International Journal of Pharmaceutics, 61 (1990) 261–264
Elsevier

IJP 02121

Notes

Colorimetric determination of dihydralazine sulfate using paramolybdate anion as an oxidizing agent

Prodromos B. Issopoulos

Laboratory of Analytical Chemistry, Department of Inorganic and Analytical Chemistry, University of Ioannina, Ioannina (Greece)

(Received 15 August 1989)
(Modified version received 18 February 1990)
(Accepted 21 February 1990)

Key words: Dihydralazine sulfate; Paramolybdate anion; Pharmaceutical analysis; Colorimetry

Summary

A suitable procedure for the colorimetric assay of dihydralazine sulfate (DZLN) using paramolybdate anion (PMA) as oxidizing agent was developed. Samples of DZLN, either in pure form or in injections, were treated with PMA in a warm (98 ± 0.5°C), acidified aqueous medium for 30 min. The blue solution formed exhibits an absorption maximum at $\lambda = 688$ nm and Beer's law is obeyed over the range of 1.0–50.0 ppm of DZLN. The apparent molar absorptivity is 1.01×10^4 l mol^{-1} cm^{-1} and the Sandell's sensitivity for log $I_0/I = 0.001$ is equal to 28.4 ng cm^{-2} (both referred to DZLN analysed). The accuracy and the precision of the method were considered as very satisfactory. The results obtained from the determination of DZLN using the proposed new method and the chloramine T procedure, both applied on samples of the same batch, were analysed statistically by means of Student's *t*-test and by the variance ratio *F*-test and found to show no significant difference.

Dihydralazine sulfate (1,4-dihydralazinophthalazine sulfate (DZLN) is an antihypertensive agent which acts predominantly by causing direct peripheral vasodilation, tending to impove renal, uterine and cerebral blood flow and its effect on diastolic pressure is more marked than on systolic pressure.

For about 25 years, various methods for the analysis of DZLN, either in the pure form or in pharmaceutical preparations (Grecu and Curea, 1965; Kalinowski, 1965; Issopoulos, 1970; Youssef et al., 1977; Duan et al., 1987; Salman, 1987) or in human body fluids (Degen et al., 1982) have been reported.

Meanwhile, the use of ammonium paramolybdate (PMA) for the determination of DZLN, either as an auxiliary substance for volumetric estimation or as a principal reagent for spectrophotometric assay, has been reported previously (Grecu and Curea, 1965; Issopoulos, 1970). Certainly, the conception and the methodology of the above-mentioned analytical uses of PMA are quite different from those of the procedure which is developed in the present work.

However, this paper describes a simple, accurate and sensitive method for the colorimetric determination of DZLN, which is based on the observation that, in a warm acidic medium, the drug analysed reduces Mo(VI) to 'molybdenum blue'.

Correspondence: P.B. Issopoulos, Laboratory of Analytical Chemistry, Department of Inorganic and Analytical Chemistry, University of Ioannina, Ioannina (Greece).

A standard solution of 100 ppm of DZLN (Ciba-Geigy, batch no. 5530) was freshly prepared, as required, by dissolving the appropriate amount of anhydrous drug in water. The exact concentration of the resulting solution was checked spectrophotometrically (Clarke, 1974). PMA solution (1.0% w/v) and sulfuric acid (1.0 M) were prepared by dilution, as required, of analytical grade reagents. Deionized, freshly double-distilled water was used throughout. A Hitachi model 100-80, double-beam spectrophotometer with matched 10-mm quartz cells was used for all absorbance measurements and a thermostated constant temperature water-bath with a sensitivity of $\pm 0.5\,^{\circ}C$ was employed.

The recommended procedure involved 0.25–12.5-ml aliquots of the standard solution of DZLN (equivalent to 25–1250 μg DZLN) being pipetted into a 50 ml test-tube. Into the same test-tube were added 5.0 ml of 1% PMA solution and 5.0 ml of 1.0 M H_2SO_4 solution. The mixture was diluted with water to 20–23 ml, mixed by shaking and then placed in a water bath, thermostatted at 98 °C, for 30 min. After the heat treatment, the solution was immediately cooled to room temperature using a cold water bath. The resulting blue solution was quantitatively transferred into a 25 ml calibrated flask and diluted to the mark with water prior to mixing, followed by being allowed to stand for 15 min at room temperature in the dark for stabilization of the color, which remains unchangeable for at least 12 h. The absorbance of the solution was measured at $\lambda = 688$ nm against a blank sample which had been treated similarly. The concentration of DZLN in the examined solution could be determined by reference to a corresponding calibration graph, which had been traced according to the regression line equation:

$$A = 3.5 \times 10^{-2} C + 3.9 \times 10^{-3}$$

$$(r = 0.9996;\ n = 6).$$

It is a well-established fact in analytical chemistry that a blue solution is obtained by reduction of an acidified solution of Mo(VI) or by oxidation of an acidified solution of Mo(V). These solutions of Mo(VI), which are commonly used for this purpose, are those of iso- and heteropolyanions of this metal.

