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Fluorimetric determination of oxamniquine in biological fluids

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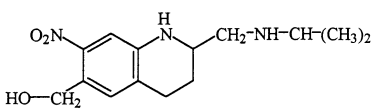
Abstract

A highly sensitive and specific fluorimetric method was developed for the determination of oxamniquine in biological fluids (urine and plasma). The proposed method is based on the reduction of oxamniquine using zinc/calcium chloride to obtain its nitroso derivative. The latter is then allowed to react with 2-cyanoacetamide to get a highly fluorescent product. The different experimental parameters affecting the intensity of the fluorescence were carefully studied and incorporated into the procedure. Under the described conditions, the method is applicable over the concentration range of 0.08–0.88 μg/ml with a minimum detectability ($S/N = 2$) of 8 ng/ml. The percentage recoveries from spiked urine and plasma were 99.75 ± 1.58 and $97.46 \pm 0.44\%$, respectively. A proposal of the reaction pathway is suggested.

Keywords: Oxamniquine; 2-Cyanoacetamide; Fluorometry; Biological fluids

1. Introduction

Oxamniquine(1,2,3,4-tetrahydro-2-[(isopropylamino)-methyl]-7-nitro-6-quinoline methanol) is an antischistosomal agent that is indicated for the treatment of *S. mansoni* (intestinal schistosomiasis) infection. It has been shown to inhibit DNA, RNA and protein synthesis in schistosomes. The oral bioavailability of oxamniquine is good, effective plasma levels are achieved in 1–1.5 h [1].



Oxamniquine is the subject of a monograph in the US Pharmacopoeia (USP XXIII) [2] whereby a spectrophotometric method is recommended for its determination, whether in its pure form or in capsules. Other reported methods include spectrophotometry [3,4], non-aqueous titration [5], gas chromatography [6], high-performance liquid chromatography [7–11], polarography [12], capillary electrophoresis [9], flow-injection analysis [13] and cyclic voltammetry [14]. The above-mentioned methods are either not sufficiently sensitive or are tedious and require highly sophisticated instrumentation. Therefore, there is still a need for a much more sensitive and simple method for the determination of oxamniquine, especially in biological fluids. Fluorimetry, by virtue of its high sensitivity, meets this requirement. Reviewing the literature revealed that no fluorimetric methods were reported for oxamniquine. As for 2-cyanoacetamide, it has been recently used for the determination of aminoglycosides [15], reducing carbohydrates [16–18], enzymatic degradation products of reducing polysaccharides [19] and catecholamines [20,21].

The aim of the present work is to study the reaction of the reduced derivative (nitroso) of oxamniquine with 2-cyanoacetamide in an attempt to develop a sensitive fluorimetric method to be used for its determination in biological fluids.

2. Experimental

2.1. Apparatus

An Aminco-Bowman Model J_4-9860 spectrofluorometer was used with the excitation and emission slits set at 5 mm. A 1-cm quartz cell was used for all measurements.

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2.2. Materials and reagents

All the chemicals used were of analytical reagent grade, while the solvents were of the spectroscopic grade. A pure drug sample of oxamniquine was kindly supplied by Pfizer (Sandwich, UK) and was used as received. 2-Cyanoacetamide was purchased from Aldrich Chemical Co. Plasma was obtained from Mansoura University Hospital, Mansoura (Egypt) and urine was obtained from healthy volunteers. Freshly prepared 10% aqueous solution of calcium chloride and 4% aqueous solution of 2-cyanoacetamide were prepared and used throughout the study.

2.2.1. Stock solution

Dissolve 20 mg of oxamniquine in 100 of methanol. Make serial dilutions with water to obtain a final concentration of 20 μg/ml.

