Determination of dipyrone in pharmaceutical preparations based on the chemiluminescent reaction of the quinolinic hydrazide–H_2O_2–vanadium(IV) system and flow-injection analysis

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ABSTRACT: A rapid, economic and sensitive chemiluminescent method involving flow-injection analysis was developed for the determination of dipyrone in pharmaceutical preparations. The method is based on the chemiluminescent reaction between quinolinic hydrazide and hydrogen peroxide in a strongly alkaline medium, in which vanadium(IV) acts as a catalyst. Principal chemical and physical variables involved in the flow-injection system were optimized using a modified simplex method. The variations in the quantum yield observed when dipyrone was present in the reaction medium were used to determine the concentration of this compound. The proposed method requires no preconcentration steps and reliably quantifies dipyrone over the linear range 1–50 µg/mL. In addition, a sample throughput of 85 samples/h is possible.

Keywords: quinolinic hydrazide; vanadium(IV); dipyrone; chemiluminescence; FIA

Introduction

Dipyrone, also known as metamizol, methanesulphonic acid or analgin, is a drug of the pyrazolone (phenylpyrazolone) family with analgesic, antipyretic and anti-inflammatory effects. The pyrazolones have been widely used in medicine, and dipyrone, which was first synthesized in 1920, is now the group's main representative (1). Sodium dipyrone (2,3-dihydro-1,5-dimethyl-3-oxo-2-phenyl-1*H*-pyrazol-4-yl-methylamino-methanesulphonate) is one of its most used salts, since it is soluble in water and is an effective non-opioid analgesic for treating acute pain. The data available on the clinical efficacy of single dose dipyrone in postoperative pain management have been analysed by the Cochrane Collaboration (2). Compared to a placebo, dipyrone showed an NNT value (i.e. the number needed to treat for pain relief of at least 50% for 4–6 h in patients with moderate to severe pain) of 2.5 for 500 mg and 1.9 for 1 g. Intramuscularly-injected dipyrone (2 g) has been reported to show a greater analgesic effect than 100 mg pethidine, 30 mg ketorolac or 10 mg morphine. In addition, oral doses of 500 mg and 1 g dipyrone are reported to be more effective than 400 mg ibuprofen, 600 mg aspirin or 1 g paracetamol (3).

Like many substances, dipyrone has been associated with side-effects, in this case agranulocytosis and anaphylactic shock reactions (4–6). Its use remains popular in some parts of Europe, the Middle East, Asia, Africa and Latin America, but in others it is not registered, and in the UK, Sweden and the USA its use is forbidden (2,3). Some research on dipyrone has shown, however, that the risks of agranulocytosis are minimal (7), and shock reactions are common to all antipyretic analgesics. Further, the available evidence suggests that its use is associated with no gastric or renal side-effects – common problems of analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) (8). Indeed, the risk of agranulocytosis should be understood in the context of the risks associated with the use of other analgesics. Andrade *et al.* (9) report the estimated excess mortality due to severe side-effects of aspirin to be 185/100 million and 592/100 million for diclofenac, but only 20/100 million for paracetamol and 25/100 million for dipyrone.

Most of the methods used for the determination of this drug are based on titrimetry (10,11), amperometry (12–15), spectrophotometry (16–21), polarography (22), HPLC (23–26) and chemiluminescence (27–30). The spectrophotometric and chemiluminescent methods, especially when combined with flow-injection analysis (FIA), provide economic, simple, sensitive and rapid assessment procedures, e.g. the methods of Gregorio Alapont *et al.* (30), Huang *et al.* (29) and Song and Zhang (28) are

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routinely used in pharmaceutical analysis. However, the concentration range that the method of (30) is capable of measuring is reduced (2.5–12.5 µg/mL), that of (29) requires preconcentration procedures be performed, and the throughput of (28) (60/h) could be improved.

