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DEVELOPMENT AND VALIDATION OF METHODS USING DERIVATIVE SPECTROPHOTOMETRY FOR DETERMINATION OF DIPYRONE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT: Dipyron (sodium noramidopyrine methanesulfate), a member of the pyrazolone group, is a drug that possesses analgesic, antipyretic, anti-inflammatory, and spasmolytic properties. Traditionally, iodimetric methods have been recommended for the quantitative determination of dipyron in all its pharmaceutical forms. This work describes the development of zero order and fourth order ($\Delta\lambda = 10$) derivative spectrophotometric methods for the determination of dipyron in different pharmaceutical formulations, using water as solvent. The analytical curves presented correlation coefficients greater than 0.999, limits of detection varying from 0.23 to 0.49 mg L⁻¹, and limits of quantification varying from 0.78 to 1.63 mg L⁻¹. The average recoveries ranged from 98 to 103 % for the zero order method and from 96 to 104 % for third and fourth order derivative methods. Intra and inter-day precisions, expressed as relative standard deviations, were below 4.2 %. The excipients present in dipyron tablets and solutions did not interfere in the analyses. The method was shown to be linear, reproducible, specific, sensitive and rugged.

INTRODUCTION: Dipyron (1-phenyl-2, 3-dimethyl-5-pyrazolone-4-methylaminomethane sulfonic acid) is the sodium sulfate derivative of aminopyrine¹. It is also known as sodium noramidopyrine methanesulfate, pitophenone and methyl-2[4-(2-piperidinoethoxy)benzyl] benzoate². **Fig. 1.** It belongs to the pyrazolone group and has analgesic, antipyretic, anti-inflammatory, and spasmolytic properties^{3,4}.

In its sodium mono-hydrate form, it is an almost white, odorless, crystalline powder. It is soluble in water and methanol, weakly soluble in ethanol, and almost insoluble in ethyl ether, acetone, benzene, and chloroform. It should be stored in containers protected from light^{5,6}. Dipyron, in combination with other compounds, is useful for relieving fever and pain, and is employed for the treatment of post-surgical pain, renal and biliary colic, cancer pain, and osteoarthritic pain⁷. Its peripheral, central, and anti-inflammatory activities are associated with inhibition of the cyclooxygenase enzyme system⁴.

However, use of dipyron can present a risk to human health⁸, with adverse effects including agranulocytosis^{9,10} and sometimes reactions such as transient disorders and inflammation of renal

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tissue, mainly in patients with history of renal disease or in cases of overdose¹¹.

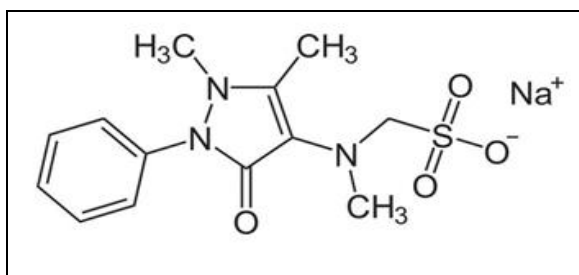


FIG. 1: STRUCTURE OF THE DIPYRONE MOLECULE

According to the Brazilian Pharmacopoeia⁵, dipyrone is marketed in the form of the sodium monohydrate, as tablets and in solution for oral administration. Iodimetry is the method recommended for the quantitative determination of dipyrone in all its pharmaceutical forms. Spectrophotometry is indicated for tablet dissolution assays, where the formulation should be dissolved in hydrochloric acid solution, followed by measurement of the absorbance at 258.0 nm.

Various other methods are reported in the literature for the determination of dipyrone, alone or associated with other active agents in different pharmaceutical formulations. These techniques include capillary electrophoresis¹², flow injection analysis^{13, 14}, voltammetry¹⁵, liquid chromatography^{16, 17}. Many of these methods are unattractive because they require expensive equipment, reagents that are toxic or high purity, and/or several analytical steps.

