical samples involve a number of high-performance liquid chromatographic [2-4], gas chromatographic [5], electrochemical [6], potentiometric methods [7-11] and amperometry [12, 13], voltammetry [14] and flow injection chemiluminescence spectrophometry [15]. The literature was reported three spectrophotometric methods differed from our described work [16-18].

Morphine (5α, 6α-didehydro-4, 5-epoxy-17-methylmorphinan- 3, 6-diol) (Scheme 1b), is a therapeutic drug that is used commonly for the control of pain and also abused as an illicit drug. Moreover, heroin is hydrolyzed in the organism to morphine; therefore, the determination of morphine content of biological samples is helpful for clinical and forensic purposes [19, 20]. Various analytical methods have been developed for the determination of morphine and its major metabolites. The most common analytical techniques currently used include gas chromatography [21, 22], high-performance liquid chromatography [23-28], and capillary electrophoresis [29, 30], chemiluminescence [31], voltammetric [32, 33] and electrochemical [34]. To the best of our knowledge, only a report was found on the determination of morphine by spectrophotometry [35].

Nalbuphine (-)-17-(cyclobutylmethyl)-4, 5α-epoxymorphinan-3, 6α, 14-triol (Scheme 1c) is a semisynthetic narcotic agonist-antagonist of the phenanthrene series. As an analgesic agent, it is almost as potent as morphine and has widely used in the treatment of acute and chronic pain [36-38]. Its main advantages over morphine are a ceiling effect of respiratory depression, low tolerance liability and a lack of significant withdrawal symptoms [39]. As to our best knowledge, there is no official analytical method for analyzing of NLB in ampoule, in pharmacopoeias and the literatures. A few methods have been qualified to detect nalbuphine in pharmaceutical formulations and in biological fluids; they include gas chromatography coupled to electron-capture detection [40], or mass spectrometry [41], high-performance liquid chromatography with electrochemical detection [42, 48], and LC-MS/MS [49].

Naltrexone (17-cyclopropylmethylmethyl-6-deoxy - 7, 8 - dihydro-14-hydroxy-6-oxo-17-normorphine) (Scheme 1d), is a long-acting synthetic opiate antagonist with few side effects that is efficacious when administered orally, either daily or three times a week for a sustained period of time. Naltrexone has been determined by using a wide variety of analytical techniques, particularly chromatographic, such as gas chromatography [50, 51], high-performance liquid chromatography with electrochemical detection [52-54] and electrophoresis [55].

Check out the literature revealed that, up to the present time nothing manuscript has been published concerning the spectrophotometric determination of nalbuphine and naltrexone and little detection has been reported for the determination of morphine and tramadol by spectrophotometric methods. For these reasons, the present study describes simple, sensitive and economical spectrophotometric methods for the analysis TRM, MRF, NLB and NLT in pure, pharmaceutical formulations and biological fluids. Analytical gauge including linearity, sensitivity, precision, accuracy and recovery are discussed.

Experimental

Apparatus

All the absorbance spectral measurements were made using spectroscan 80 D double-beam UV/Vis spectrophotometer (Biotech Engineering Ltd., UK), with wavelength range 190–1100 nm, spectral bandwidth 2 nm, with 10 mm matched quartz cells. An Orion Research Model 601 A/digital analyzer, pH-meter with a combined saturated calomel glass electrode was used for pH measurements.

Reagents and materials

All reagents and chemicals used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily.

i. Standard solution of pure drugs

A stock standard solutions containing 20 mg of tramadol (TRM), morphine (MRF), nalbuphine (NLB) and naltrexone (NLT) were prepared by dissolving appropriate weight of pure drugs in distilled water and made up to the mark in a 100 ml calibrated. The analytical standard solutions of the studied drugs were prepared daily by appropriate dilution of the stock standard solution in water.

ii. Bromate – bromide mixture

A bromated – bromide solution equivalent 100 µg ml^{-1} $KBrO_3$ and 10-fold excess of KBr was prepared by dissolving accurately weighed 10 mg of $KBrO_3$ and 0.1 g of KBr in water and diluting to the mark in a 100 ml calibrated flask.

iii. Ferrous ammonium sulfate

A stock solution of ferrous ammonium sulfate with concentration of 5×10^{-3} M was freshly prepared by dissolving 1960 mg from $(NH_4)_2Fe(SO_4)_2.6H_2O$ in 20 ml distilled water containing 1.0 ml of 1.0 M H_2SO_4 and then diluted to 100 ml in a calibrated flask with distilled water.

iv. 1, 10-phenanthroline

A stock solution of 0.2% (w/v) of 1, 10-phenanthroline monohydrate (Sigma Chemical Company, St. Louis, USA), was made up by dissolving the solid in 1.0 ml of 2.0 M HCl and then diluted to 100 ml in a calibrated flask with distilled water.

v. 2,2' Bipyridyl

A stock solution of 0.5% w/v of 2, 2'-bipyridyl (Sigma Chemical Company, St. Louis, USA), was made up by dissolving the solid in 1.0 ml of 2.0 M HCl and then diluted to 100 ml in a calibrated flask with distilled water.

vi. Ammonia solution

A stock solution of 1: 1 v/v ammonia was prepared by diluting 50 ml of concentrated ammonia with 50 ml of distilled water in 100 ml calibrated flask.

vii. Hydrochloric acid

A 2.0 M of HCl was prepared by diluting 41.8 ml of concentrated acid (Merck, Darmstadt, Germany, sp. gr. 1.18, 37%) to 250 ml with water.