The substances responsible for the blue color are various oxides and hydroxides of Mo, which appear as a common characteristic: the mean oxidation state of the metal is between 5 + and 6 +, reported examples being $MoO_{2.0}(OH)$ and $MoO_{2.5}(OH)_{0.5}$. However, these 'blue compounds' appear to represent an entire series of compounds having the same basic structure but differing in the charges of cations and anions, i.e. they are 'genotyping' compounds, with $MoO(OH)_2$ (olive-green) as one limit and MoO_3 (white) as the other (Cotton and Wilkinson, 1980).

It was observed by the same author (Issopoulos, 1989) that the color of a reduced solution of Mo(VI) changes between emerald blue and deep ultramarine blue, as a function of the nature and concentration of the absorbed species, which arises from the reduction of Mo(VI). This variation in color results in the simultaneous change of λ_{max} of the treated solution, owing to the different conditions for reduction of Mo(VI), e.g. nature of the reducing agent, temperature of the reaction, acidity of the solution. Another very important factor which affects the tone as well as the intensity of the color is the sequence of addition of the solutions for reaction. The optimum sequence which should be constant and unchangeable is: drug solution (reducing agent) – Mo(VI) solution (oxidizing agent) – sulfuric acid (acidifying agent).

The absorption spectrum of the colored reaction products formed was measured in the range 500–820 nm against a reagent blank. Maximum absorption occurred at $\lambda = 688$ nm. Beer's law was obeyed over the concentration range of 1.0–50.0 ppm, with an optimum region of 2.0–30.0 ppm. The apparent molar absorptivity and the Sandell's sensitivity, referred to DZLN analysed, were calculated to be 1.01×10^4 l mol^{-1} cm^{-1} and 28.4 ng cm^{-2}, respectively (average of five determinations).

Meanwhile, to determine the optimum conditions for maximum and faster color development of the reaction product, the rate of formation of molybdenum blue under the reducing effect of DZLN was investigated and the rate was found to be very slow at room temperature (20 °C). In

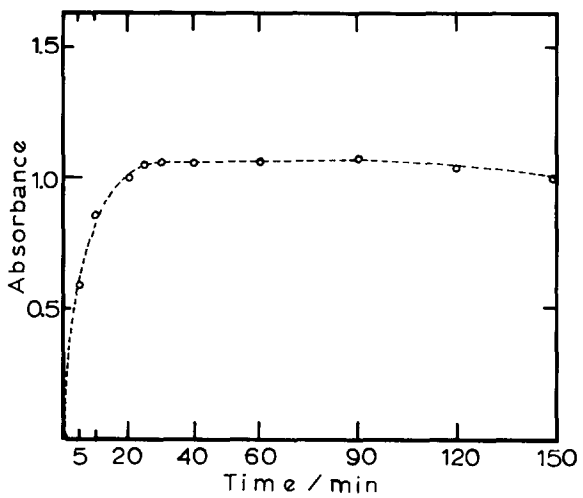

Fig. 1. Effect of heating time on formation of the colored product of DZLN-PMA (concentration of DZLN, 30 μg ml^{-1}; temperature, 98 ± 0.5 °C; λ = 688 nm).

order to accelerate it, higher temperature was used. Thus, after heating for at least 30 min at 98 ± 0.5 °C, the oxidation of DZLN had reached completion and maximum absorbance of the treated solution was achieved. (Prolongation of heating for more than 90 min is not only without effect but also will probably give undesirable results. Fig. 1 shows the effect of heating time on the formation of molybdenum blue.

In order to determine the accuracy and the precision of the proposed method, solutions containing four different concentrations of DZLN were prepared and five absorbance measurements were made on each reaction product obtained according to the above-described procedure. The results of this study are listed in Table 1. The results tabulated above were so advantageous as to be considered very satisfactory especially for the concentration levels examined.

Subsequently, the new method was applied for the determination of DZLN in the pure form and in 'Nepresol' powder for injection – 25 mg of DZLN per ampoule (Ciba-Geigy), a pharmaceutical formulation widely circulated in Greece.

Therefore, the same batches of pure form of DZLN and of Nepresol injection were each analysed five times, using the suggested method and also simultaneously by the chloramine T method (Pinzauti et al., 1974), since no official method exists in the British or U.S. Pharmacopoeias.