Molecular absorption spectrophotometry is widely used for the determination of pharmaceuticals, providing reliable results and offering advantages including speed and low cost. However, conventional (zero order) spectro-photometric techniques may be unsuitable for the analysis of drug mixtures without employing a previous separation step, due to overlapping electronic transition bands^{18, 19}. Analysis can also be problematic when there is interference from the excipients, or when the analyte presents weak absorbance²⁰.

Derivative spectrophotometry is useful when there is a signal overlap or interference, improving sensitivity and selectivity in the qualitative and quantitative analysis of pharmaceutical mixtures^{21, 22}. Its use helps to resolve the components of a

mixture, including excipients and degradation products, because the absorption bands become thinner, and it also provides better detection of small spectral signals¹⁸. Various methods have recently been reported that use derivative spectrophotometry for individual and simultaneous determination of drugs²³⁻²⁶.

The aim of the present work was to develop and validate zero order and derivative spectrophotometry methods in the determination of dipyrone in different pharmaceutical formulations, using deionized water as solvent. Validation of the proposed methods was based described parameters by ANVISA²⁷ and ICH²⁸.

MATERIALS AND METHODS:

Instrumentation and Data Treatment: The spectrophotometric measurements employed a Hitachi U-3000 dual-beam instrument, and the manufacturer's UV00 software was used for derivation of the data. The spectra were acquired in the range from 190 to 400 nm, with a slit width of 2 nm and scan speed of 300 nm/min, employing a quartz cell with a 1 cm optical path length. Origin 7.5 software was used for data processing, construction of graphs, and statistical treatments. Other items of equipment used were an analytical balance (model APX-200, Denver Instrument), a digital pH meter (model Tec-3MP, Tecnal), and a Purelab Option-Q water purification system that provided water with resistivity of 18.2 MΩ cm.

Reagents and Samples: All the reagents used were analytical grade. Distilled or deionized water was used as solvent. Hydrochloric acid (Merck) and sodium hydroxide (Synth) were used to adjust the pH of the dipyrone solution.

Sodium dipyrone monohydrate (Genix, batch 1001026, 99.95%) was employed as a standard. The excipients studied were talc (Henrifarma, batch N-113190-2, 99.0%), magnesium stearate (Valdequímica, batch 1444471, 98.7%), sorbitol 70% (Fragon and Deg, batch BC-05200611, 68.1%), carboxymethylcellulose (Attivos Magistrais, batch ACX01122F4, 99.6%), and colloidal silicon dioxide 200 (Aerosil) (Henrifarma, batch 3150111714, 99.9%).

The following pharmaceutical products containing dipyrone in tablet or oral solution forms were used:

Medley[®] 500 mg (tablet: batch 12061284; oral solution: batch 228526) and Anador[®] 500 mg (tablet: batch 8295; oral solution: batch 7936).

Optimization of Derivation Conditions: The derivatives of the zero order spectra were obtained with the UV00 software. The spectrum obtained for a freshly prepared 50 mg L⁻¹ standard solution of dipyrone was treated to obtain the 1st, 2nd, 3rd and 4th order derivative spectra. For each derivative spectrum, the wavelength ($\Delta\lambda$) was varied from 1 to 10 nm.

Methodology:

Robustness: For evaluation of the influence of the solvent, 125 mg portions of dipyrone were weighed out and quantitatively transferred to 250 mL volumetric flasks, with the volumes being completed using distilled or deionized water, resulting in 50 mg L⁻¹ solutions. The spectra obtained for each solvent, immediately after preparation of the solutions, were compared using the paired t-test applied at the wavelength of interest and at greater and smaller λ values, for a total of 11 points in the spectrum. A significance level of 5 % was adopted in the t-tests.

The stability of dipyrone was evaluated using different conditions of light, temperature, and solvent. Solutions of dipyrone at a concentration of 50 mg L⁻¹ were prepared in distilled or deionized water in volumetric flasks protected from light. Each solution was dispensed into one transparent polyethylene flask and two amber flasks. One of the amber flasks was then placed in a refrigerator at 4.0 ± 0.5 °C and the other was left on the laboratory bench at room temperature (25.2 ± 0.6 °C).