Recommended procedures

Appropriate volumes of solutions prepared from the standard drug solution of TRM, MOR, NLB and NLT (200 µg ml^{-1}), in the concentration range stated in Table (1) were placed in a series of 25 ml volumetric flasks using a micro pipette. To each flask 1.0 ml of 2.0 M HCl and 1.4 ml of bromate–bromide mixture solution (100 µg ml^{-1} in $KBrO_3$) were added. The flasks were stoppered, content mixed and allowed to stand for 15 min with occasional shaking. Then, added 0.8 ml of 5×10^{-3} M FAS, then set aside for 10 min for each drug with occasional shaking. Chelating agent added by constant concentrations, at using 1,10- phenanthroline was 2.0 ml of 0.2% w/v, but in case of 2,2' bipyridyl was 2.0 ml of 0.5% w/v, then set aside for 10 min with occasional shaking. At last pH of acidic medium was raised by adding 1.0 ml of 1:1 (v/v) ammonia solution and after 5 min; the volume was adjusted to the mark with distilled water and mixed well. The absorbance of each solution was measured at 510 or 522 nm for 1,10- phenanthroline or 2,2' bipyridyl, respectively against a reagent blank. In either method, the concentration of the unknown was read from the calibration graph or calculated using the regression equation obtained by using the Beer's law.

Procedure for the tablets

Ten tablets of tramundin (Manufactured by Mundi Pharmaceuticals Co., Egypt) each containing 100 mg of TRM and deltrexone (Manufactured by Delta Pharmaceuticals Co., Egypt) each containing 50 mg of NLT were completely powdered. An accurately weighed portion, equivalent to 20 mg was dissolved in about 10 ml of distilled water and any remaining residue was removed by filtration. The filtered solution was then transferred into a 100 ml calibrated flask and diluted to 100 ml with water. Suitable dilution was made to fit the applicable concentration range and the above described procedures were followed. The nominal content of the tablet was assayed from the calibration curves.

Procedure for ampoules

The content of five morphine ampoules labeled to contain (20 mg ml^{-1}) (Manufactured by Misr Pharmaceuticals Co., Egypt) and five nalufin ampoules (20 mg ml^{-1}) of nalbuphine were mixed. A volume equivalent to 20 mg of MOR and NLB was transferred to a 100 ml volumetric flask and made up to the mark with water. Suitable dilution was made to fit the applicable concentration range and the above described procedures were followed. The nominal content of the ampoules was calculated either from calibration graph or using the regression equation.

Procedure for spiked biological fluids

For the determination of the studied drugs in spiked urine and serum, 0.5 ml of diluted urine or serum were put in a 25 ml calibrated flasks. The solutions were prepared and following the same procedure as that for standard solutions. The absolute recovery was determined by comparing the representative recovery of the treated urine or serum samples with the standard drugs at the same concentration.

Results and Discussion

The proposed methods involves two steps, namely reaction of drugs with bromine generated, *in situ* by the action of acid on a bromate–bromide mixture, giving oxidation products, followed by the determination of residual bromine by reacting it with excess of iron(II) and the remaining iron(II) is complexed with 1,10- phenanthroline or with 2,2' bipyridyl and measuring the absorbance at 510 and 522 nm (Fig. 1), respectively. The provisional reaction schemes for the two methods are given in Scheme 2.

Optimum reaction conditions

The effect of reagent concentration (acidity, BrO_3^-, 1,10- phenanthroline and 2,2' bipyridyl), mixing time in each step with respect to maximum sensitivity, minimum blank, committing to Beer's law, reproducibility and stability of final color were studied by means of controlled experiments varying one parameter at a time.

Effect of bromate – bromide mixture

The optimum reaction conditions for the quantitative determination of each drug were established through a number of preliminary experiments. The results obtained show that at least 1.4 ml of bromate-bromide mixture (0.01% w/v $KBrO_3$) is required for maximum color development for each drug.

Selection of acid type and acid concentration

The reactions were tested in HCl, H_2SO_4, HNO_3 and CH_3COOH solutions. The results showed that the reaction is suitable in HCl medium. A 2.0 M HCl was found to be adequate for the oxidation of the drugs. The variation in HCl concentration indicated that constant absorbance was obtained with 0.4–2.0 ml of 2.0 M HCl for each drug; so subsequent studies were performed with 1.0 ml of 2.0 M HCl for each drug.

Effect of 1,10 phenanthroline and 2,2' bipyridyl

The effects of 1,10-phenanthroline or 2,2'bipyridyl were studied by measuring the absorbance of solutions containing a fixed concentration of each drugs and varied amounts of the reagents separately. It was observed that the maximum color intensity was obtained with 2.0 ml of 1,10 phenanthroline and 2, 2' dipyridyl (Figs. 2, 3), after which further increase in volume resulted in no change in the absorbance for both methods. So the same volume of both the reagents was used throughout the assay.

