Spectrophotometric Assay of Dipyrone in Pharmaceutical Preparations Via Oxidative Coupling Reaction with m-Toluidine and Potassium Hexacyanoferrate (III)

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الملخص

تم وصف طريقة طيفية بسيطة وسريعة وعالية الحساسية لتقدير الدايبرون بهيئته النقية وفي مستحضراته الصيدلانية . اعتمدت الطريقة على تفاعل الاقتران التأكسدي العضوي للدايبرون مع كاشف ميتا–تولويدين بوجود بوتاسيوم سداسي سيانو الحديد (III) في المحلول المائي لتكوين ناتج ارجواني اللون يقاس امتصاصه عند 530 نانوميتر ، وكانت حدود تطبيق قانون بير 0.4–10 مايكروغرام مللتر$^{-1}$ بدقة وتوافق مرضيين . إذ بلغ معدل نسبة الاسترجاع 99.71% والانحراف القياسي النسبي اقل من 2.0% . بلغت الامتصاصية المولية 40770 لتر.مول$^{-1}$.سم$^{-1}$ بحدود كشف 0.051 وتقدير كمي 0.167 مايكروغرام. سم$^{-1}$. طبقت الطريقة بنجاح في تقدير الدايبرون في المستحضرات الصيدلانية بشكل حقن ، إذ وجد ان نتائج الطريقة متفقة مع الطريقة القياسية المعتمدة (الطريقة اليوديمترية) ومع المحتوى الاصيل للمستحضرات الصيدلانية .

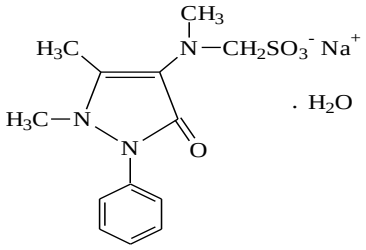
Abstract

A simple, rapid and highly sensitive spectrophotometric method has been described for the determination of dipyrone in a bulk and in dosage forms. The method depends on the organic oxidative coupling reaction of dipyrone with m-toluidine in the presence of potassium hexacyanoferrate (III) in aqueous solution to form purple product measured at 530 nm. Beer's law is obeyed in the concentration range 0.4-10.0 μg.ml^{-1} with accuracy (average recovery) of 99.71% and precision (RSD) is less than 2%. The molar absorptivity is 40770 l.mol^{-1}.cm^{-1} with LOD 0.051 μg.ml^{-1} and LOQ 0.167 μg.ml^{-1}. The analytical results agree favourably with official method (iodimetric method) and also with certified values.

Introduction

The dipyrone [(sodium salt of the 1-phenyl-2,3-dimethyl-4-methyl aminomethane sulfonate-5-pyrazolone), (Scheme 1)] is widely used in therapeutics as an analygesic, antipyretic and antispasmodic drug (1). Dipyrone acts at the central and outlying level simultaneously, and its absorption is fast, uniform and almost complete about 58% of the dose

links to plasma proteins, and the effect of this drug occur approximately fifteen minutes after its administration. The biotransformation of the dipyrone takes place at the hepatic level and the duration of its effect is approximately 4-6 h and its elimination is at renal level (2). The drug can cause occasional or rare reactions as transitory disturbances and inflammation of the renal tissue, mainly in patients with renal disease or in cases of overdose (3, 4).



Scheme 1. Chemical structure of dipyrone.

Several analytical methods have been reported for the estimation of dipyrone in pharmaceutical preparations and/or biological fluids including titrimetry in aqueous (5) and non aqueous media (6), spectrophotometry (7-9), fluorimetry (10), chemiluminesence (11, 12), turbidimetry (13), ion-selective electrode based on potentiometry (14), amperometry (15), polorography (16) and voltammetry (17). The derivative spectrometry (18-20), HPLC (21, 22) and HPTLC (23) methods are frequently used for dipyrone determination in combination with other drugs. However, some of these procedures suffer from one or the other disadvantage such as poor sensitivity, time consuming, instability of the coloured species, or require extraction step and complicated apparatus.