Zero order, third order (3D) and fourth order (4D) derivative spectra ($\Delta\lambda = 10$) of the dipyrone solutions were acquired immediately after preparation and after 30 min, 1 h, 2 h, 3 h, 4 h, 24 h, and 48 h. The intervals in the spectra at which there was the least temporal variation of the absorbance were then compared by applying the paired t-test to the data at eleven consecutive points, with the analysis wavelength as the central value. The effect of pH was determined by comparing the spectra obtained for 50 mg L⁻¹ dipyrone solutions at different pH values. Aliquots of 1 mL of 500 mg L⁻¹ dipyrone solution were

placed in eleven 25 mL volumetric flasks, to which were added aliquots of 0.01 mol L⁻¹ hydrochloric acid or 0.01 mol L⁻¹ sodium hydroxide ranging from 10 to 1000 μ L, followed by completion of the volumes with deionized water. The pH values of the solutions was measured and the spectra were acquired.

Selectivity: Stock 200 mg L⁻¹ solutions of each excipient studied were prepared and then diluted to concentrations of 2.5, 5.0 and 10 mg L⁻¹. Spectra were obtained for these solutions and for solutions containing the mixture of excipients at concentration levels of 2.5, 5.0, and 10 mg L⁻¹, without dipyrone and with dipyrone at 50 mg L⁻¹. The zero order and derivative spectra of the standard solution of dipyrone and the mixtures of dipyrone and excipients, in the ranges of interest, were analyzed by comparison using the paired t-test, applied at eleven consecutive points including the median analytical wavelength.

Linearity: The linearity of the methods was evaluated using the zero order and derivative spectra for fifteen standard solutions at concentrations between 10 and 150 mg L⁻¹. The analytical curve for dipyrone was obtained from the spectra of seven solutions at concentrations in the range from 10 to 70 mg L⁻¹. The same standards were used to construct curves using the 3D ($\Delta\lambda = 10$) and 4D ($\Delta\lambda = 10$) derivative spectra.

Detection and Quantification Limits: The limits of detection (LD) and quantification (LQ) were calculated using Equations 1 and 2, respectively:

$$LD = 3 s/SC \quad (1)$$

$$LQ = 10 s/SC \quad (2)$$

Where s is the standard deviation obtained for measurements of 21 blank solutions and SC is the slope of the analytical curve²⁷.

Accuracy: Accuracy was determined as the percentage recovery of a known amount of analyte added to the sample, considering the linear range of the analytical curve. The assay was performed at three concentrations (low, medium, and high), with three replicates at each level²⁷. For each tablet formulation, six tablets were homogenized using a mortar and pestle. The mass required to prepare a stock solution containing 100 mg L⁻¹ of dipyrone

was weighed out (in triplicate) and transferred quantitatively to 100 mL volumetric flasks, followed by completion of the volumes with deionized water. For the oral solution formulations, volumes corresponding to the mass required to prepare a 100 mg L⁻¹ dipyrone solution were used.

A 2.0 mL volume of the 100 mg L⁻¹ Medley[®] or Anador[®] tablet solutions was added to each of four 10.0 mL volumetric flasks, giving final concentrations of 20 mg L⁻¹. Aliquots of 1.0, 2.0, and 3.0 mL of the 100 mg L⁻¹ dipyrone standard solution were then added to three of the flasks, giving dipyrone concentrations of 10, 20, and 30 mg L⁻¹, respectively. One of the flasks remained without addition of dipyrone standard solution. The volumes were completed with deionized water.