Effect of ammonia

The formation of ferroin and iron(II)-bipy complex was slow at room temperature and at low pH and required longer time for completion. Hence efforts were made to accelerate by carrying out the reaction at higher pH range (4.0-6.0). The pH of acidic medium employed for the redox reaction was raised by adding 1.0 ml of 1: 1 ammonia solution which was found to be optimum for both methods. The volume of 1: 1 ammonia was not critical, since the stability and sensitivity of complexes are unaffected over a wide pH range. However, 1.0 ml of 1:1 ammonia was used to raise the pH to about 4. Under the described experimental conditions, ferroin and iron (II)-bipy complex were found to be stable for 6.0 h.

Effect of order of addition

After fixing all other parameters, a few experiments were performed in order to achieve the influence of the order in which reagents were added. The maximum absorbance and highest stability were obtained when the order of addition was: drugs, HCl, bromated – bromide mixture, Fe(II) solution, 1,10 phenanthroline or 2,2' bipyridyl and ammonia solution. The same order of addition was followed throughout the investigation.

Analytical data

A linear correlation was found between absorbance at λ_{max} and concentration of the selected drugs. The graphs showed negligible intercept and are described by the equation:

$$Y = a + b\,X$$

(Where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in µg ml^{-1}). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 1. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity values of both methods are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [56], are also presented in (Table 1) and reveal the very high sensitivity of the methods.

Accuracy and precision

The precision of the proposed methods was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of the studied drugs were analyzed in five replicates during the same day (intra-day precision) and for seven consecutive days (inter-day precision). The analytical results obtained from the investigation are summarized in (Tables 2, 3). The percentage relative error (Er %) and the percentage relative standard deviation (RSD %) are considered very satisfactory. This level of precision of the proposed methods was adequate for the quality control analysis of the studied drugs.

Robustness

Robustness was examined by evaluating the influence of a small variation of the methods variables including the concentration of analytical reagents and reaction time on the performance of the proposed methods. In these experiments, one parameter was changed whereas the others were kept unchanged and the recovery percentage was calculated for each time. It was found that small variations in these variables did not affect the method significantly. This was an indication of the reliability of the proposed method during its routine application for analysis of the investigated drug and so the proposed spectrophotometric methods are considered robust.

Analysis of pharmaceutical formulations

The proposed methods were applied to the determination of TRM in tramundin tablets, MRF in morphine injection, NLB in nalufin and NLT in deltrexone. The results obtained are satisfactorily accurate and precise as indicated by the excellent % recovery and RSD% less than 1.98% (Table 4).

Analysis of spiked serum and urine samples

The high sensitivity of the proposed methods allowed the determination of the studied drugs in spiked urine and serum samples. The recovery studies were carried out on the sample, where known amounts of the studied drugs were added and the results of spiked urine and serum samples are given in (Tables 5, 6). Recovery was from 100.90 to 101.58 for urine and 100.74 to 101.60 for serum. This indicates good level of precision and accuracy.

Conclusions

The proposed methods have the advantages of simplicity and rapidity for the determination of four analgesic drugs in pure, pharmaceutical preparations and in biological fluids. The assay methods involve less stringent control of experimental parameters such as the stability of the colored species, time of analysis and temperature independence. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any tedious sample preparation. These advantages encourage the application of the proposed methods in routine quality control analysis of the selected drugs in pharmaceutical formulations.

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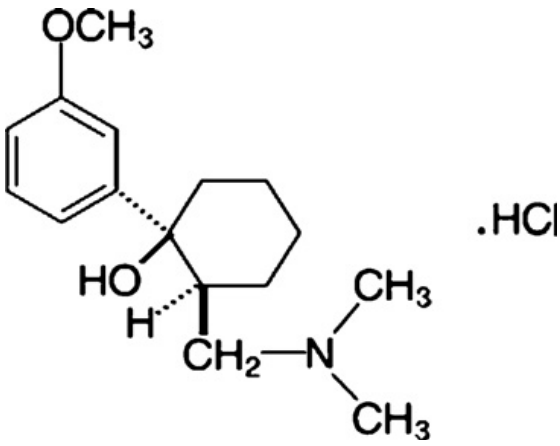
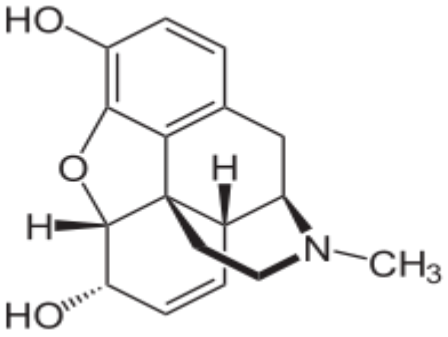
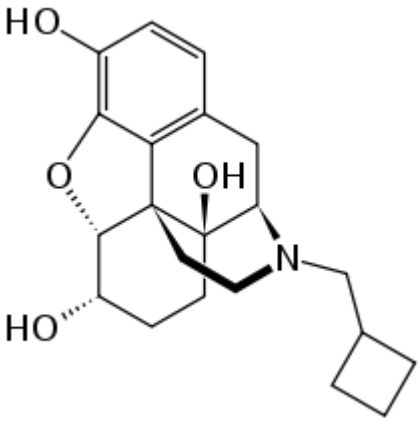


