Colorimetric Determination of Dipyrone

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Abstract □ The reaction product of dipyrone with nitrous acid was utilized for its colorimetric determination in the pure state and in tablets and injections. The derivative, so formed, obeys Beer's law in ammoniacal medium and had a maximum absorbance at 403 nm. The proposed method has the advantage over the iodometric USSR Pharmacopoeia IX method in that: (*a*) it can be applied to a much lower concentration of dipyrone, and (*b*) sodium sulfite, which is used as an antioxidant in dipyrone injections, does not interfere with the determination.

Keyphrases □ Dipyrone—colorimetric determination, bulk and formulations □ Colorimetry—analysis of dipyrone, bulk and formulations

Various methods have been described for the determination of dipyrone [sodium (antipyrinylmethylamino)methanesulfonate monohydrate]. It was determined gravimetrically as sodium sulfate after ashing with sulfuric acid (1). Several iodometric methods have been commonly used (2–4). Wirth (5) recommended that the sample be weighed instead of measured in the iodometric titration of dipyrone in parenteral preparations, and he proposed the determination of specific gravity as a method of assay. Iodometric methods have also been described for the determination of dipyrone in mixtures (6–8). One iodometric method (9) depends on the fact that dipyrone is split into sulfite by potassium cyanide in moderately alkaline solution and then the liberated sulfite is titrated with standard iodine solution in an acid medium. Trials were made (10) to explain the mechanism of the iodometric titration.

Dipyrone was also determined complexometrically after oxidation of the sulfite group to sulfate (11). Alternatively, the sulfate ion produced was titrated with barium chloride solution (12). Other methods include the titration of dipyrone with chloramine solution (13) and potentiometric titration with 0.1 *N* perchloric acid solution in nonaqueous solvents (14, 15).

The color produced when dipyrone reacted with sodium β-naphthoquinone-4-sulfonate was employed (16) for its identification and determination. This method was modified (17) to eliminate the interference by primary amines, and the reaction mechanism was explained (18). The color reactions of dipyrone with a Schiff reagent (19), 4-dimethylaminocinnamaldehyde (20, 21), quinone (22), and *p*-diazobenzenesulfonic acid (23) were used for its determination. Dipyrone was also determined colorimetrically by measuring the absorbance of its complex with copper chloride (24).

A spectrophotometric method was also described in which dipyrone was determined at 272 nm. (25). A rapid determination of dipyrone in solution by IR spectroscopy was also proposed (26). Furthermore, a polarographic method was suggested (27), in which a polarographically active derivative of this compound was determined.

In this report, a colorimetric method for determining dipyrone in the pure state and in tablets and injections is described.

EXPERIMENTAL

Apparatus and Reagents—The following were used: a spectrophotometer[1]; dipyrone, USSR Pharmacopoeia IX grade; sodium nitrite, chemical reagent; 2.0 *N* hydrochloric acid solution; and dilute ammonium hydroxide solution, prepared by diluting 33.3 ml. of concentrated ammonium hydroxide solution to 100.0 ml. with distilled water.

Construction of Standard Absorbance Graph—Prepare a series of solutions containing 40.0, 60.0, 80.0, 100.0, and 120.0 mg. dipyrone/5.0 ml. solution by dissolving 0.400, 0.600, 0.800, 1.000, and 1.200 g., respectively in distilled water in a series of five 50-ml. volumetric flasks and dilute to volume. Accurately measure 5.0 ml. of each solution and transfer into a series of five 50-ml. beakers. To each of the five beakers, add 0.5 g. of sodium nitrite followed by 20.0 ml. of 2.0 *N* hydrochloric acid solution. Heat gently with continuous stirring for 7 min. until the solutions begin to boil. Cool, transfer the contents of each beaker to a 100-ml. volumetric flask, and dilute to volume with 2.0 *N* hydrochloric acid solution. Accurately measure 2.0 ml. of each solution, transfer to a 25-ml. volumetric flask, and dilute to volume with dilute ammonium hydroxide solution. Shake well and measure the absorbance of each prepared solution at 403 nm. against a blank prepared by substituting 5.0 ml. of distilled water for 5.0 ml. of the dipyrone solution. Results are shown in Table I. The equation of the straight line was determined by the method of least squares, and the slope of the line was found to be 0.066.

Pure Compound—Weigh accurately about 0.750 g. of dipyrone, place in a 50-ml. volumetric flask, dissolve in distilled water, and dilute to volume. Measure accurately 5.0 ml. of the prepared solution, place in a 50-ml. beaker, and proceed as described previously. Measure the absorbance at 403 nm. against a blank prepared by substituting 5.0 ml. of distilled water for 5.0 ml. of the dipyrone solution. Determine the concentration from the straight-line equation using the measured absorbance. Results are shown in Table II.