In previous work (31) our group reported that, under certain conditions, cyclic hydrazides such as cinchomeronic hydrazide (31,33) and quinolinic hydrazide (34,35) undergo chemiluminescent reactions that can be used for the determination of compounds such as vanadium and H_2O_2. The present work describes a method for dipyrone determination involving the quinolinic hydrazide–H_2O_2–vanadium(IV) system. The proposed method is based on the reduction, caused by dipyrone, in the quantum yield of the vanadium(IV)-catalysed quinolinic hydrazide chemiluminescent reaction ($\lambda_{max} = 420$ nm). The most important reaction conditions and FIA variables of the reaction were optimized using a super-modified simplex method (32). The simplicity and selectivity of the proposed method allows the determination of dipyrone concentrations in pharmaceutical preparations without the need for prior separation or preconcentration processes. It also uses relatively inexpensive equipment and has a high sample throughput (85 samples/h).

Experimental

Chemicals and reagents

Quinolinic hydrazide (QH; 5,8-dione-6,7-dihydro-pyrido(2,3δ)pyridazine) is a cyclical hydrazide with a structure similar to luminol; that used in the present work was synthesized in our laboratory following the procedure described by Toro *et al.* (36). Briefly, quinolinic acid, hydrazine and water were mixed and heated under reflux for 2 h. The solid obtained was purified by heating until reaching 250 °C. It was then cooled and the product crystallized in water. The crystals were characterized by infra-red spectrometry, which confirmed them to be the desired hydrazide (74% purity).

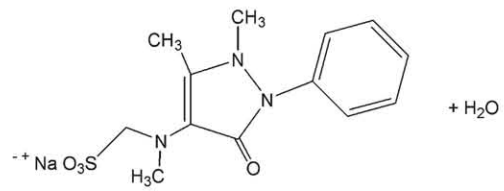

Figure 1. Chemical structure of dipyrone.

A quinolinic hydrazide stock solution of 2.0×10^{-2} mol/L was prepared by dissolving 0.82 g of these crystals in 10 mL 1 mol/L NaOH (Merck, Spain) and then diluting to 250 mL with deionized water. A vanadium(IV) stock solution of 1.0×10^{-2} mol/L was prepared from 2.53 g vanadyl sulphate (Merck, Spain) by dilution with 10 mL 0.1 mol/L H_2SO_4 (Panreac, Spain) and diluting to 100 mL with deionized water and adjusting to pH 1.9. Hydrogen peroxide solutions of 0.25 mol/L were prepared daily by dilution of 2.84 mL 30% H_2O_2 (Foret, Spain; final volume 100 mL) with deionized water.

A 300 mg/L dipyrone stock solution (Fig. 1) was prepared by dissolving 150 mg of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$ (Sigma-Aldrich, Germany) in 500 mL deionized water and adjusting to pH 1.9 with 0.1 mol/L H_2SO_4. This stock solution was maintained under refrigeration at 5 °C. Working standards within the 0.005 and 200 mg/L dipyrone range were prepared daily by water dilution of the above stock.

The pharmaceutical formulations of dipyrone analysed were those commercially available in Spain: Nolotil capsules (575 mg), injectable Nolotil (2 g/5 mL) and injectable Metamizol Normon (2 g/5 mL). For the analysis of the capsules, the material from 10 capsules was removed, weighed and finely powdered. A sample equivalent to the contents of two capsules was homogenized and dissolved in 60 mL water and filtered (0.45 µm). The residue was washed three times with 10 mL water. The pH of the resulting solution was adjusted to 1.9 with 0.1 mol/L H_2SO_4 before diluting to 100 mL with deionized water. For the analysis of the injectable Nolotil and Metamizol Normon formulations, five samples from single boxes were separately mixed. An aliquot of the mixture equivalent to the contents of one vial (2 mL) was diluted to 100 mL with deionized water and adjusted to pH 1.9 with 0.1 mol/L H_2SO_4. All the reagents used, with the exception of the quinolinic hydrazide, were of analytical grade. Deionized water was prepared using a Milli-Q system (Millipore).

Apparatus

A Perkin-Elmer LS-50 B spectrofluorimeter equipped with a Labsphere (FSA-PE-50 L) luminescence accessory (an integrating sphere that detects emissions from chemiluminescent reactions) was used to measure the light signals produced in reactions.

Figure 2 shows the flow-injection manifold used for the detection of chemiluminescence. This included a Gilson peristaltic pump (Minipuls 2), an Omnifit six-way injection valve, a three-way Omnifit connector and an in-house spiral reaction coil made of polytetrafluoroethylene (PTFE) tubing (1 m long, i.d. 0.8 mm). This was connected to the integrating sphere. The pH of all solutions was recorded using a Metrohm 654 pH meter.