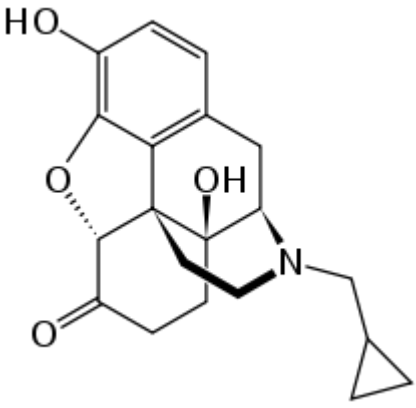
a- Tramadol



b- Morphine



c- Nalbuphine



d- Naltrexone

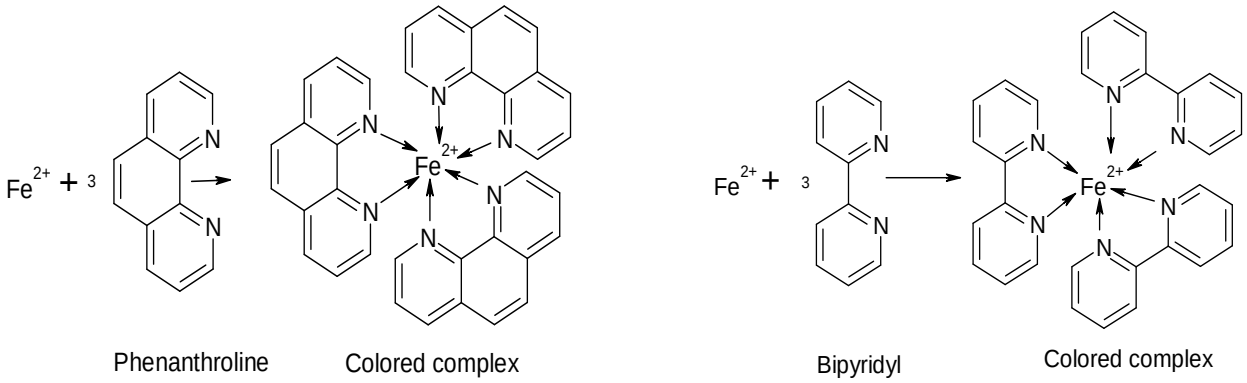
Scheme 1: Chemical structure of the selected drugs.

Step 1:

Drugs + BrO_3^- + Br^- + H^+ → Oxidation product of drugs + Unreacted Br_2

Unreacted Br_2 + excess Fe^{2+} → Fe^{3+} + Unreacted Fe^{2+}

Step 2:



Scheme: 2. Possible reaction pathway of methods A and B.