Oxidative coupling organic reactions are now a well established methods that when applied to the analysis of pharmaceutical preparations (24-26), can be considered to be an advantageous alternative to others normally used, owing to its simplicity, sensitivity and versatility of applications. To the best of our knowledge, the only spectrophotometric method for dipyrone determination via oxidative coupling reaction described in the literature to date (27) is based on the coupling of dipyrone with aniline in the presence of potassium hexacyanoferrate (III) and in a neural medium. In this work, m-toluidine was utilized as coupling reagent to develop a simple, sensitive and accurate method for the spectrophotometric determination of dipyrone, either in pure form or in pharmaceutical preparations.

Experimental

Apparatus

Shimadzu UV-1650PC and UV-210 digital double beam spectrophotometers, both with 1-cm quartz cells were used for all spectra and absorbance measurements.

Reagents

All chemicals were of analytical grade and used without further purification. Dipyrone sodium salt was provided from SDI-Iraq, m-toluidine and potassium hexacyanoferrate (III) were obtained from Fluka company. All solutions were prepared in distilled water except m-toluidine, which was dissolved in a minimum amount of ethanol and the volume was completed with distilled water.

Solutions

A stock standard solution of dipyrone (10000 μg/ml) was prepared by dissolving 1.00 g of pure drug in distilled water and then diluted to the mark in a 100 ml volumetric flask. The solution was stable for 7 days when kept in a refrigerator at 5 °C. A working standard solution of the drug containing 100 μg/ml was prepared by further dilution. A 0.05 M m-toluidine solution and 0.005 M potassium hexacyanoferrate (III) solution were used.

Recommended procedure

A liquots of working dipyrone standard solution containing 10-250 μg were transferred into a series of 25 ml volumetric flasks, to each flask, 2 ml of 5 x 10^{-2} M m-toluidine and 2 ml of 5 x 10^{-3} M potassium hexacyanoferrate (III) were added and the mixture was diluted to the mark with distilled water and mixed well. The absorbance values were measured at 530 nm after 15 min from final addition against a reagent blank which was treated similarly. The calibration graph was constructed by plotting the measured absorbance versus the drug concentration (Fig. 1). The unknown concentration of dipyrone sample was computed from the regression equation.

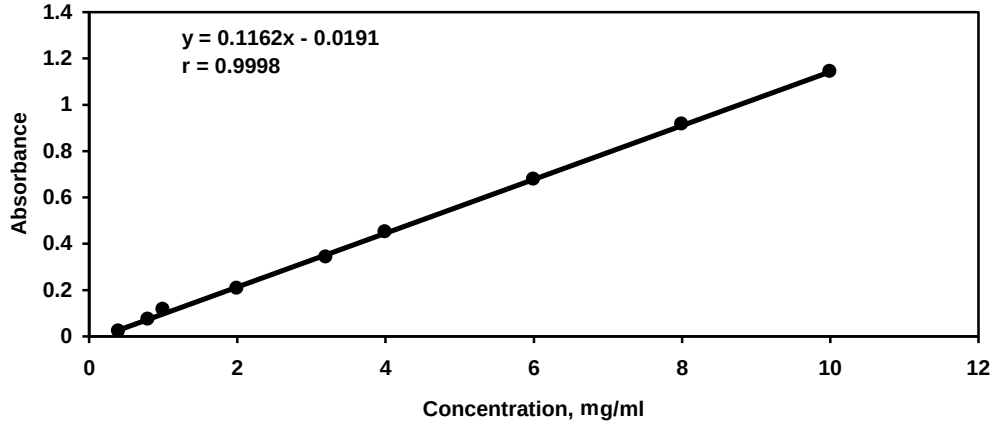


Fig. 1. Calibration graph for the determination of dipyrone.

Procedure of injection

The contents of five ampoules (each contain 1 gm dipyrone per 2 ml) were mixed and a 2.0 ml was accurately transferred into a 100 ml volumetric flask and diluted to the mark with distilled water. An accurate volume was appropriately diluted to get 100 μg ml^{-1} of dipyrone solution. Aliquots of the diluted drug solution were treated as described under the recommended procedure.