The results obtained with the proposed method were compared to those obtained by reverse-phase liquid chromatography (HPLC) (24,25)

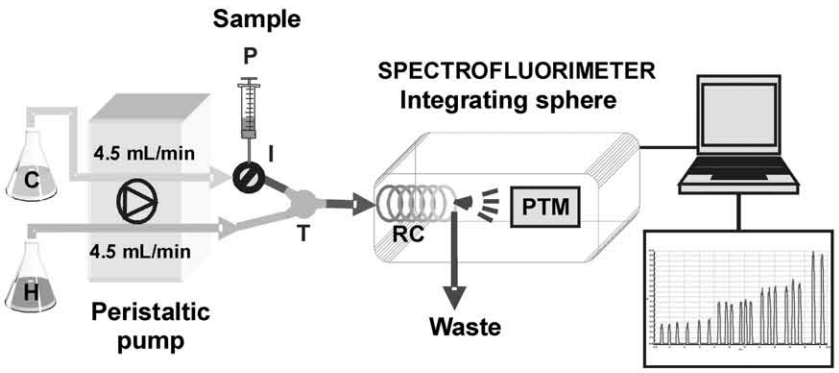

Figure 2. Flow-injection manifold. I, injection valve; RC, reaction coil; C, carrier solution; P, vanadium–dipyrone solution; H, hydrazide solution; PTM, photomultiplier; T, connector.

Table 1. System optimization by the simplex method

	Quinolinic hydrazide pH	Vanadium conc. (mol/L)	Vanadium pH	H_2O_2 conc. (mol/L)	Flow rate (mL/min)
Study range	10–14–(H_ 16)	5×10^{-5}–10^{-1}	1.5–3.5	7.5×10^{-4}–4.5×10^{-2}	3.2–6.5
Starting condition	11.5	10^{-3}	1.7	5×10^{-3}	3.5
Step size	1.3	2.4×10^{-2}	0.5	1.1×10^{-2}	0.8
Optimum conditions	13.2 (H_)[a]	3.3×10^{-2}	1.9	1.7×10^{-2}	4.5

[a]Equivalent NaOH concentration = 0.17 mol/L.
H_, alkaline function.

(reference method), involving an Agilent Technologies 1200 Liquid Chromatograph equipped with a diode array detector (DAD), a thermostatic column module, a quaternary pump and an Agilent Eclipse XDB-C_{18} LC column (5 µm particle size, 4.6 mm × 150 mm i.d.). The injection volume was 100 µL and the DAD wavelength 254.4 nm.

Quantum yield of the system and dipyrone estimation

To study the quantum yield of the HQ–H_2O_2–vanadium(IV) [V(IV)] system in the absence of dipyrone, sample solutions of V(IV) (in the range 2×10^{-3}–1 µg/mL) were prepared by serial dilution of the stock solution. All solutions were adjusted to the optimum working pH of 1.9. Samples (1.5 mL) were introduced into the chemiluminescence cell though an injection valve and in an aqueous carrier solution (distilled water at pH 1.9; flow rate, 4.5 mL/min).

A solution of quinolinic hydrazide was prepared at 2×10^{-3} mol/L by dilution of the stock solution. Hydrogen peroxide (1.7×10^{-3} mol/L) was pH-adjusted to 13.2 with 0.17 mol/L NaOH. The quinolinic hydrazide–H_2O_2 solution was pumped at a flow rate of 4.5 mL/min and mixed at the junction of the T-piece, with the carrier solution also flowing at a rate of 4.5 mL/min. The mixture was allowed to immediately enter the reaction coil. Chemiluminescent emissions were then recorded by the detection system. All solutions were tested in triplicate.

For the determination of dipyrone, sample solutions were prepared by adding the compound to the V(IV) solution. The dipyrone concentration varied in the range 5×10^{-3}–1 µg/mL; the final V(IV) concentration in all dipyrone solutions was 3.29×10^{-3} mol/L. Light emissions were then recorded again.

The HPLC comparison determinations were undertaken using methanol and water (65:35, v/v) as mobile phases A and B. The flow rate was 0.6 mL/min. Under these conditions, the retention time of dipyrone was 3.25 min.