2.3. Procedure

2.3.1. Preparation of the calibration curve

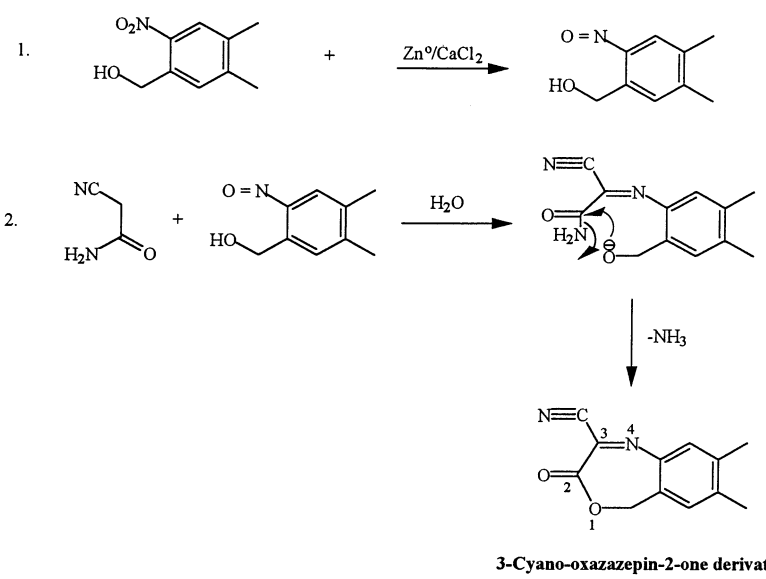
Transfer aliquots of oxamniquine final stock solution into a series of 25 ml standard flasks so that the final drug concentration is in the range 0.08–0.88 μg/ml. Add to each flask 120 ± 5 mg of zinc dust and 12 ml of calcium chloride solution. Shake well and leave to stand for 20 min. Filter the excess zinc powder. Add 1 ml of 2-cyanoacetamide solution to the filtrate, then heat on a boiling water bath for 1 h. Cool and fill to the mark in 25 ml standard flask with water. Measure the fluorescence of the filtrate using 370 nm as the excitation wavelength and 450 nm as the emission wavelength. A blank reagent is prepared simultaneously. Plot the values of the % relative intensity against the final concentration in μg/ml to get the calibration graph. Alternatively, the regression equation is derived.

2.3.2. Determination of oxamniquine in spiked human plasma and urine

A standard calibration curve was prepared daily by spiking blank plasma or urine with varying amounts of oxamniquine. A stock solution containing 10 μg/ml of oxamniquine was freshly prepared. Control samples of plasma or urine were spiked with different quantities of oxamniquine to give a final drug concentration of 0.2–0.8 μg/ml. Add 0.8 ml of 1 M NaOH with gentle shaking to 1.0 ml of the spiked urine or plasma. The solution was then vortexed with 3 × 5 ml of ether for 2 min then centrifuged at 2500 rpm for 5 min. The resulting supernatant was then evaporated to dryness under nitrogen at ambient temperature. The resulting residue was dissolved in 5 ml of methanol and then made up to 10 ml with water in a volumetric flask. The procedure described in Section 2.3.1 was then applied. A blank experiment using plasma or urine without the drug was carried out simultaneously. The content of oxamniquine was then determined from the previously plotted calibration graphs.

3. Results and discussion

The nitro group is reported to be reduced into the corresponding nitroso group upon reduction with Zn/$CaCl_2$ [22]. This process was confirmed by testing for the nitroso group using sodium nitroprusside [23]. The nitroso compounds were reported to react with active methylene containing compounds with the production of the corresponding imines [24]. The latter is proposed to undergo a ring-closing reaction with the elimination of one molecule of ammonia and the production of the 3-cyanooxazazepin-2-one derivative, which is the fluorescent product. A proposal of reaction pathway is suggested in Scheme 1. A highly fluorescent product



3-Cyano-oxazazepin-2-one derivative

Scheme 1. Proposal of the reaction pathway between 2-cyanoacetamide and the nitroso derivative of oxamniquine.

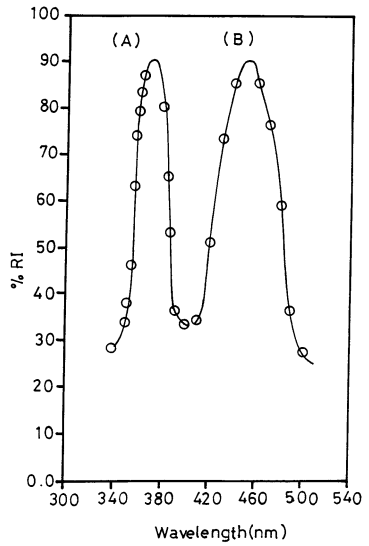


Fig. 1. Fluorescence spectrum of the reaction product of oxamniquine (0.86 μg/ml). (A) Excitation and (B) emission spectra.

with λ_{max} excitation at 370 nm and λ_{max} emission of 450 nm was obtained (Fig. 1).

3.1. Study of the experimental conditions

The factors affecting the production of nitroso derivative were first studied. All factors were kept constant except one, which was changed in turn to study its effect. Any amount of zinc powder in the range 100–300 mg was found to give the same results; therefore 120 ± 5 mg were used throughout the work. As for calcium chloride solution 12 ± 1 ml of 10% (wt./vol.) aqueous solution was found to give maximum fluorescence. Larger volumes caused slight decrease in fluorescence. Increasing the reduction time (at room temperature) was found to increase the fluorescence up to 20 min, after which time the fluorescence intensity remained unchanged.