Results and Discussion

Spectral characteristics

Dipyrone react with m-toluidine in the presence of potassium hexacyanoferrate (III) in a neutral media to form an intense purple coloured product measurable at 530 nm, where as the reagent blank has negligible absorption at this wavelength (Fig. 2).

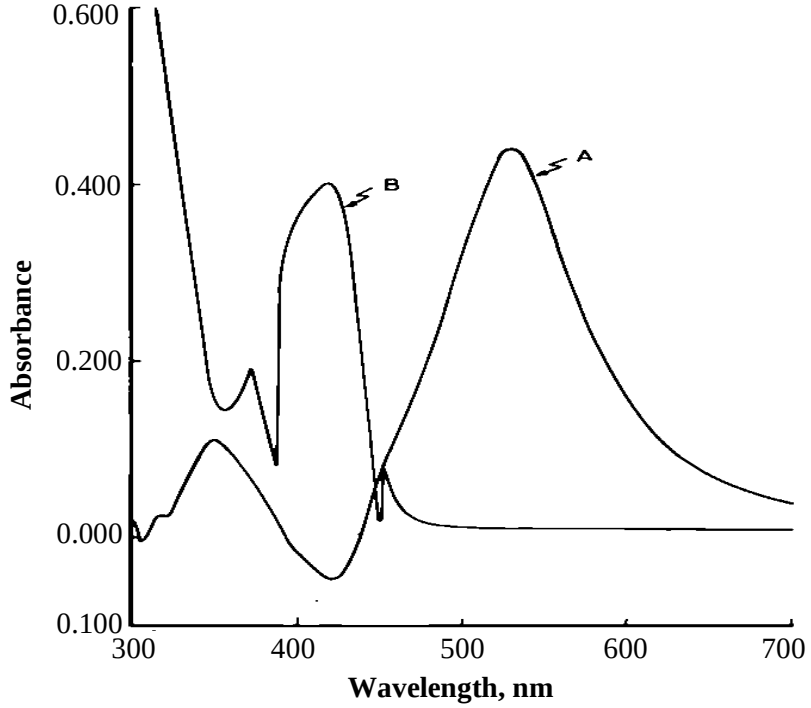


Fig. 2. Absorption spectra of 4 μg ml^{-1} of dipyrone measured against reagent blank (A) and the reagent blank measured against distilled water (B).

Optimization of variables

The optimum conditions for the development of colour were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the coloured species. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in recommended procedure.

However; optimization of conditions were carried out on using 4 μg ml^{-1} of dipyrone.

Effect of buffer solution

The effect of buffer solution with pH in the range of 2.2-8 using citric acid and disodium hydrogen phosphate on the absorbance of 4 □g ml^{-1} dipyrone were studied. As shown in Table 1, it was observed that these buffers were decreased the absorbance and high sensitivity of the coloured product was achieved in neutral medium of pH 6.59 without using of buffer solutions.

Table 1. Effect of pH on the sensitivity of product.

pH*	Absorbance	Molar absorptivity $l.mol^{-1}.cm^{-1}$	Final pH of the solution
2.2	0.037	3251	3.31
3.0	0.051	4482	3.52
4.0	0.369	32425	4.95
5.0	0.410	36028	5.48
6.0	0.419	36819	6.15
7.0	0.231	20299	7.04
8.0	0.079	6942	7.50
without buffer	0.459	40334	6.59

* Citric acid, disodium hydrogen phosphate buffer.

Effect of reagent and oxidant concentrations

The effects of various concentrations of m-toluidine and potassium hexacyanoferrate (III) were investigated. It was found that m-toluidine (5 x 10^{-2} M) solution in the range 1.5-2.5 ml and potassium hexacyanoferrate (III) (5 x 10^{-3} M) solution in the range 1.5-3.0 ml showed maximum colour intensity of the product. The colour intensity was decreased above and below this limit (Fig. 3). Therefore, 2 ml for each of m-toluidine and hexacyanoferrate (III) were recommended for all subsequent measurements.

