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Journal of Chromatography B, 698 (1997) 312–316

JOURNAL OF CHROMATOGRAPHY B

Short communication

Contribution to the determination of the chemotherapeutic drug G1 in biological fluids

Lizette Gil del Valle*, Daniel Gebru W/Michael, Alina Martinez Rodríguez, Rolando Tápanes Peraza, Francisco Ramos, Jorge Pérez Avila

Department of Clinical Pharmacology, Institute of Tropical Medicine "Pedro Kourí" (IPK), Box 601, Marianao 13, Ciudad de la Habana, Cuba

Received 10 July 1996; received in revised form 27 March 1997; accepted 18 April 1997

Abstract

A sensitive gas chromatographic method for the quantitative determination of the new antibacterial and antifungal drug G1, 1-(5-bromofuran-2-yl)-2-bromo-2-nitroethene, has been optimized. The method involves a fast and single extraction step from spiked serum and urine samples. The G1 drug was quantified using an internal standard method and by means of a nitrogen-selective detector. The results are statistically significant and show that mean levels of G1 as low as 1 $\mu g\ ml^{-1}$ can be measured accurately. © 1997 Elsevier Science B.V.

Keywords: 1-(5-Bromofuran-2-yl)-2-bromo-2-nitroethene

1. Introduction

The furanosic derivative G1, 1-(5-bromofuran-2-yl)-2-bromo-2-nitroethene, is a new pharmaceutical product with a wide range of antifungal and antibacterial actions [1].

This product could be used to treat some opportunistic infections affecting AIDS patients; however, further analysis of the drug G1 requires standardization to evaluate both its pharmacological and its therapeutic effects. Good extractive and analytical methods are therefore of the utmost importance. Some analytical methods have been described for the quantitation of this drug using UV spectrophotometry combined with a solid–liquid extraction procedure [2] and by gas–liquid chromatography (GC) using a packed GC SE-30 column and an electron capture detector combined with a liquid–liquid extraction procedure [3].

This paper describes a fast and efficient extraction method with a high recovery from serum and urine. GC analysis was performed using a nitrogen-selective detector [4].

2. Experimental[1]

G1 (I, Fig. 1) and UC-245 (II, Fig. 1) [1-(5-bromofuran-2-yl)-2-methyl-2-nitroethene], the latter

[1]The manufacturer's names and products are given as scientific information only and do not constitute an endorsement by the Cuban Government.

*Corresponding author.

PII S0378-4347(97)00260-0

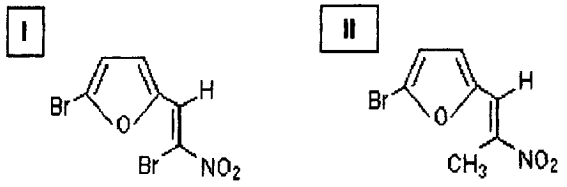

Fig. 1. Chemical structures of G1 (I) and the internal standard (II).

was used as the internal standard (I.S.), were supplied by the Bioactive Chemical Centre from the Central University of Villa Clara. All chemicals were of analytical or equivalent grade and were used without further purification. Methanol was from BDH (Poole, UK) and ethyl acetate was from Merck (Darmstadt, Germany).

Glassware and centrifuge tubes were pre-cleaned in an ultrasonic bath and were rinsed with double-distilled water.

The standard solutions of G1 (0.5–50 μg ml^{-1}) and UC-245 (100 μg ml^{-1}) were both prepared in methanol and ethyl acetate. Sequential dilutions were done starting from 1 mg ml^{-1} and 100 μg ml^{-1} solutions.

A Unicam Model 610 gas chromatograph, equipped with a nitrogen-selective detector, and the 4880 computer program (Unicam) were used. The glass column (1.5 m×4 mm I.D.) was packed with 3% OV-1–OV-17 (1:3) on Chromosorb G (100–120 mesh, AW/DMCS). The column temperature was 155°C and the injector and detector temperatures were 230 and 280°C, respectively. The flow-rate of the carrier gas (Argon) was 33.3 ml min^{-1} [4,5].

G1-free serum samples, used for spiked extractions, were supplied by the Marianao Blood Bank (Havana, Cuba).

Urine samples were collected from two normal volunteers who had never before taken G1. The serum and urine blanks were made with 2 ml of each media and these were analyzed by GC.

The samples for the calibration curve were prepared using 2 ml of each G1 standard solution in ethyl acetate and 0.1 ml of the I.S. (100 μg ml^{-1}) in ethyl acetate.