The procedure used for the oral solution formulations was similar to that described for the tablets. 2.5 mL aliquots of the 100 mg L⁻¹ Medley[®] sample were added to four 10.0 mL flasks, followed by addition of 1.25, 2.50 and 3.75 mL of the 100 mg L⁻¹ dipyrone standard solution, giving concentrations of 12.5, 25.0 and 37.5 mg L⁻¹, respectively. No standard solution was added to the fourth flask. The volumes were completed with deionized water. The same procedure was performed for the Anador[®] oral solution, adding the standard solution at concentrations of 15.0, 30.0, and 45 mg L⁻¹. The recovery assays were performed in triplicate for all the samples.

Precision: Intra-day and inter-day precisions were determined at three concentration levels for each formulation. Solutions were prepared containing dipyrone at concentrations in the range from 30 to 60 mg L⁻¹ (according to the information provided by the manufacturers). The precisions were evaluated using the relative standard deviations (RSD), and paired t-tests were used to compare the values obtained using the different methods.

RESULTS AND DISCUSSION: Dipyrone dissolved in deionized water absorbs in the ultraviolet region of the electromagnetic spectrum, with an intense band at around 193.0 nm **Fig. 2**. This band exhibits a bathochromic shift as the concentration increases^{29,30}, so Beer's law does not apply and therefore, this band was not used for the quantitative determination of dipyrone.

The spectrum also contains a less intense molecular absorption band in the range from 220 to 270 nm. Many organic compounds exhibit broad bands that often appear to be continuous, resulting from the overlap of vibrational transitions with electronic transitions, leading to complex combinations of superimposed lines³⁰. The wavelength range 220-270 nm was employed in the stability and robustness assays, with derivatives of the zero order spectra being used to obtain clearer absorption bands and improved quantitative analysis of the drug.

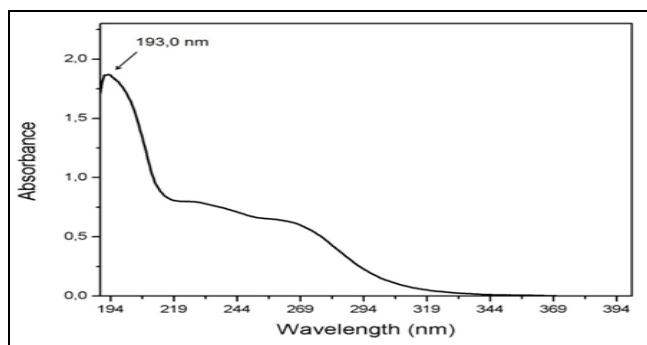


FIG. 2: SPECTRUM OF ABSORPTION OF DIPYRONE IN DEIONIZED WATER AT 50 mg L⁻¹

Robustness: Robustness is a measure of the ability of a method to tolerate variations in analytical parameters, providing an indication of the stability of the technique. In the case of a spectrophotometric method, factors that should be considered in determining robustness include the nature of the solvent, temperature, and solution pH²⁷.

Selection of the Solvent: The dipyrone solutions were prepared in water, because the compound is highly water soluble, hence minimizing costs and avoiding the use of toxic solvents. Dipyrone is also soluble in methanol, which can reduce its degradation³¹, but this solvent was not used here because it is toxic, difficult to handle, and its improper disposal can harm the environment. Spectral scans of the solutions of dipyrone prepared in distilled water and deionized water were performed in the wavelength range from 190 to 400 nm. The spectra obtained soon after preparation of the solutions presented similar profiles, but were significantly different (paired t-test, $p = 0.05$) in the wavelength range used for the analysis. Deionized water was selected as the solvent, since its quality could be controlled more easily, compared to distilled water.

Stability of Dipyrone: The stability of the dipyrone solution was evaluated considering the presence of light and different temperatures and solvents. Dipyrone undergoes hydrolysis in an aqueous medium³², which resulted in temporal changes in its spectral profile for all the storage conditions tested. Effects on the spectral profile due to variation of temperature and the presence of light were similar for the two solvents (distilled water and deionized water).