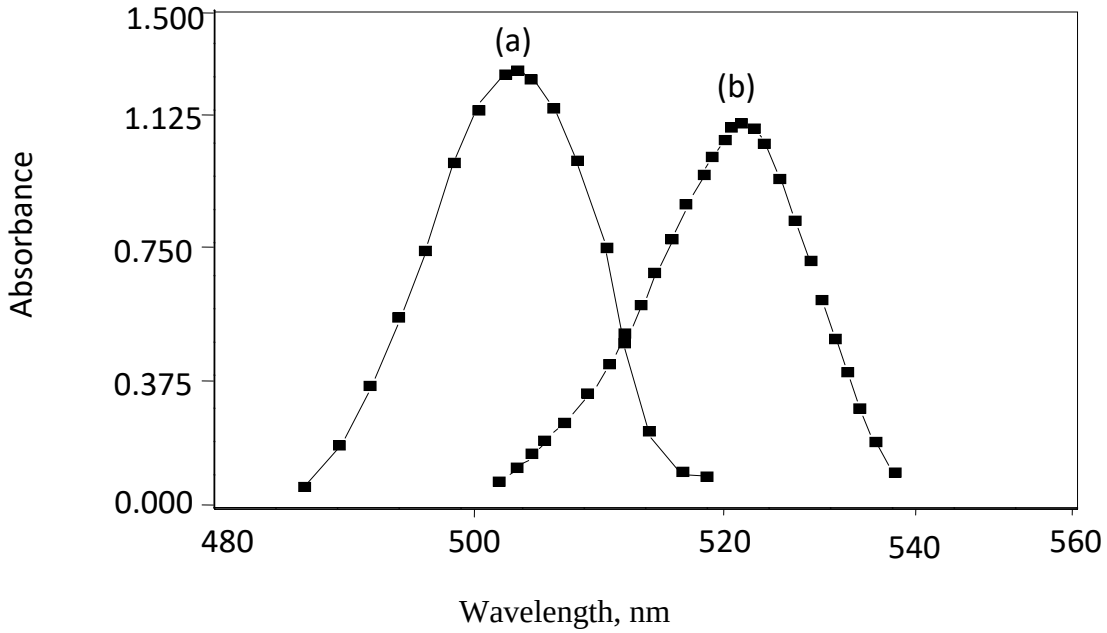
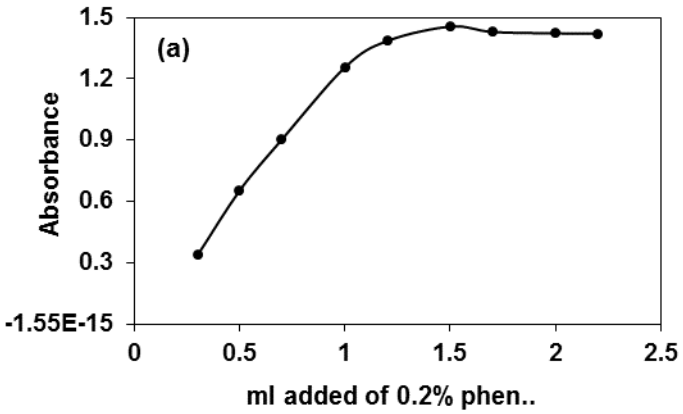
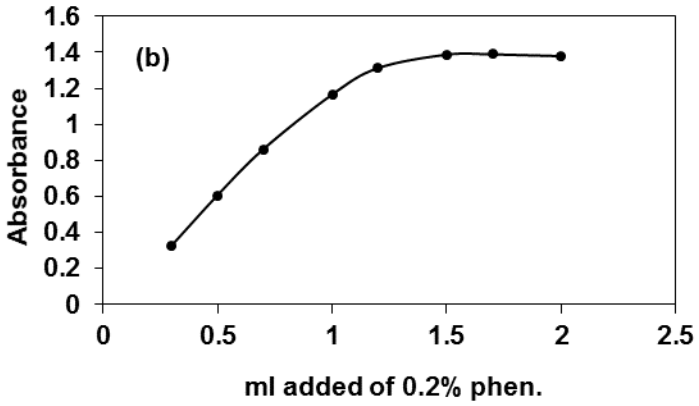
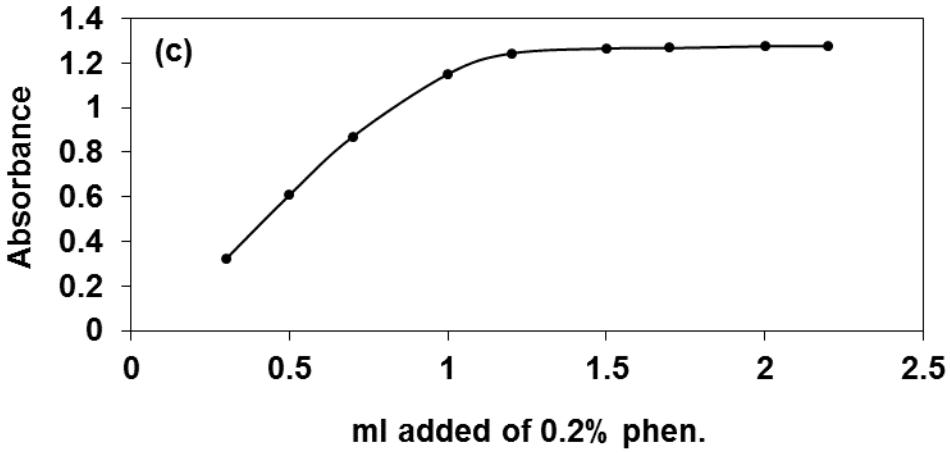


Fig. 1: Absorption spectra of the oxidation product with: (a) 1,10- phenanthroline and (b) 2,2' bipyridyl.




(b)
Absorbance
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
2
2.5
ml added of 0.2% phen.


(c)
Absorbance
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
2
2.5
ml added of 0.2% phen.

