Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, No. 5, pp. 417–420, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00
Pergamon Press plc

Short Communication

Determination of the chromonar hydrochloride metabolite in urine using derivative spectroscopy

ABDEL FATTAH M. EL WALILY,* MAHMOUD A. ELSAYED, MAGDA H. BARARY and SUZY M. SABRY

Faculty of Pharmacy, Pharmaceutical and Analytical Chemistry Department, El-Mesallah, Alexandria 21521, Egypt

Keywords: *Chromonar hydrochloride; metabolism; acid metabolite;* in vivo *determination; urine; derivative spectroscopy.*

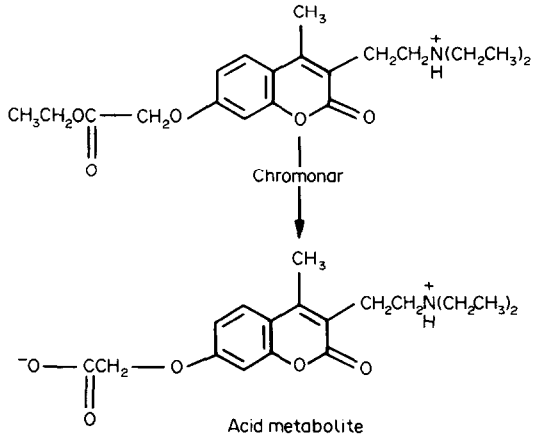
Introduction

Chromonar, 3-(-diethylamino-ethyl)-4-methyl-7-carbethoxy-methoxy-2-oxo-(1,-2-chromene), is a coumarin compound that is used for the treatment of angina pectoris in Germany, Japan and Egypt. When the drug comes into contact with biological tissues, it is rapidly hydrolysed to the corresponding acid (Scheme 1), which exists as a zwitterion at the pH of blood and urine [1]. Only two fluorimetric procedures have been reported for the quantitative determination of the drug metabolite in biological fluids [1, 2].

It was necessary to develop a simple, fast and accurate method for the determination of chromonar metabolite in urine which could provide the information required to assess how the drug was handled by the body.

The rapidly developing technique of UV derivative spectrophotometry has been applied to forensic toxicology [3, 4] and has also been used for the determination of benzodiazepines [5], amitriptyline, perphenazine [6], nitrofurantoin [7] and amoxycillin [8] in either urine or plasma.

The present study describes the application of first-(^{1}D) and second-(^{2}D) derivative spectroscopy for the determination of the acid metabolite of chromonar in urine.



Scheme 1
Metabolism of chromonar hydrochloride.

Experimental

Materials

Chromonar hydrochloride (CR) was kindly supplied by Hoechst-Orient S.A.A. (Cairo, Egypt). The acid metabolite (CRM) was prepared by incubating freshly collected human plasma with CR for 10 min. The hydrolysed product was recrystallized in ethanol–petroleum ether analysed and characterized using TCL and IR techniques [2]. The purity of the CRM was determined using a Hewlett–Packard gas chromatograph (model 8590) with a cross-linked methylsilicone gum capillary column (12 m × 0.2 mm i.d.; film thickness 0.33 μm). The GC was operated at 180–280°C at a heating rate of 10°C min^{-1} using nitrogen as the carrier gas. The retention times of CR

*Author to whom correspondence should be addressed.

and CRM were 3.47 and 5.25 min respectively. The purity of the CRM was found to be 98%. All solutions were prepared in double-distilled water. The commercial product (Intensain tablets each containing 150 mg of CR) was purchased locally.

Apparatus

A double beam UV–vis spectrophotometer model 550s (Perkin–Elmer) was used in the derivative mode with 10-mm quartz cuvettes and a Hitachi model 651 recorder. Instrument settings were: derivative mode (first and second); scan speed 120 nm min^{-1}; chart speed 60 nm min^{-1}; response time 6 s; and wavelength range 220–360 nm.

Standard solutions

A standard solution was prepared in distilled water to contain 0.5 mg ml^{-1} of CRM. Aliquots of 0.2–1.2 ml (in 0.2-ml increments) of the standard solution were transferred to 50-ml volumetric flasks. To each flask, 1 ml of blank urine was added and the solution was diluted to volume with distilled water. The ^1D and ^2D derivative spectra were recorded against a blank of 1 ml of urine diluted to 50 ml with distilled water.

Sample solutions

A single dose of one CR tablet (150 mg) was administered to a healthy male volunteer (19 years, 60 kg) with informed consent; no other drugs were taken during the study. Urine samples were collected at 1.5, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 h with complete emptying of the bladder after the medication had been taken. The volume of the urine was measured and recorded after each collection. A 1-ml aliquot of the urine was analysed for CRM by the proposed method.