The decomposition of dipyrone was slower under refrigeration ($4.0 \pm 0.5^\circ\text{C}$) than at room temperature ($25.2 \pm 0.6^\circ\text{C}$), in agreement with the literature, since the hydrolysis of dipyrone is thermodynamically favored at higher temperatures³². Decomposition was slower in the solutions protected from light, compared to those exposed to light.

The spectral profile of dipyrone prepared in deionized water changed over time, at room temperature and in the presence of light **Fig. 3**, while the absorbance at 244 nm remained almost invariant. Comparison of the spectra at ten

wavelengths adjacent to 244 nm revealed no statistically significant differences (paired t-test, $p = 0.05$) for a period of up to 24 h. Therefore, this wavelength was selected for the determination of dipyrone by zero order spectrophotometry.

Derivative dipyrone absorption spectra were obtained in order to achieve well-defined bands, hence facilitating selection of the best analysis wavelength. The noise in the derivative spectra increased as the derivative order increased.

This difficulty was resolved by increasing the value of $\Delta\lambda$ in calculation of the derivative. It was therefore, important to optimize $\Delta\lambda$ experimentally in order to improve selectivity, sensitivity, and the signal-to-noise ratio. The lowest noise and highest sensitivity of the spectra were obtained using $\Delta\lambda = 10$, which was adopted for all derivative orders. The 1st, 2nd, 3rd and 4th order derivatives of the zero order dipyrone spectra, according to time, were obtained for solutions prepared in deionized water, at room temperature and in the presence of light, using $\Delta\lambda = 10$ **Fig. 4**.

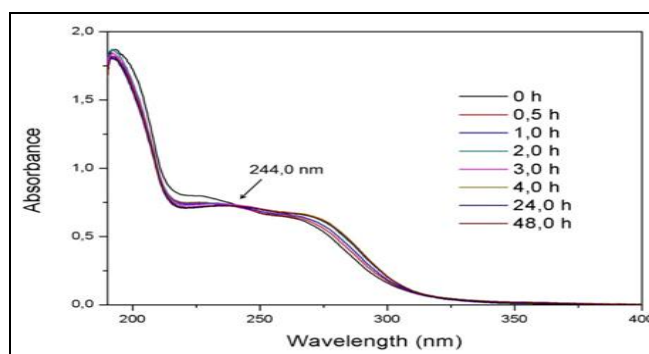


FIG. 3: SPECTRUM OF ABSORPTION OF DIPYRONE IN DEIONIZED WATER AT AMBIENT TEMPERATURE AND IN THE PRESENCE OF LIGHT

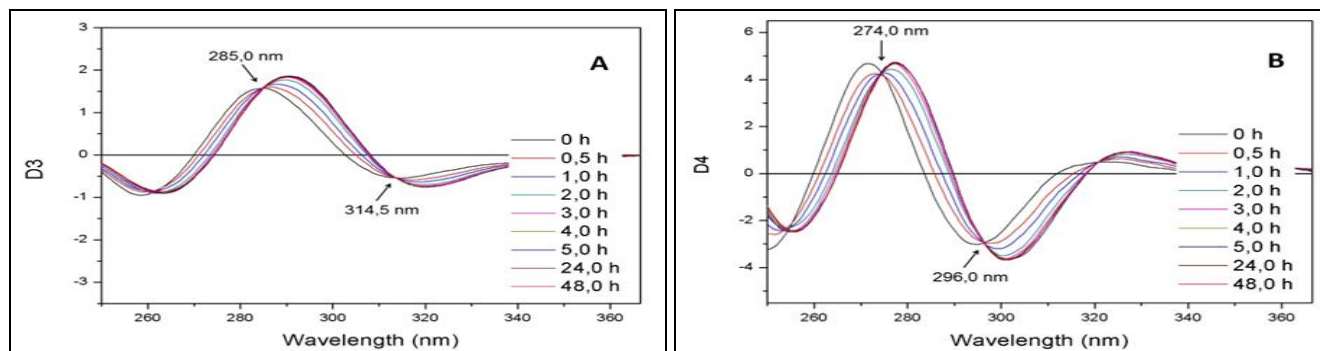


FIG. 4: DIPYRONE SPECTRA DERIVED FROM 3D (A) AND 4D (B) WITH $\Delta\lambda$ 10 IN DEIONIZED WATER AT ROOM TEMPERATURE AND IN THE PRESENCE OF LIGHT