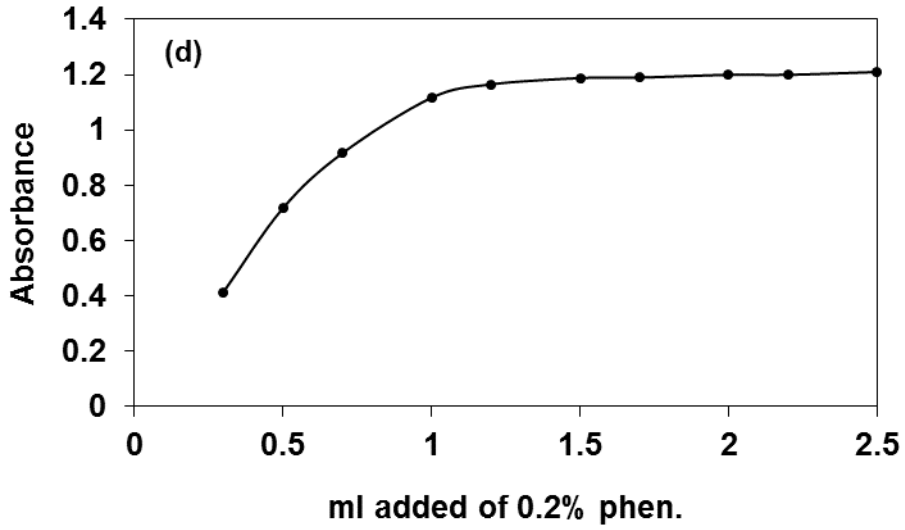
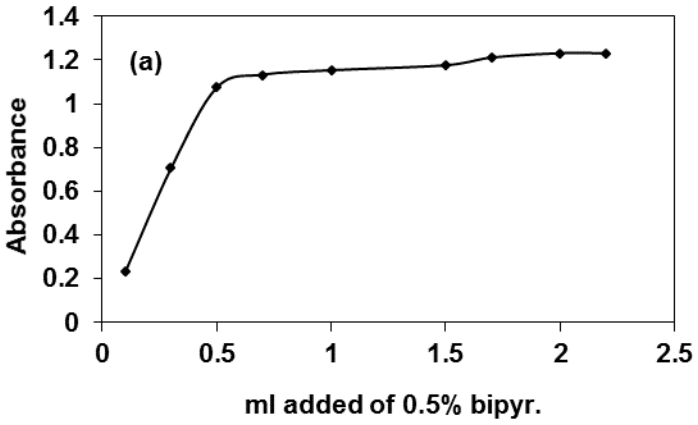
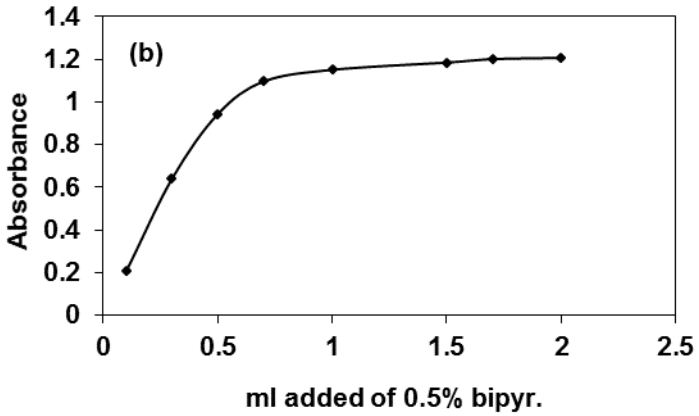
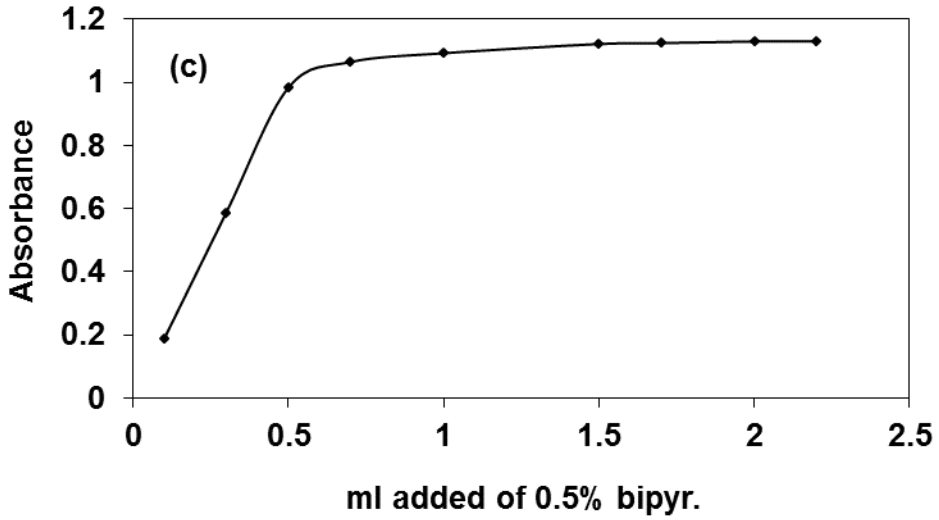


Fig. 2. Effect of added 0.2%1,10- phenanthroline on residual bromine from oxidation of:
a- TRM (11.2 µg ml^{-1}), b- MRF (8.8 µg ml^{-1}), c- NLB (14.4 µg ml^{-1}) and d- NLT (12.8 µg ml^{-1}).


(a)
Absorbance
ml added of 0.5% bipyr.
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0.5
1.5
2
2.5


(b)
Absorbance
ml added of 0.5% bipyr.
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0.5
1.5
2
2.5