Tablets—Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder, equivalent to 0.750 g. of dipyrone, to a 50-ml. volumetric flask. Dissolve in distilled water, dilute to volume, and filter. Transfer 5.0 ml. of the filtrate, accurately measured, to a 50-ml. beaker and proceed as described previously. Measure the absorbance at 403 nm. against a blank prepared by substituting 5.0 ml. of distilled water for 5.0 ml. of the dipyrone solution. Determine the concentration from the straight-line equation using the measured absorbance. Results are shown in Table II.

Injections—Accurately measure 3.0 ml. of the mixed contents of injections, equivalent to 1.500 g. of dipyrone, and transfer to a 100-ml. volumetric flask. Dilute to volume with distilled water and shake well. Measure 5.0 ml. of this solution, place in a 50-ml. beaker, and proceed as usual. Measure the absorbance at 403 nm. against a blank prepared by substituting 5.0 ml. of distilled water for 5.0 ml. of the dipyrone solution. Determine the concentration from the straight-line equation using the measured absorbance. Results are shown in Table II.

RESULTS AND DISCUSSION

The nitrosation of dipyrone with sodium nitrite and 2.0 *N* hydrochloric acid solution produced a yellowish derivative, which was

[1] Unicam SP600.

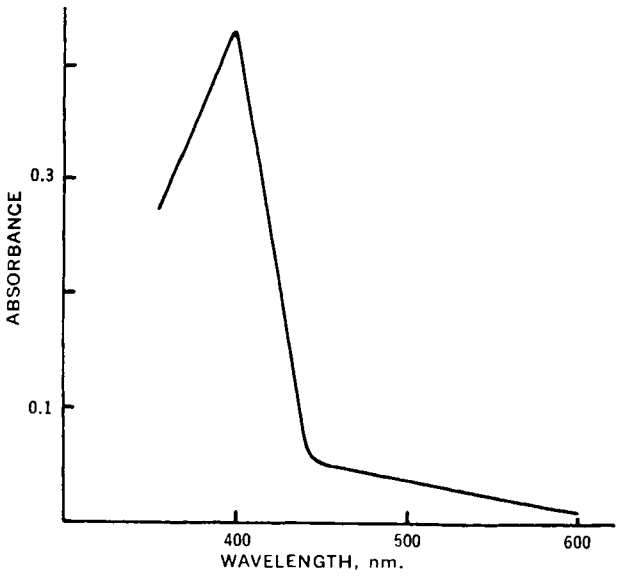


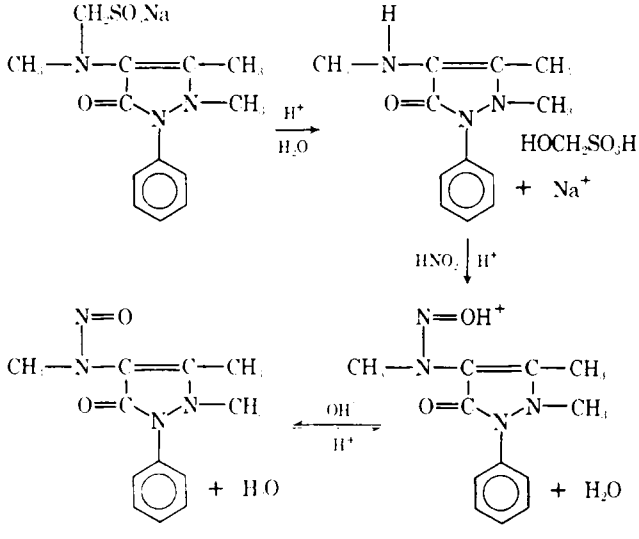
Figure 1—*Absorbance spectrum of dipyrone.*

intensified when the medium was made alkaline with ammonium hydroxide solution. This reaction was put on a quantitative basis to determine dipyrone in the pure state as well as in tablets and injections. The produced color had an absorbance peak at 403 nm. (Fig. 1) and obeyed Beer's law in a concentration range of 3.2–9.6 mg./100.0 ml. For a 72-hr. study period, the color was stable and gave no change in absorbance over a wide concentration range.

The scope of the method for control purposes was demonstrated by its application to a series of samples of dipyrone in the pure form and in different pharmaceutical forms such as tablets and injections (Table II). The presence of tablet excipients such as lactose, starch, and magnesium stearate did not interfere with the determination. The iodometric method adopted by USSR Pharmacopoeia IX also was used to determine dipyrone in the pure state and in tablets, and the results (Tables II and III) indicate that the proposed method is equivalent in accuracy and precision. However, the proposed method is more sensitive and selective.