3.2. Factors affecting the development of the fluorophore

The effect of the concentration of 2-cyanoacetamide was studied by using increasing volumes of 4% aqueous solution. It was found that 1.0 ± 0.1 ml is appropriate for maximum fluorescence intensity; larger volumes caused no further increase in fluorescence. The temperature and time of heating were also studied. It was concluded that heating in a boiling water-bath for 1 h gave the highest fluorescence readings. Longer heating time caused a decrease in fluorescence as shown in Fig. 2.

After optimizing the conditions, it was found that the relation between the % relative intensity (% RI) and final concentration of oxamniquine was rectilinear over the range 0.08–0.88 μg/ml with a minimum detectability ($S/N = 2$) of 8 ng/ml (Fig. 3). The linear regression analysis of the results gave the following equation:

$$\%\ \mathrm{RI} = 0.0358 + 107.4C \quad (r = 0.9999)$$

where C = final concentration in μg/ml, % RI = relative fluorescence intensity and r = correlation coefficient.

Statistical analysis of the results of analysis of authentic sample of oxamniquine obtained by both the proposed method and the official method [2] revealed no significant difference between the two methods regarding the accuracy and precision, as indicated by the F-test and Student's t-test, respectively [25].

Oxamniquine is readily absorbed following oral ingestion, and a peak concentration in plasma occurs within about 3 h. The presence of food significantly delays absorption and limits the concentration achieved

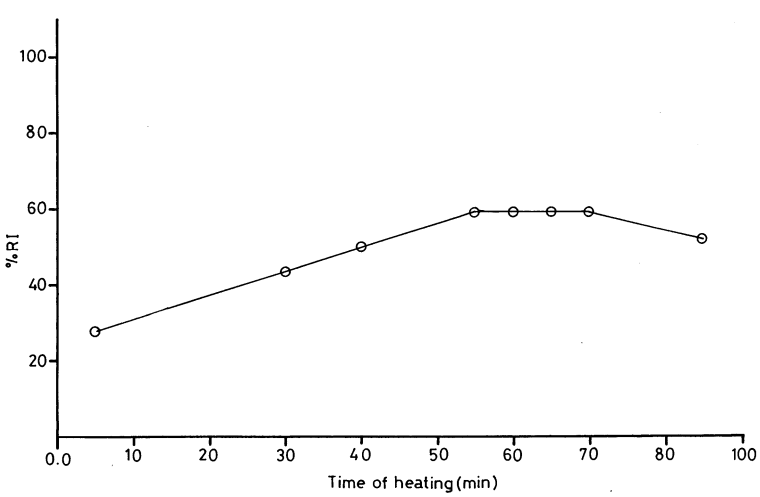


Fig. 2. Effect of the reaction time on the fluorescence intensity of oxamniquine (0.56 μg/ml).

in plasma during the first several hours after administration. Urinary excretion is the major route of elimination in humans [26]. Oxamniquine is given orally in a dose of 250 mg three times daily; this leads to a final blood level concentration of about 5 μg/ml, i.e. higher than the upper limit of the working range of the proposed method. Thus, the high sensitivity of the proposed method allowed the determination of oxamniquine in biological fluids. Table 1 shows the results for the fluorimetric determination of a pure sample of oxamniquine, while Table 2 shows the results of the fluorometric determination of oxamniquine in spiked urine and plasma samples, respectively. The extraction procedure described by Woolhouse and Wood [6] was adopted here. The results are satisfactorily accurate and precise.

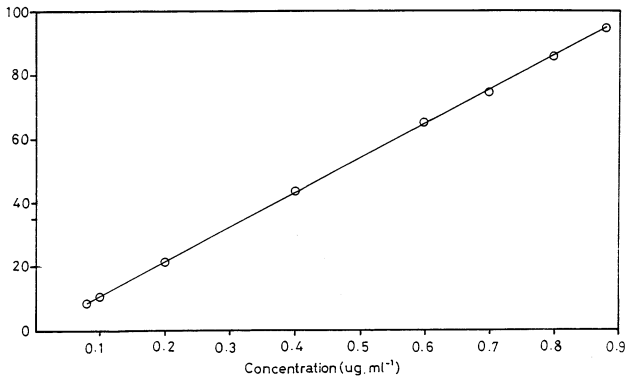


Fig. 3. Relation between the fluorescence intensity and the final concentration of oxamniquine at 370 nm (λ_{max} excitation) and 450 nm (λ_{max} emission).