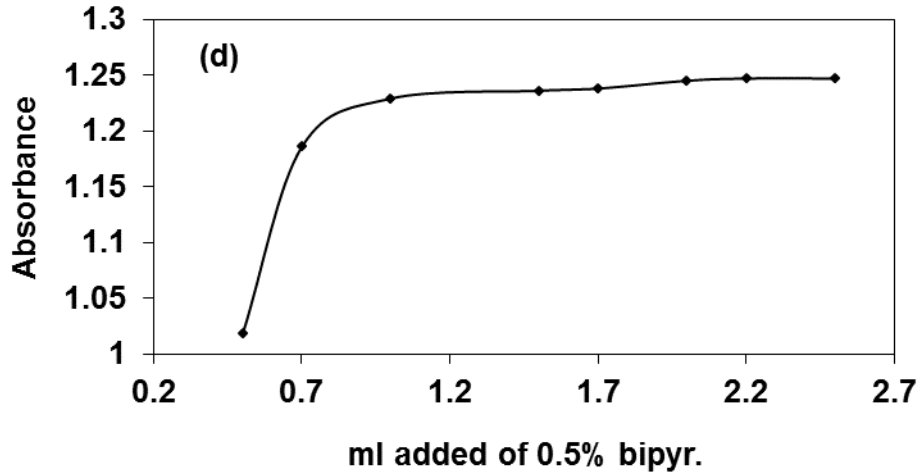


Fig. **3**: Effect of ml added of 2, 2'bipyridyl (0.5%) on residual bromine from oxidation of: a- TRM (11.2 µg ml^{-1}), b- MRF (9.6 µg ml^{-1}) c- NLB (12.8 µg ml^{-1}) and d- NLT (12.8 µg ml^{-1}).

Table 1. Optical characteristics, statistical data of the regression equations and validation parameters for the selected drugs

Parameters	1,10-phen.				2,2ˈ bipyr.			
	TRM	MRF	NLB	NLT	TRM	MRF	NLB	NLT
λ_{max}, nm	510	510	510	510	522	522	522	522
Beer's law limit, µg ml^{-1}	3.2-12.8	2.4-8.8	4.8-14.4	3.2-12.8	1.6-12.8	1.6-9.6	3.2-12.8	3.2-12.8
Molar absorptivity, L mol^{-1} cm^{-1}	3.00×10^4	1.06×10^5	3.33×10^4	3.70×10^4	2.63×10^4	8.95×10^4	3.24×10^4	3.41×10^4
Sandell's sensitivity, ng cm^{-2}	9.9	7.1	11.8	10.1	11.3	8.4	12.1	33.8
Correlation coefficient (r)	0.9997	0.9997	0.9997	0.9995	0.9998	0.9995	0.9992	0.9993
$S_{y/x}$	0.0107	0.0 115	0.0105	0.0173	0.0075	0.0136	0.0138	0.0136
Intercept (a)	-0.3135	-0.3468	-0.4552	-0.2219	-0.0232	-0.0677	- 0.2587	- 0.1753
Slope (b)	0.1254	0.1800	0.1208	0.1164	0.0892	0.1259	0.1033	0.1064
SD of slope (S_b)	0.0014	0.0023	0.0017	0.0002	0.0009	0.0022	0.0021	0.0022
SD of intercept (S_a)	0.0291	0.0240	0.0318	0.0389	0.0160	0.0301	0.0467	0.0333
LOD, µg ml^{-1}	0.0257	0.0294	0.0324	3.95×10^{-3}	0.0232	0.0402	0.0468	0.0476
LOQ, µg ml^{-1}	0.1116	0.1277	0.1407	0.0172	0.1008	0.1747	0.2032	0.2067

Table 2. Evaluation of intra-day and inter-day accuracy and precision by using 1,10-phen. Reagent

Drugs	Taken µg ml^{-1}	Intra-day accuracy and precision				Inter-day accuracy and precision			
		Found µg ml^{-1}	Recovery, %	RSD, %	Er, %	Found µg ml^{-1}	Recovery, %	RSD, %	Er, %
TRM	4.8	4.799	99.996	0.456	-0.004	4.799	99.996	0.617	-0.004
	8.0	7.999	99.994	0.194	-0.006	7.999	99.996	0.217	-0.004
	12.8	12.799	99.994	0.187	-0.006	12.799	99.994	0.067	-0.006
MRF	4.8	4.799	99.996	0.284	-0.004	4.799	99.994	0.295	-0.008
	6.4	6.399	99.996	0.185	-0.004	6.399	99.996	0.154	-0.004
	8.0	7.999	99.998	0.117	-0.002	7.999	99.996	0.144	-0.004
NLB	8.0	7.999	99.998	0.317	-0.002	7.999	99.994	0.229	-0.006
	11.2	11.199	99.996	0.173	-0.004	11.199	99.996	0.126	-0.004
	14.4	14.397	99.996	0.098	-0.014	14.399	99.996	0.069	-0.004
NLT	6.4	6.398	99.98	0.310	-0.020	6.399	99.994	0.232	-0.007
	9.6	9.600	100.004	0.176	0.004	9.599	99.994	0.125	-0.006
	12.8	12.800	100.006	0.127	0.006	12.798	99.992	0.091	-0.008

Table 3. Evaluation of intra-day and inter-day accuracy and precision by using 2,2'-bipyr. Reagent