The mixture was evaporated to dryness at 4°C under a stream of argon. The residue was dissolved in 50 μl of methanol, and 0.5–2 μl were injected into the gas chromatograph [6].

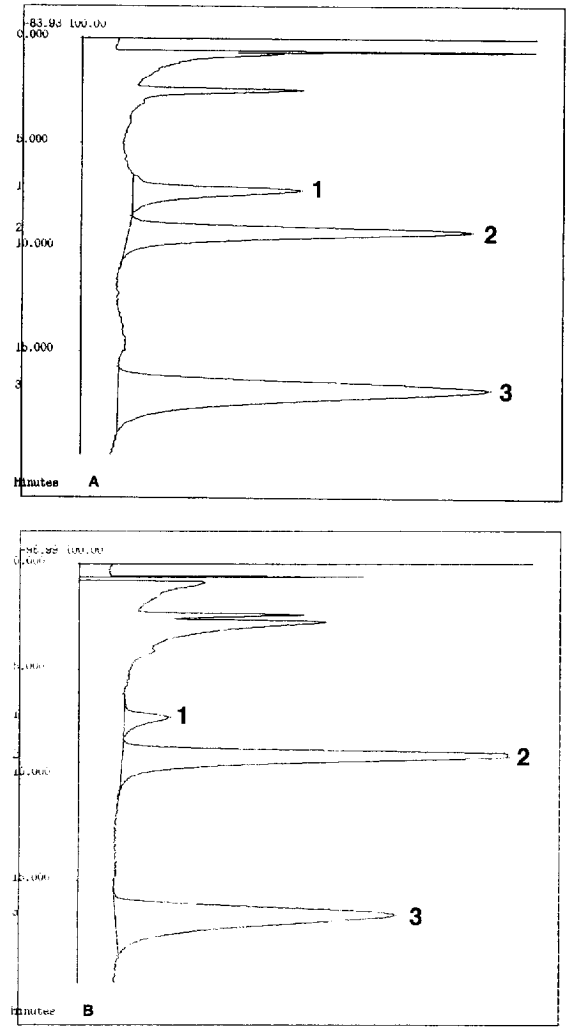

Fig. 2. (A) Chromatogram of G1 (3) and the I.S. (2) in a 30 μg/ml solution (standard). Peak (1) is an impurity of G1 (5.3%). (B) Chromatogram of G1 (3) and the I.S. (2) in a 20 μg/ml solution (matrix, serum). peak (1) is an impurity of G1 (5.4%).

For spiked extraction, 2 ml of serum or urine were combined with 2 ml of each G1 standard solution in methanol and with 0.1 ml of the I.S. (100 μg ml^{-1}) in methanol in a 200-ml screw-cap centrifuge tube, then the mixtures were shaken for 1 min.

These mixtures were extracted with 3 ml of ethyl acetate for 5 min with gentle mixing on a platform shaker. After centrifugation for 10 min at 1500-2000 *g*, the ethyl acetate layer was transferred to another tube and evaporated to dryness at 45°C under a

stream of argon. The residue was dissolved in 500 μl of methanol, then transferred to a micro-tube and evaporated to dryness once more. This residue was dissolved in 50 μl of methanol, of which 0.5–2 μl were injected into the gas chromatograph.

Statistical and regression studies were employed to fit plots of peak-area ratio (G1/I.S.) versus G1 concentration. Statistica and MicroCal Origin programs (Windows 3.11) for the SyncMaster 2 computer (486 DX 2, 66 MHz, 8 Mb RAM) were used [5].

3. Results and discussion

UC-245 was chosen as the I.S. because it is a furanosic derivative, with structural similarity and extractive characteristics that are similar to G1 and because they may be resolved efficiently by a chromatographic analysis resolution of 1.19 (Fig. 2A).

The assay was carried out using seven standard calibration points in the concentration range of 0.5–50 μg ml^{-1} for the calibration curve.

This curve exhibited excellent linearity over the concentration range of 1–50 μg ml^{-1}, with a determination coefficient (R^2) that was at least 0.97. The correlation coefficient (r) was 0.99, the standard error was 0.0935 and p was 0.00017 [7].

The equation of the calibration curve of the form $y=a+bx$ is described for $a=0\pm5.3524E-215$ and $b=0.0648\pm0.00157$ (mean±SD). The intercept did not differ significantly from zero.