Results and Discussion

The aim of this work was to develop a rapid, simple and sensitive assay for monitoring excreted CRM in human urine, for use in clinical studies.

The zero-order spectrum did not permit the detection and determination of CRM in urine owing to lack of sensitivity and to interference from the urine matrix (Fig. 1). Derivative spectroscopy has been used to eliminate broad absorption bands resulting from turbidity and matrix interference [9].

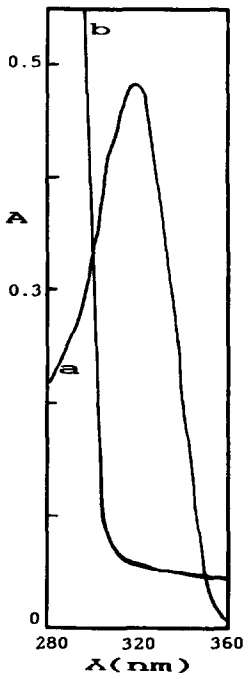


Figure 1
Absorption spectra of (a) 10 μg ml^{-1} of acid metabolite and (b) blank diluted urine.

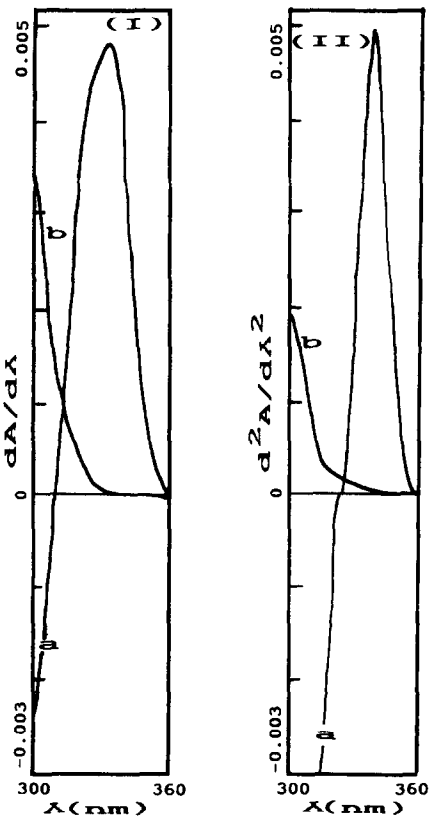


Figure 2
(I) First- and (II) second-derivative spectra of (a) 10 μg ml^{-1} of chromonar acid metabolite and (b) blank diluted urine.

The ^1D and ^2D derivative spectra of CRM showed maxima at 334 and 342 nm, respectively (Fig. 2). The graphs of the amplitudes of ^1D and ^2D at these wavelengths against concentration were linear within a concentration range of 1–12 μg ml^{-1}. The corresponding regression equations were:

$$^1D = 0.00108 + 0.00669c \quad (r = 0.9994)$$
$$^2D = -0.00002 + 0.00042c \quad (r = 0.9996)$$

where c was the concentration of the CRM in μg ml^{-1} and r was the correlation coefficient. The intercepts were negligibly small for both calibration curves.

The within-day precision was evaluated by replicate analysis of pooled urine samples spiked with CRM. The mean recoveries of CRM were 99.4 and 99.7% with relative standard deviations (RSD) of 0.87 and 0.96% ($n = 4$) for ^1D and ^2D, respectively. The between-day precision was similarly evaluated on several days up to 4 days. In this case the mean recoveries for the CRM were 99.6 and 100% with RSD of 0.49 and 0.77% ($n = 4$) for ^1D and ^2D, respectively. In both cases the results indicated a high precision since the RSD did not exceed 1%.

To describe the urinary excretion kinetics, two parameters were evaluated (Table 1): the cumulative amounts of CRM excreted after 7.0 h following oral administration; and the elimination rate constant K was calculated from the slopes of the straight lines obtained

Table 1
Pharmacokinetic parameters of the urinary excretion of the chromonar acid metabolite

Pharmacokinetic parameters	ARE method* ^1D	ARE method* ^2D	ER method† ^1D	ER method† ^2D
Excretion rate (h^{-1})	1.098	1.059	1.025	1.142
Half-life (h)	0.632	0.655	0.677	0.607
	^1D		^2D	
Cumulative amount excreted (μg)	33.89		34.25	

*ARE = amount of the metabolite remaining to be excreted.
†ER = excretion rate.