The derivative spectra presented better defined bands, compared to the zero order spectra, with invariant signals at 285.0 and 314.5 nm for the 3D

spectra and at 274.0 and 296.0 nm for the 4D spectra. Application of the paired t-test ($p = 0.05$) for ten neighboring points at each wavelength

revealed no significant differences up to 48 h. These wavelengths were therefore selected for the determination of dipyrone using the derivative methods. The first and second order derivative spectra, obtained using $\Delta\lambda = 10$, were rejected because the signals showed significant differences over time, at all wavelengths, and were therefore unsuitable for use in the analyses. In order to minimize the effects of dipyrone degradation, the prepared solutions were protected from light and stored under refrigeration where appropriate.

Effect of pH: The 50 mg L⁻¹ solution of dipyrone in deionized water was slightly acidic (pH 6.2). The spectral profile altered with increasing acidity. This effect was studied by varying the pH in a broad range, from pH 3.5 to pH 9.6, and in a reduced range, from pH 5.9 to pH 6.3. At pH 4.4, the spectral profile of dipyrone in an aqueous medium was altered, with the appearance of a weak band at 258.0 nm, which became better defined as the acidity was increased further. No changes in the spectral profile were observed in basic media (up to pH 9.6).

The pH is one of the factors that can contribute to rapid hydrolysis of dipyrone, especially in acidic media³². Hydrolysis occurs due to a shift in the chemical equilibrium, with the acid reacting with the secondary amine of the 4-methylamino-antipyrine product, which exists in equilibrium with dipyrone in aqueous solution, forming a protonated compound³¹. Acid-catalyzed hydrolysis has been suggested previously as the main mechanism of dipyrone hydrolysis³². No changes in the spectral profiles were observed when the dipyrone solution was subjected to small pH variations (from pH 5.9 to pH 6.3). The determination of dipyrone in an aqueous medium

could therefore be performed without the need for pH adjustment.

Selectivity: The excipients studied were those commonly found in dipyrone formulations. The spectra for solutions containing the same concentration of dipyrone, alone or together with the excipients, were compared using the paired t-test ($p = 0.05$) for ten wavelengths close to 244.0 nm, revealing no statistically significant differences. Similarly, no significant differences were found for the derivative spectra at wavelengths of 285.0 nm and 314.5 nm (3D, $\Delta\lambda = 10$), and 296.0 nm and 294.0 nm (4D, $\Delta\lambda = 10$). The zero order and derivative spectra for the solutions containing the mixture of excipients showed zero signals at the wavelength for dipyrone analysis, with no differences for concentrations of 2.5, 5.0 and 10.0 mg L⁻¹. The selectivity study showed that the excipients did not interfere in the determination of dipyrone.

Linearity and Limits of Detection and Quantification: Linearity was evaluated from the relation between the absorbance and the concentration of the standard. The equation of the analytical curve was obtained by the least squares method³⁰. Analytical curves for the different methods were constructed using the wavelengths identified in the study of robustness. The methods showed good linearity, with correlation coefficients (r) higher than 0.999 **Table 1**, exceeding the minimum value of 0.99 recommended by ANVISA²⁷. The methods showed linear ranges between 70 and 90 mg L⁻¹, with working curves obtained in the range from 10 to 70 mg L⁻¹, as well as good sensitivity, with low limits of detection and quantification **Table 1**.