System optimization

In this study the systematic super-modified simplex multivariate method was used, which varies all the parameters to find the shortest path to the optimum. It is generally recommended in the simplex that the number of variables does not exceed five or six, so as not to create a very complex system. Initially, experiments were conducted to control the temperature, the presence of oxygen and buffer addition. Because these variables had no significant influence, they were removed from the Simplex system. There was a univariate optimization of injection volume, photomultiplier voltage and concentration of hydrazide and a simplex multivariate optimization of the five main variables: the concentration and pH of the vanadium solution, the pH of the quinolinic hydrazide solution, the H_2O_2 concentration and the carrier flow rate, making a total of 135 experiments in this part.

Five main system variables were optimized using the super-modified simplex method (32) (Table 1) before preparing the calibration curve or performing dipyrone estimations. Multivariate sequential optimization methods, such as the simplex method, allow the simultaneous variation of the different variables affecting the response of the system, providing the shortest route to the optimal conditions. This is more efficient and accurate than univariate optimization methods. The latter are more simple to perform and are probably the most widely used in chemistry, but univariate methods suffer from strong interactions between variables, increasing the probability of a false optimum being returned (indeed, this is quite common). The loop volume of the injection valve (1.5 mL) and the voltage of the photomultiplier (830 V) were previously optimized and therefore were not included among these variables. The injection volume that provided the highest quantum yield of the system was determined by injecting different times at a constant flow rate. The ranges of the above five variables were optimized by taking into account the data obtained in previous quinolinic hydrazide chemiluminescence studies (34,35), in which FIA techniques were not used. The fixed variables were: quinolinic hydrazide concentration, 2.0×10^{-3} mol/L; vanadium injection volume, 1.5 mL; and photomultiplier voltage, 830 V.

For quinolinic hydrazide solution pH values > 13.0, the alkaline function (H_) was used (37) (expressed as NaOH concentration).

Table 2 shows the variation in chemiluminescence intensity with pH. These results confirm that the reaction is dependent on pH and that a strongly alkaline medium is required for the best results.

Table 2. Variation of chemiluminescence intensity with pH

pH range	Chemiluminescence (%)
10–11.5	6
11.5–12.5	43
12.5–13.2 (H_)	82
Optimum 13.2 (H_)	100
> 13.2 (H_)	90

H_, alkaline function.

Results and discussion

Figure 3 shows the vanadium(IV)-catalysed mechanism of reaction between quinolinic hydrazide and H_2O_2. Dipyrone reduces the quantum yield by interfering with reaction (A), i.e. the formation of the semidione radical. This radical is an intermediate compound in the formation of excited phthalate ions, those responsible for light emissions.

Analytical performance and validation

The calibration curve for dipyrone determination showed a linear interval between 1 and 50 µg/mL. The experimental data

fitted a straight line, obtained by the least squares method, with a correlation coefficient of $R = 0.998$ for $n = 7$. The equation of this line was: $I = 879.24\ (\pm\ 6.67) - 5.73\ (\pm\ 0.29)\ C$, where I is the relative intensity of the emission and C is the dipyrone concentration in µg/mL. The detection limit, calculated by the method of Miller (38), was 2.52 µg/mL for a signal:noise (S:N) ratio of 3. The quantitation limit (S:N = 10) was 8.4 µg/mL. The relative standard deviation (RSD) for seven dipyrone injections (15 µg/mL) was 3%. Eighty-five samples can be processed in 1 h.

Application of the method

For the injectable formulations, the results and recoveries were in good agreement with those provided by the HPLC reference procedure. No significant differences in dipyrone concentration were seen between the proposed method and HPLC at the 95% confidence level ($f_{exp} < f_{tab}$), comparing variances by the procedure described by Miller (38) (Table 3). However, for the solid formulations the results were not very good, probably due to the excipients present. In the Nolotil capsules, magnesium stearate is a major excipient. Indigo carmine, erythrosine, titanium dioxide and gelatin are also present in lesser amounts (Table 4).

Compared to the current chemiluminescent/FIA procedures for dipyrone estimation (28–30), the proposed method offers certain advantages: (a) the range of dipyrone concentrations that can be detected is greater than that offered by the (30) method; (b) there is no need for preconcentration, as in the (29) method; and (c) the throughput is greater than that of the (28) method (85 samples/h compared to 60/h). Table 5 compares the characteristics of the proposed method with those of the above and several other methods for determining dipyrone (15,20,24,25,28–30).