Drugs	Taken μg ml^{-1}	Intra-day accuracy and precision				Inter-day accuracy and precision			
		Found μg ml^{-1}	Recovery, %	RSD, %	Er, %	Found μg ml^{-1}	Recovery, %	RSD, %	Er, %
TRM	3.2	4.7998	99.998	0.600	-0.002	3.197	99.936	0.420	-0.064
	6.4	7.999	99.994	0.336	-0.006	6.399	99.994	0.273	-0.006
	9.6	12.799	99.994	0.210	-0.006	9.599	99.996	0.215	-0.004
MRF	3.2	4.799	99.994	0.320	-0.006	3.199	99.998	0.217	-0.002
	6.4	6.399	99.994	0.145	-0.006	6.399	99.994	0.152	-0.006
	9.6	*7.999*	99.996	0.136	-0.004	9.599	99.994	0.100	-0.006
NLB	6.4	7.999	99.994	0.300	-0.006	6.399	99.994	0.371	-0.006
	11.2	11.199	99.992	0.093	-0.008	11.199	99.996	0.131	-0.004
	14.4	14.397	99.996	0.186	-0.004	14.401	100.012	0.175	0.012
NLT	6.4	6.398	99.996	0.335	-0.004	6.399	99.996	0.440	-0.004
	11.2	9.600	99.996	0.258	-0.004	11.199	99.994	0.128	-0.006
	16	12.800	99.994	0.095	-0.006	15.999	99.994	0.156	-0.006

Table 4. Determination of NLT, NLB, TRM and MRF in pharmaceutical preparations

Drugs	Name of preparation	1,10-phen.					2,2-bipyr.				
		Taken μg ml^{-1}	Found μg ml^{-1}	Recovery, %	RSD, %	Er, %	Taken μg ml^{-1}	Found μg ml^{-1}	Recovery, %	RSD, %	Er, %
TRM	Tramundin 100 mg/tablet	4.8	4.864	101.333	1.692	1.333	3.2	3.149	98.426	1.972	-1.573
		8.0	8.096	101.212	1.518	1.212	6.4	6.499	101.555	1.946	1.555
		12.8	12.883	100.650	0.815	0.650	9.6	9.706	101.108	1.392	1.108
MRF	Morphine Injection 20 mg ml^{-1}	4.8	4.862	101.292	1.708	1.292	3.2	3.150	98.461	1.927	-1.538
		6.4	6.444	100.701	0.886	0.701	6.4	6.357	99.329	0.845	-0.670
		8.0	8.034	100.432	0.544	0.432	9.6	9.561	99.594	0.509	-0.405
NLB	Nalufin 20 mg ml^{-1}	8.0	8.107	101.349	1.692	1.346	6.4	6.485	101.329	1.666	1.329
		11.2	11.308	100.974	1.217	0.972	11.2	11.322	101.095	1.375	1.095
		14.4	14.540	100.96	1.223	0.976	14.4	14.545	101.007	1.260	1.007
NLT	Deltrexone 50 mg/tablet	6.4	6.304	98.500	1.882	-1.498	6.4	6.312	98.629	1.719	-1.370
		9.6	9.475	98.709	1.628	-1.293	11.2	11.048	98.644	1.697	-1.355
		12.8	12.680	99.065	1.172	-0.935	16	15.922	99.516	0.622	-0.483

Table 5. Determination of NLT, NLB, TRM and MRF in urine

Drugs	1,10-phen.					2,2-bipyr.				
	Taken μg ml^{-1}	Found μg ml^{-1}	Recovery, %	RSD, %	Er, %	Taken μg ml^{-1}	Found μg ml^{-1}	Recovery, %	RSD, %	Er, %
TRM	6.4	6.464	101.012	1.268	1.011	6.4	6.499	101.556	1.946	1.555
	8.0	8.072	100.901	1.131	0.901	9.6	9.729	101.348	1.678	1.348
MRF	8.0	8.107	101.343	1.683	1.343	6.4	6..475	101.179	1.477	1.179
	12.8	12.958	101.241	1.577	1.240	9.6	9.731	101.374	1.720	1.374
NLB	8.0	8.127	101.591	1.996	1.591	11.2	11.321	101.086	1.369	1.086
	11.2	11.370	101.524	1.913	1.524	14.4	14.542	100.987	1.238	0.987
NALT	6.4	6.501	101.583	1.981	1.583	11.2	11.339	101.241	1.555	1.241
	9.6	9.749	101.560	1.957	1.560	16	16.241	101.337	1.679	1.337

Table 6. Determination of NLT, NLB, TRM and MRF in serum

Drugs	1,10-phen.					2,2-bipyr.				
	Taken μg ml^{-1}	Found μg ml^{-1}	Recovery, %	RSD, %	Er, %	Taken μg ml^{-1}	Found μg ml^{-1}	Recovery, %	RSD, %	Er, %
TRM	6.4	6.484	101.317	1.649	1.317	6.4	6.501	101.592	1.996	1.592
	8.0	8.124	101.551	1.941	1.550	9.6	9.727	101.325	1.678	1.325
MRF	8.0	8.109	101.373	1.724	1.373	6.4	6.449	100.777	0.978	0.777
	12.8	12.96	101.257	1.572	1.257	9.6	9.671	100.740	0.930	0.740
NLB	8.0	8.133	101.673	2.098	1.673	11.2	11.295	100.855	1.073	0.855
	11.2	11.360	101.436	1.808	1.436	14.4	14.536	100.951	1.197	0.950
NLT	6.4	6.501	101.583	1.992	1.583	11.2	11.314	101.021	1.278	1.021
	9.6	9.751	101.582	1.979	1.582	16	16.208	101.304	1.633	1.304