TABLE 1: ANALYTICAL PARAMETERS FOR THE QUANTIFICATION OF DIPYRONE

Method	λ (nm)	Linear equation	R	LOD (mg L ⁻¹)	LOQ (mg L ⁻¹)	LOL (mg L ⁻¹)
ZO	244.0	A=0.0368+0.0250C	0.9999	0.49	1.63	90.0
D3	285.0	D3=-0.0055+0.0660C	0.9998	0.23	0.78	90.0
D3	314.5	D3=0.0184-0.0240C	0.9994	0.40	1.35	80.0
D4	274.0	D4=-0.1561+0.1829C	0.9997	0.28	0.94	70.0
D4	296.0	D4=0.0695-0.1247C	0.9998	0.35	1.18	90.0

ZO - Zero order, D3 - Third order derivative, D4 - Fourth order derivative, LOD - Limit of detection, LOQ - Limit of quantification, LOL - Limit of linearity, r - linear correlation coefficient.

The methods showed linear ranges between 70 and 90 mg L⁻¹, with working curves obtained in the range from 10 to 70 mg L⁻¹, as well as good

sensitivity, with low limits of detection and quantification **Table 1**. The LQ value indicates the smallest amount of the analyte present in a sample

that can be determined with precision and accuracy, under the experimental conditions employed^{27, 28}. The LD and LQ values for the derivative methods were smaller than for the zero order method, reflecting greater sensitivity of the derivative spectrophotometric methods, compared to conventional spectrophotometry.

Accuracy: Evaluation of accuracy, using standard addition and recovery, enabled determination of the influence of the excipients on the dipyrone analyses, as well as the agreement between the analytical results and the true values^{27, 28}.

The accuracy was assessed using two different brands of pharmaceuticals containing dipyrone in tablet and solution forms. The recoveries obtained for the formulations at three concentration levels were in the ranges 98 - 103% for the zero order method (244.0 nm) and 96 - 104% for the 3D (285 nm) and 4D (296 nm) methods **Table 2**. These results were indicative of satisfactory analytical performance, since the recovery values were close to the optimum (100%). Lower values than these are permissible, given adequate accuracy and precision²⁷.

TABLE 2: RESULTS OF ACCURACY TESTS FOR THE SAMPLES AT THREE CONCENTRATION LEVELS

Method λ (nm)	Sample	Added (mg L ⁻¹)	Recovered (mg L ⁻¹)	Recovery (%)	RSD (%)
ZO 244.0	MT	10.0	9.9	99	1.9
		20.0	19.5	98	2.5
		30.0	29.8	99	1.2
D3 285.0	MT	10.0	9.9	100	1.1
		20.0	20.1	101	1.1
		30.0	30.4	102	0.3
D4 296.0	MT	10.0	9.8	98	4.1
		20.0	19.8	99	3.3
		30.0	30.7	102	0.8
ZO 244.0	MS	12.5	12.3	98	6.2
		25.0	24.7	99	1.4
		37.5	36.6	98	2.0
D3 285.0	MS	12.5	12.0	96	7.3
		25.0	24.3	97	1.6
		37.5	36.0	96	2.5
D4 296.0	MS	12.5	12.0	96	7.1
		25.0	24.6	98	1.4
		37.5	36.1	96	2.2
ZO 244.0	AT	10.0	10.2	102	3.4
		20.0	20.5	103	0.6
		30.0	30.6	102	1.3
D3 285.0	AT	10.0	10.2	102	1.7
		20.0	20.4	102	1.5
		30.0	30.4	101	0.7
D4 296.0	AT	10.0	9.7	97	4.1
		20.0	19.8	99	1.3
		30.0	29.8	99	1.4
ZO 244.0	AS	15.0	15.0	100	4.9
		30.0	30.1	100	2.7
		45.0	45.0	100	2.3
D3 285.0	AS	15.0	15.3	102	4.9
		30.0	31.1	104	1.4
		45.0	46.5	103	3.3
D4 296.0	AS	15.0	14.8	99	4.8
		30.0	29.5	98	3.0
		45.0	44.8	100	2.8

ZO - Zero order; D3 - Third order derivative; D4 - Fourth order derivative; MT - sample Medley® (tablet); MS - sample Medley® (solution); AT - sample Anador® (tablet); AS - sample Anador® (solution); RSD - Relative standard deviation

The results were comparable to those obtained elsewhere using liquid chromatography to determine dipyrone, where recoveries in the range

93-100% were obtained¹. Poor recoveries were obtained here using wavelengths of 314.5 nm (3D) and 294.0 nm (4D).