Conclusion

This new method for determining dipyrone concentrations in injectable pharmaceutical preparations provides results comparable to those obtained using an HPLC reference technique – but more quickly and cheaply. It also provides advantages over

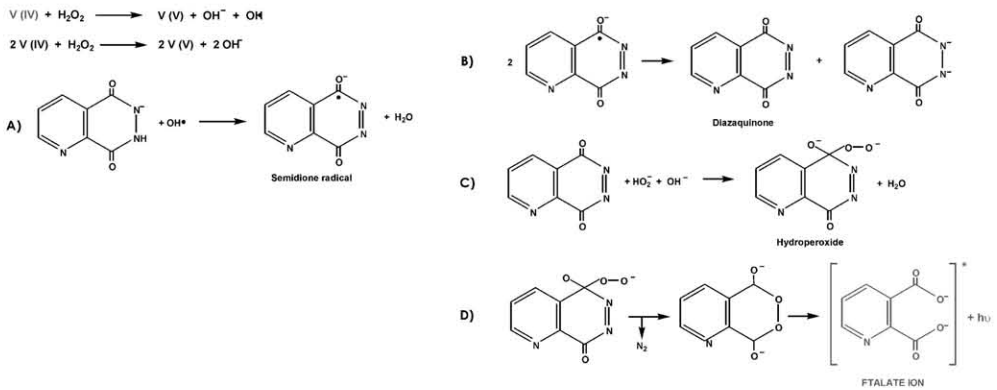

Figure 3. Reaction mechanism between quinolone hydrazide and H_2O_2, catalysed by V(IV).

Table 3. Validation of method for dipyrone determination

	Hydrazide method			HPLC method				
Dipyrone added (µg/mL)	Dipyrone found (µg/mL)	S	n	Dipyrone found (µg/mL)	S	n	F_{exp}	F_{tab} (95%)
5	5.21	–0.48	8	4.93	0.27	4	3.16	14.62
10	10.21	–0.5	7	9.50	0.26	4	3.69	14.73
15	14.93	–0.44	7	14.63	0.29	4	2.30	14.73

F_{exp}: F experimental; F_{tab}: F tabulated.

Table 4. Dipyrone determination in pharmaceutical formulations

Sample	Content on label	Dipyrone concentration ± SD		RSD %	
		QH – H_2O_2–V(IV) [a]	HPLC [b]	QH–H_2O_2–V(IV)	HPLC
Nolotil (injection)	400 (mg/mL)	395.2 ± 0.5 (mg/mL)	392 ± 0.3 (mg/mL)	3.56	6
Normon (injection)	400 (mg/mL)	400.5 ± 0.5 (mg/mL)	397 ± 0.3 (mg/mL)	3.51	2.14
Nolotil (capsule)	575 (mg)	1123.7 ± 1.8 (mg)	570 ± 1.3 (mg)	1.79	2.6

[a]Average ± SD of 4 determinations.
[b]Average ± SD of 3 determinations.

Table 5. Comparison with other methods

Reaction system	Detection	FIA	Linear range (LOD, µg/mL)	Throughput (samples/h)	Ref. no.
PDAB	Spectrophotometric	Yes	10–400 (1)	50	20
Luminol–dichromate	CL	Yes	0.0005–0.05 (2×10^{-5})	60	28
Rodamine 6 G–Tween 80	CL	Yes	0.05–10 (0.003)	–	29
Luminol–H_2O_2–$Fe(CN)_6^{3-}$	CL	Yes	2.5–12.5 (2.1)	87	30
Pt electrode	Amperometric	Yes	10–50 (0.009)	48	15
Reverse phase–C_{18}-DAD	HPLC	No	4.5–38 (1.5)	–	24
Reverse phase–C_{18}-DAD	HPLC	No	0.5–100 (0.0004)	–	25
QH–H_2O_2–V(IV)	CL/FIA	Yes	1–50 (2.5)	85	This study

CL, chemiluminescent.

the currently used chemiluminescent/FIA methods. These advantages may render the proposed system very suitable for the routine analysis of dipyrone in injectable pharmaceutical formulations.

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