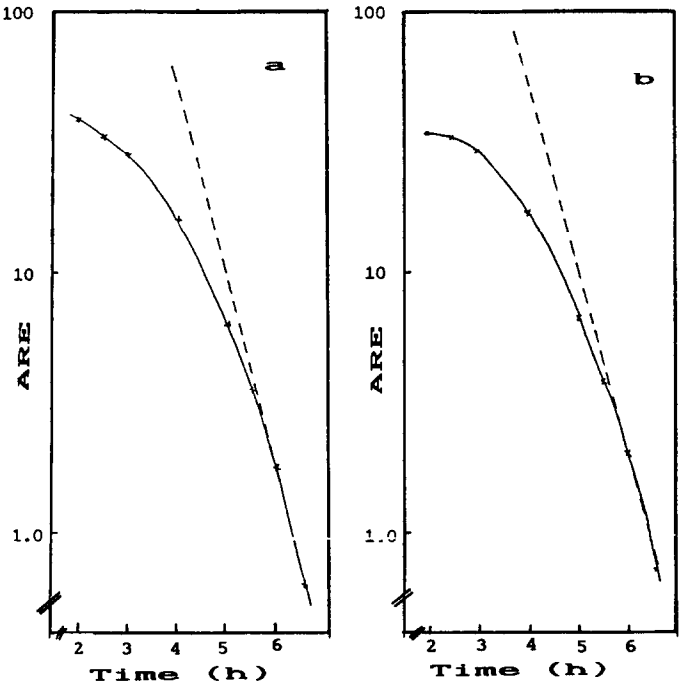


Figure 3
Semilogarithmic plots of $M_u^\infty - M_u$ (ARE) versus time after oral administration of chromonar hydrochloride, from (a) ^1D and (b) ^2D measurements.

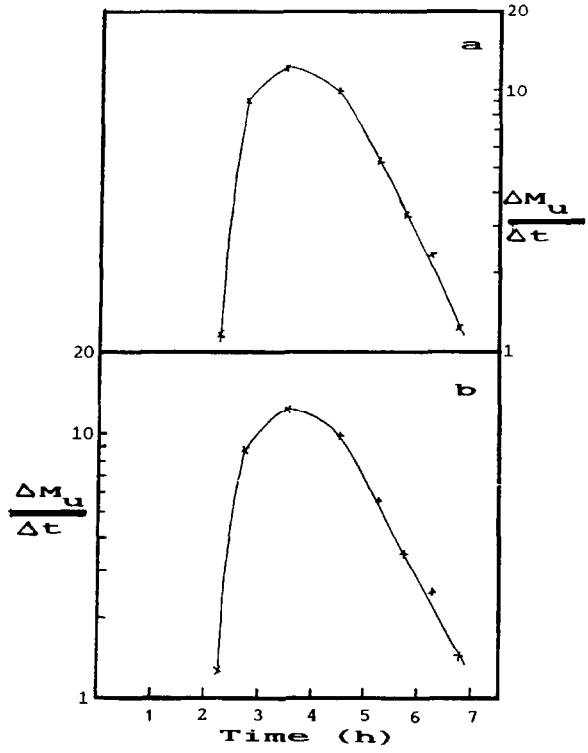


Figure 4
Semilogarithmic plots of excretion rate versus time (mid-point of the urine collection interval) after oral administration of chromonar hydrochloride, from (a) ^{1}D and (b) ^{2}D measurements.

either by plotting the amount of CRM remaining to be excreted versus time (ARE method) [10] (Fig. 3) or by plotting the rate of excretion of CRM versus the midpoint of the urine collection time (ER method) [10] (Fig. 4).

First- and second-derivative spectroscopic methods are simple and rapid. They have a satisfactory precision and accuracy for CRM concentrations of 1–12 μg ml^{-1} and can be used routinely for monitoring the urinary excretion of the chromonar acid metabolite.

References

[1] M. Klarwein and R.E. Nitz, *Arzneim-Forsch.* **15**, 555 (1965).
[2] Y.C. Martin and R.G. Wiegand, *J. Pharm. Sci.* **59**, 1313–1318 (1970).
[3] R. Gill, T.S. Bal and A.C. Moffat, *J. Forensic Sci. Soc.* **22**, 165–171 (1982).
[4] A.F. Fell, D.R. Jarvie and M.J. Stewart, *Clin. Chem.* **27**, 286–292 (1981).
[5] D. Martinez and M.P. Gimenez, *J. Anal. Toxicol.* **5**, 10–13 (1981).
[6] P. Fernandez, A.M. Bermejo and M. Lopez-Rivadulla, *Anal. Lett.* **21(B)**, 1045–1054 (1988).
[7] M. Poulou and P. Macheras, *Int. J. Pharm.* **34**, 29–34 (1986).
[8] A.M. El Walily, M.A. El Sayed, M.A. Korany and S.M. Galal, *J. Clin. Pharm. Ther.* In press.
[9] P.G. Green and J. Hadgraft, *Int. J. Pharm.* **46**, 193–195 (1988).
[10] M. Gibaldi and D. Perrier, *Pharmacokinetics* 2nd edn, pp. 7–10, Marcel Dekker, New York (1982).

[Received for review 24 November 1989; revised version received 11 January 1991]