Precision: The intra-day and inter-day precisions were determined for the formulations at three different concentration levels. The intra-day precision, for two determinations, was expressed by the agreement between data obtained on the same day, by the same analyst, while the inter-day precision considered analyses performed on different days by different analysts²⁹. For all the formulations, the RSD values obtained using the proposed methods were below 5% **Table 3** and were therefore in compliance with the recommen-

dations of ANVISA²⁷ and ICH²⁸. The precisions of the developed methods were compared using the paired t-test ($p = 0.05$), which showed that there were no significant differences among the results obtained for the intra-day and inter-day precisions of the different methods.

Overall, the results obtained for the validation parameters demonstrated that the proposed analytical methods were suitable for the quantitative analysis of dipyrone.

TABLE 3: INTRA-DAY AND INTER-DAY PRECISIONS FOR THE PROPOSED METHODS

Method λ (nm)	Sample	Concentration (mg L ⁻¹)	Intra-day precision		Inter-day precision
			RSD (%)	RSD (%)	RSD (%)
ZO 244.0	MT	20.0	2.7	3.1	3.3
		30.0	2.3	3.4	3.9
		40.0	1.8	2.6	3.8
D3 285.0	MT	20.0	1.7	2.5	3.4
		30.0	1.9	2.6	3.7
		40.0	2.2	2.5	3.8
D4 296.0	MT	20.0	1.6	3.2	3.6
		30.0	1.9	2.3	3.9
		40.0	3.0	2.8	3.8
ZO 244.0	MS	40.0	0.5	1.5	3.3
		50.0	1.4	0.9	1.1
		60.0	0.7	0.3	0.8
D3 285.0	MS	40.0	0.4	0.4	1.7
		50.0	1.1	1.0	0.9
		60.0	0.8	0.5	1.6
D4 296.0	MS	40.0	0.4	1.3	1.5
		50.0	1.2	0.1	0.9
		60.0	0.9	0.4	1.8
ZO 244.0	AT	40.0	3.4	1.6	1.7
		50.0	2.8	1.2	0.8
		60.0	1.2	2.0	1.8
D3 285.0	AT	40.0	1.7	1.5	1.4
		50.0	2.8	1.7	1.3
		60.0	1.5	2.0	1.3
D4 296.0	AT	40.0	3.1	1.1	1.6
		50.0	2.6	2.7	1.3
		60.0	1.9	2.5	1.3
ZO 244.0	AS	40.0	0.7	0.9	3.4
		50.0	1.3	2.0	2.7
		60.0	0.3	0.4	4.2
D3 285.0	AS	40.0	0.7	0.7	3.7
		50.0	1.5	1.4	2.5
		60.0	0.4	0.4	4.1
D4 296.0	AS	40.0	0.3	1.2	3.2
		50.0	1.3	2.1	2.4
		60.0	0.3	0.4	4.2

ZO - Zero order; D3 - Third order derivative; D4 - Fourth order derivative; MT - sample Medley® (tablet); MS - sample Medley® (solution); AT - sample Anador® (tablet); AS - sample Anador® (solution); RSD - Relative standard deviation

The derivative methods had the advantage of being more sensitive than the zero order method. All the techniques were robust, selective, and showed good precision.

CONCLUSION: The zero order and derivative spectrophotometry techniques developed for the determination of dipyrone in pharmaceutical formulations were found to be selective, with no

interference of excipients in the analyses. The methods presented good linearity, sensitivity, accuracy, and precision. Other advantages included simplicity, speed, an absence of toxic reagents, and the ability to determine dipyrone in different pharmaceutical forms.

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