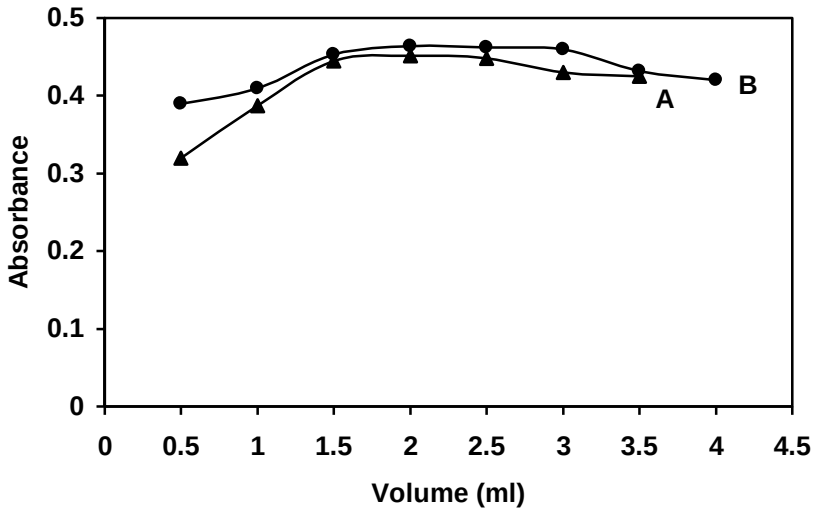


Fig. 3. Effect of the volume of 5 x 10^{-2} M m-toluidine (A) and 5 x 10^{-3} M of $K_3Fe(CN)_6$ (B) on 4 $mg.ml^{-1}$ dipyrone.

Effect of temperature on coloured product

The reaction between dipyrone and m-toluidine in the presence of hexacyanoferrate (III) was studied at 0 °C and 45 °C, in addition to room temperature (25 ± 5 °C). It was found that the reaction is completed within 10 min at room temperature, but 15 min was sufficient to get maximum intensity and stable colour for at least 3h which is used in all subsequent experiments.

Effect of order of additions

To obtain optimum results the order of additions of reagents should be followed as given under the general procedure, otherwise a loss in colour intensity was observed.

Validation of assay procedures

Under the optimized experimental conditions, straight-line calibration graph was obtained over the calibration ranges 0.4-10 mg ml^{-1} of dipyrone with molar absorptivity of 40770 $l.mol^{-1}.cm^{-1}$ and Sandell sensitivity 8.62 $ng.cm^{-2}$, indicating the method is sensitive. The detection and quantification limits were calculated from the standard deviation of the absorbance measurements obtained from a series of 10 blank solutions (28). The limits of detection (0.051 $\mu g/ml^{-1}$) and quantification (0.167 $\mu g/ml^{-1}$) were below the lower limit of Beer's law range. Regression analysis revealed very small intercept (-0.0191) and a perfect linearity (correlation coefficient, r=0.9998) between the absorbance and concentration of drug in the Beer's law limit studied.

Accuracy and precision

The accuracy and precision of the method were evaluated by performing six replicate analyses of dipyrone in pure form at four different concentration levels (2, 4, 6, 8 $\mu g.ml^{-1}$). The mean recovery (99.71%) and relative standard deviation (<2.0) can be considered to be very satisfactory.

Nature and stability constant of the dye product

The stoichiometry of the reaction was studied adopting Job's method of continuous variation (29). The results obtained (Fig. 4) show that a 1:1 drug to the analytical m-toluidine reagent was formed.