Table 1
Fluorimetric determination of oxamniquine in its pure form[a]

Added (μg)	Found (μg)	Recovery (%)	
		Proposed method	Official method [2]
0.08	0.078	98.50	
0.10	0.098	98.00	
0.20	0.199	99.50	
0.40	0.404	101.00	
0.60	0.604	100.66	
0.80	0.796	99.50	
0 88	0.879	99.89	
$\bar{X}$		98.73	100.09
±S.D.		1.42	0.87

[a] Each result is the average of three separate determinations.

Table 2
Fluorimetric determination of oxamniquine in spiked biological fluids[a]

Sample	Added (μg)	Found (μg)	Recovery (%)
Urine	0.10	0.097	97.00
	0.20	0.202	101.00
	0.40	0.403	100.75
	0.60	0.593	98.83
$\bar{X}$			99.75
±S.D.			1.58
Plasma	0.20	0.195	97.50
	0.40	0.395	98.13
	0.60	0.390	96.63
	0.80	0.780	97.56
$\bar{X}$			97.46
±S.D.			0.44

[a] Each result is the average of three separate determinations.

In conclusion, a highly sensitive and accurate method was developed for the determination of oxamniquine in urine and plasma. Although the previously reported methods [6,7] for its determination in biological fluids may be less time-consuming, the proposed method is, however, more sensitive and does not need complicated instrumentation.

References

[1] Wilson, Gisvold, in: J.N. Delgado, W.A. Rames (Eds.), Textbook of Organic Medicinal and Pharmaceutical Chemistry, 9th ed., J.B. Lippincott, Philadelphia, PA, 1991, p. 184.
[2] The US Pharmacopoeia XXIII, NF 18, The US Pharmaceutical Convention, Rockville, MD, 1995, p. 1120.
[3] S.M. Hassan, F. Belal, M. Sharaf-El-Din, M. Sultan, Analyst 113 (1988) 1807.
[4] K. Kelani, L.I. Bebawy, Anal. Lett. 30 (1997) 1843.
[5] A. Korolkovas, T. Haraguchi, Rev. Farm. Bioquim. Univ. Sao Paulo 16 (1980) 12, Chem. Abstr. 94 (1981) 78 46e.
[6] N.M. Woolhouse, P.R. Wood, J. Pharm. Sci. 66 (1977) 479.
[7] H.W. Jun, M.A. Radwan, Anal. Lett. 18 (1985) 1345.
[8] A.F. Fell, T.A.G. Notor, J.E. Mama, B.J. Clark, J. Chromatogr. 404 (1988) 377.
[9] M.A. Abushoffa, B.J. Clark, J. Chromatogr. 700 (1995) 51.
[10] T.A. Noctor, A.F. Fell, B. Kaye, Chirality 2 (1990) 269.
[11] A.F. Fell, T.A. Noctor, B. Kaye, J. Pharm. Biomed. Anal. 7 (1989) 1743.
[12] F. Belal, F.A. Aly, Electroanalytical 7 (1995) 483.
[13] M.Y. Mohamed, A.E. El-Gendy, M.G. El-Bardicy, M.S. Tawakol, A.K.S. Ahmad, Spectrosc. Lett. 29 (1966) 299.
[14] A. Radi, F. Belal, J. Electroanal. Chem. 441 (1998) 39.
[15] N.A. Zakhari, Anal. Lett. 23 (1990) 1843.
[16] S. Honda, Y. Nishimura, M. Takahashi, H. Chiba, K. Kakehi, Anal. Biochem. 119 (1982) 194.
[17] S. Honda, K. Kakehi, Y. Fujikawa, Y. Oka, M. Takahashi, Carbohydr. Res. 183 (1988) 59.
[18] J.H.H. Van den Berg, H.W.M. Horsels, R.S. Deelher, J. Liquid Chromatogr. 7 (1984) 2351.
[19] E. Bach, E. Schollmeyer, Anal. Biochem. 203 (1992) 335.
[20] S. Honda, M. Takahashi, Y. Araki, K. Kakehi, J. Chromatogr. 274 (1983) 45.
[21] S. Honda, Y. Araki, M. Takahashi, K. Kakehi, Anal. Chim. Acta 149 (1983) 297.
[22] S.R. El-Shabouri, Talanta 33 (1986) 743.
[23] H.A. Abu-Shady, S.T. Hassib, H.H. Hassanien, A.M. Abadi, Bull. Fac. Pharm. Cairo Univ. 33 (2) (1994) 193.
[24] J. Merch, Advanced Organic Chemistry, 2nd ed., McGraw-Hill, Tokyo, 1977, p. 543.
[25] R. Caulcut, R. Boddy, Statistics for Analytical Chemists, Chapmann and Hall, London, 1983.
[26] A. Iqbal, A. Tauqir, in: K. Florey (Ed.), Analytical Profile of Drug Substances, vol. 20, Academic Press, New York, 1991, p. 623.