The presence of reducing agents such as sodium sulfite, which are added to dipyrone injections as stabilizers, did not interfere with the proposed method. Such a reducing agent is expected to interfere with the iodometric method of USSR Pharmacopoeia IX because of the interaction of iodine and the reducing agent. Table IV shows the recovery data of dipyrone in prepared injections containing various amounts of sodium sulfite when the iodometric method was employed; dipyrone recoveries ranged from 100.81% in the absence of sodium sulfite to 125.79% when 48.0 mg. of this stabilizer was added.

The colored derivative formed when dipyrone is treated with sodium nitrite and hydrochloric acid solution is believed to be the



Scheme I

Table I—Absorbance of Different Concentrations of Dipyrone at 403 nm.

Concentration, mg. %	Average Absorbance
3.2	0.230
4.8	0.325
6.4	0.430
8.0	0.540
9.6	0.650

Table II—Determination of Dipyrone in Pure Form, Tablets, and Injections by the Proposed Colorimetric Method

Concentration, mg. %—Added	Found	Recovery, %
	Pure Compound	
3.6	3.68	102.22
4.2	4.27	101.69
5.2	5.33	102.50
6.6	6.67	101.06
7.8	7.91	101.41
	Average	101.77
	SD	0.59
	Tablets	
6.0	6.09	101.50
6.0	6.09	101.50
6.0	6.01	100.16
6.0	6.06	101.00
6.0	6.09	101.50
	Average	101.13
	SD	0.59
	Injections	
6.0	5.94	99.00
6.0	6.06	101.00
6.0	6.03	100.50
6.0	5.94	99.00
6.0	6.03	100.50
	Average	100.00
	SD	0.94

Table III—Determination of Dipyrone in Pure Form and Tablets by the USSR Pharmacopoeia IX Method

Sample Number	Weight, g.	Recovery, %
	Pure Compound	
1	0.4002	98.07
2	0.3989	101.01
3	0.2000	101.60
4	0.2000	99.04
5	0.2001	100.70
6	0.2007	101.20
7	0.1995	99.29
8	0.1998	99.96
9	0.1833	103.45
	Average	100.48
	SD	1.60
	Tablets	
1	0.5000	99.34
2	0.5000	103.76
3	0.5001	103.08
4	0.5000	103.92
5	0.5010	104.66
6	0.5030	100.68
	Average	102.57
	SD	2.09

Table IV—Determination of Dipyrone in Injections Containing Variable Amounts of Sodium Sulfite by the USSR Pharmacopoeia IX Method

Sample Number	Content of Dipyrone, g.	Content of Sodium Sulfite, mg.	Recovery, %
1	0.5010	00.0	100.81
2	0.5020	14.0	105.73
3	0.5030	23.0	112.67
4	0.5030	38.0	118.11
5	0.5010	48.0	126.79

nitroso derivative of one of its hydrolysis products (27). Scheme I illustrates the suggested mechanism for the formation of such a derivative.

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Optimum Conditions for GLC Analysis of Neomycin

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Abstract □ A previously described GLC method for the analysis of neomycin was critically examined to delineate the problems involved and to resolve the difficulties encountered in the procedure. The breakdown and/or adsorption of the silyl derivatives is minimized by modifying the injection port of one instrument so as to eliminate all metal and Teflon contact with the injected material.

Keyphrases □ Neomycin—GLC analysis, optimum conditions □ GLC—analysis, neomycin, optimum conditions

Neomycin is an antibiotic used extensively throughout the world. Many dermal, renal, and ototoxic reactions associated with its therapeutic use have been reported in the literature. These reports, however, do not indicate the chemical composition of the antibiotic bulk used in commercial formulations, although neomycin is known to be comprised of at least five different components in variable amounts (1). These neomycin fractions have different responses to various microorganisms and may also differ in therapeutic activity and potency as well as in pharmacological properties. Improved methods of analysis are necessary to elucidate and characterize the chemical nature of these substances.

A reported GLC method (2), which is regularly utilized at The Upjohn Company, appears to be the best, practical method available for the qualitative and quantitative analysis of neomycin fractions, particularly neomycin B and neomycin C isomers which normally predominate. The method had been under evaluation at the National Center for Antibiotic Analysis (NCAA) for some time. Test results by The Upjohn and NCAA laboratories were comparable for the potency values of commercial bulk samples containing a fairly low amount (less than about 5%) of neomycin C. However, there was poor interlaboratory correlation between results for the neomycin C content for most bulk samples. Be-