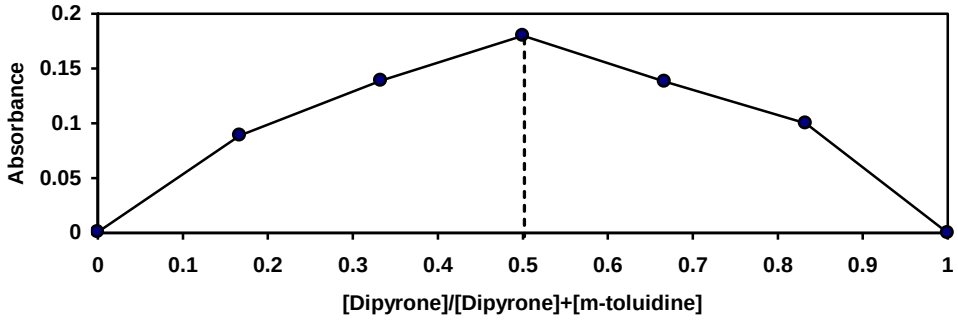
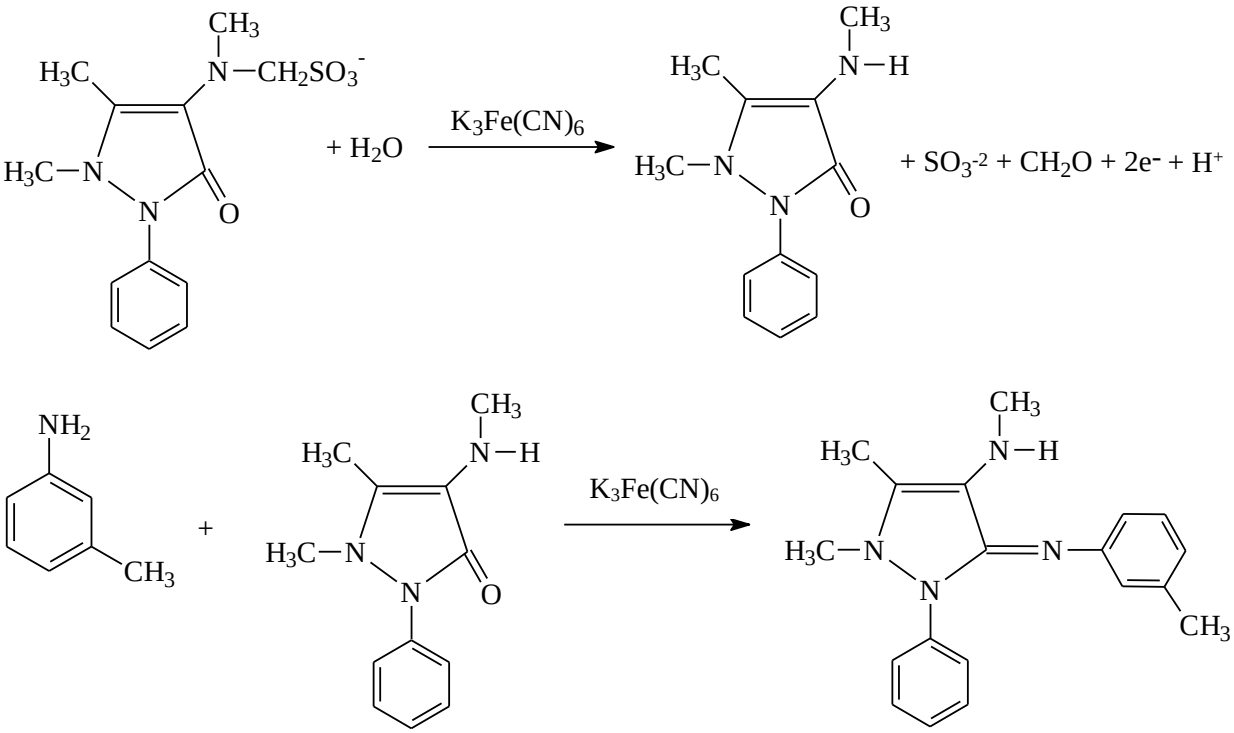


Fig. 4. Job's plot method of dipyrone-m-toluidine in the presence of $K_3Fe(CN)_6$.

Therefore, the formation of the purple coloured product (17, 30) may probably occur as shown in the following Scheme 2:



Scheme 2.