The values for recovery % and rsd% were:

(i) For DZLN in the pure form: 100.06 ± 1.075 and 1.068% (new method) and 99.76 ± 0.559 and 0.560% (chloramine T method);

(ii) For DZLN in Nepresol injection: 102.50 ± 0.500 and 0.488% (new method) and 101.40 ± 1.140 and 1.60% (chloramine T method).

TABLE 1

Accuracy and precision in the determination of dihydralazine sulfate

No.	DZLN sulfate (ppm)		rsd%	SAE [a]	Confidence limits
	Added	Found ± SD			($p = 0.05$; $(n-1) = 4$)
1	5.0	5.10			
2		4.97			
3		5.05			
4		5.00			
5		5.00			
Mean		5.024 ± 0.051	1.02	0.023	5.024 ± 0.064
6	10.0	9.93			
7		9.86			
8		10.00			
9		10.00			
10		10.07			
Mean		9.972 ± 0.080	0.80	0.036	9.972 ± 0.099
11	25.0	25.00			
12		24.69			
13		25.12			
14		25.26			
15		25.69			
Mean		25.152 ± 0.367	1.46	0.164	25.152 ± 0.456
16	40.0	40.00			
17		40.30			
18		40.40			
19		39.59			
20		39.45			
Mean		39.948 ± 0.420	1.05	0.188	39.948 ± 0.522
Mean rsd%			1.0825		
Mean SAE				0.10275	

[a] SAE, standard analytical error (SD/$\sqrt{n}$).

Subsequently, the t values obtained from a statistical analysis of the above results (t values calculated = 0.55 and 1.60, for pure form and Nepresol injection, respectively, while the tabulated t value for $p = 0.05$ and 8 degrees of freedom is equal to 2.306) show no significant difference between the two sets of the mean recoveries and the variance ratio F values (F value calculated to equal 3.69 for the pure form and 5.20 for the Nepresol injection when the tabulated F value for $p = 0.05$ and $f_1 = f_2 = 4$ is equal to 6.39) indicating that there is no significant difference between the precision of the two analytical methods applied.

References

Clarke, E.G.C., *Isolation and Identification of Drugs*, Pharmaceutical Press, London, 1974, p. 304.

Cotton, F.A. and Wilkinson, G., *Advanced Inorganic Chemistry*, 4th Edn, Wiley, New York, 1980, pp. 848, 861.

Degen, P.H., Brechbuehler, S., Schucider, W. and Zbinden, P., Determination of apparent dihydralazine in plasma by gas-liquid chromatography and electron capture detection. *J. Chromatogr.*, 233 (1982) *Biom. Appl.*, 22 (1982) 375–380.

Duan, L., Xiang, B., Sheng, L., Wu, R. and An, D., Orthogonal design-simplex method and its application to the analysis of compound antihypertensive tablets. *Yaoxue Xuebao*, 22 (1987) 761–768; *Anal. Abstr.*, 50 (1988) 6E61.

Grecu, I. and Curea, E., Determination of dihydrazinophthalazine sulfate. *Rev. Chim.*, 16 (1965) 389–390; *Chem. Abstr.*, 64 (1966) 11031a.

Issopoulos, P.B., Spectrophotometric determination of microquantities of 1,4-dihydrazinophthalazine sulfate. *Proc. 4th Natl. Chem. Conf.*, Athens, Greece, 1970, pp. 130–132; *Chem. Abstr.*, 85 (1976) 68349f.

Issopoulos, P.B., Analytical investigations of β-lactam antibiotics in pharmaceutical preparations. III. Spectrophotometric determinations of some cephalosporins using paramolybdate anion. *J. Pharm. Biom. Anal.*, 7 (1989) 619–625.

Kalinowski, K., Manganobromometric determination of dihydralazine with amperometric dead-stop and visual end-point detection. *Farm. Pol.*, 21 (1965) 914–916; *Chem. Abstr.*, 64 (1966) 17359b.

Pinzauti, S., Dal Piaz, V. and La Porta, E., Chloramine-T as titrimetric reagent in potentiometric determination of isoniazid, phenelzine and dihydralazine. *J. Pharm. Sci.*, 63 (1974) 1446–1448.

Salman, A., Spectrophotometric determination of dihydralazine sulfate with sodium 1,2-naphthoquinone-4-sulfonate. *Sci. Pharm.*, 55 (1987) 255–259.

Youssef, A.F., Ibrahim, S.A. and Elshabouri, S.R., Dihydralazine sulfate analysis using 2-methyl-3-nitropyridine-6-carboxaldehyde. *J. Pharm. Sci.*, 66 (1977) 116–118.