The average stability constant of the dye product (obtained by following the equation cited in reference 29) was 2.50 x 10^6 l.mol^{-1} which indicates that a stable dye product is formed.

Interference studies

In order to investigate the analytical application of the proposed method, the effect of some common drugs and excipients usually present in the pharmaceutical preparations was investigated by carrying out the determinations of 100 μg ml^{-1} dipyrone in the presence of each of the different excipients at concentration that can be found in the pharmaceutical preparations. Experimental results showed that there was no interference from excipients for the examined method up to 100-fold excess. Typical results are given in Table 2.

Table 2. Effect of excipients for assay of dipyrone.

Excipient	Recovery (%) of 100 mg of dipyrone per mg excipient added		
	1000	**5000**	**10000**
Paracetamol	99.53	98.78	97.32
Caffein	97.38	96.67	97.14
Saccharine	100.35	101.85	100.97
Propylene glycol	102.37	101.70	103.27
Lactose	100.03	100.88	102.62
Glucose	101.33	100.51	100.72
Fructose	102.64	103.00	103.08
Starch	101.00	102.79	103.87
Talc	100.15	100.23	104.13
Sodium chloride	100.85	100.10	102.87
Magnesium stearate	98.72	103.51	99.24

Analytical applications

The proposed method was successfully applied to determine dipyrone in six pharmaceutical preparations (injectables). The obtained results were compared statistically by a student's t-test for accuracy and a variance ratio F-test for precision (31) with the official method (1) (based on titrimetric titration in hydrochloric acid medium with a standardized solution of iodine using starch as indicator) at the 95% confidence limits for six and three degree of freedom respectively, as recorded in Table 3. The results showed that the t and F values were less than the critical values indicating that there was no significant difference between the proposed methods and official method.

Table 3. Application of the proposed and official method to the determination of dipyrone in pharmaceutical preparations.

Pharmaceutical preparations	Supplier	Certified value gml^{-1}	Recovery[(a)] (%)	
			Proposed[b] method	**Official[b] method**
Adepiron injection	Adeka Adeka Ilacsan 55020-Samsan Indian	1 g/2 ml	100.29	99.87
Analyin injection	Guj, Drugs G303 Iran	1 g/2 ml	102.75	101.91
Metamizole injection	S.A. Pharma LLC-USA	1 g/2 ml	99.72	100.38
Metamizole injection	Wuxipharm-Inter Brussel-Belgium	1 g/2 ml	102.98	100.86

[a] for three determinations of 2,4,8 □g ml^{-1}

[b] The calculated and tabulated values of t and F at the 95% confidence limit are 0.72, 3.82 and 2.45, 9.28

Finally, in comparison with previously oxidative coupling method (27) for dipyrone determination, the proposed method has a high sensitive and the coupling reagent (m-toluidine) does not need any previous purification step (Table 4).

Table 4. Comparison of results for the determinations of dipyrone by the proposed and reported methods.

Analytical parameters	m-Toluidine-$K_3Fe(N)_6$ system Present method	Aniline-$K_3Fe(CN)_6$ system (27)
λ_{max} (nm)	530	530
pH	Aqueous medium	aqueous medium
Temperature	R.T	R.T
Development time (min)	15	15
Molar absorptivity ($l.mol^{-1}cm^{-1}$)	40770	22553
Beer's law range ($\mu g\ ml^{-1}$)	0.4-10	0.4-16
LOD ($\mu g\ ml^{-1}$)	0.051	0.094
LOQ ($\mu g\ ml^{-1}$)	0.167	0.312
Average recovery (%)	99.71	100.62
RSD (%)	<2.0	<3.0
Analytical application	Injection	Injection

Conclusion

The proposed method for the spectrophotometric determination of dipyrone in pharmaceutical preparations is simple, rapid and very sensitive. The oxidative coupled product formed is fairly soluble and quick stable. Statistical analysis of the result indicates that the method has good precision and accuracy. No interferences from associated excipients and additives were observed.

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