Table 1
C.V. using standard (1), serum (2) and urine (3) ($n=7$)

Concentration (μg/ml)	C.V. (%)		
	1	2	3
50.0	4.500	3.000	2.900
30.0	5.670	5.760	3.900
20.0	6.020	6.080	5.800
10.0	7.140	7.120	7.310
5.0	8.280	8.780	8.590
1.0	9.400	9.870	8.490
0.5	32.000	41.000	35.000

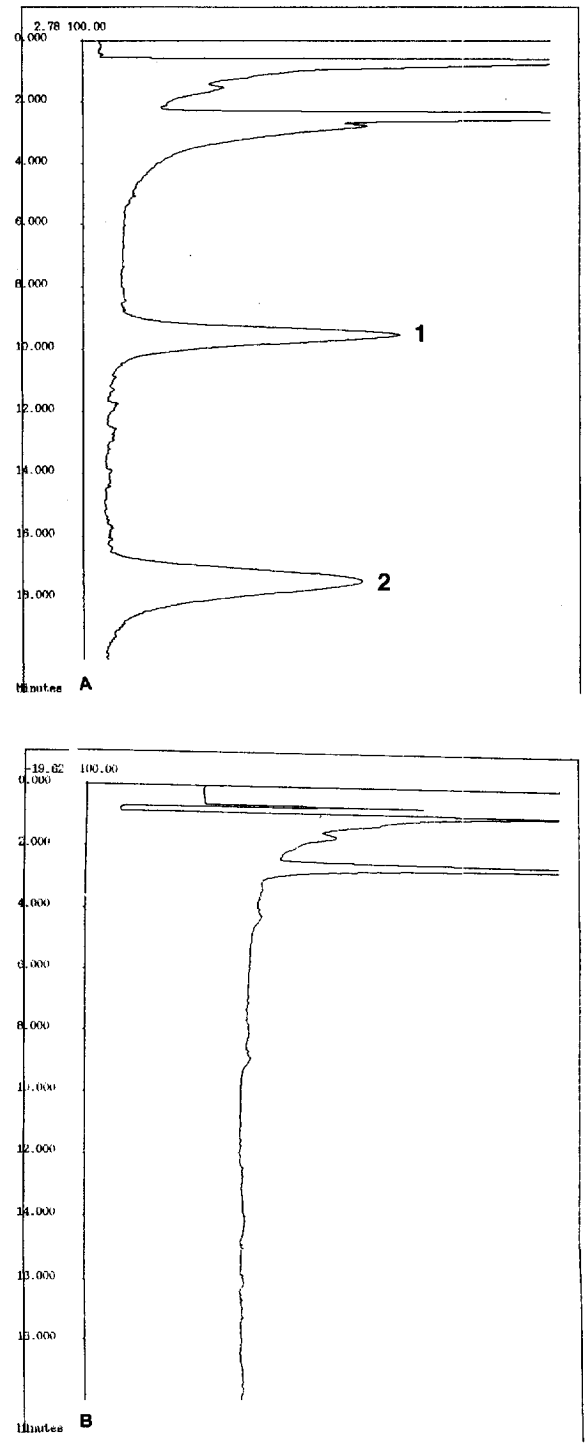

Fig. 3. Chromatograms from (A) a plasma extract 2 h after a dose of G1 and (B) a plasma blank, obtained by the proposed extraction method. Peaks: 1=UC-245 and 2=G1.

In the case of the calibration samples, intra-assay precision was determined at six concentrations for seven analyses (Table 1). Inter-assay precision was determined at the same concentrations but in four replicates ($n=4$). The samples were stored frozen for four weeks, then determinations were carried out using one aliquot each week. The within-day coefficient of variation (C.V.) for G1 was 4.6%, and the day-to-day C.V. was 6.6% [7].

Samples (in methanol) that were stored in colorless glass vessels at −70°C, in the dark, over the same time period did not show relevant instability, and recoveries during consecutive determinations were 97.15±3.1% for the first week, 94.9±4.2%, 95.26±2.6% and 94.12±5.2% for the three other weeks, respectively.

The addition of known concentrations (1–50 μg ml^{-1}) of G1 in methanol to serum and urine gave average recoveries of 95.1±4.2 and 95.4±3.8%, respectively, using seven samples of each concentration by the above described extraction procedure. There are not significant differences in the recovery when 1 μg ml^{-1} levels were evaluated for both biofluids with $\alpha=0.5$.

This method resulted in serum and urine extracts that were free of interfering endogenous substances in a shorter period of time (Fig. 2B).

Standard curves for the drug in each spiked biological fluid exhibited excellent linearity over the concentration of 1–50 μg ml^{-1}. The limit of quantitation is presented on Table 1, together with the C.V. The limit of quantitation was found to be ca. 1 μg ml^{-1}, with a C.V. that was lower than 10% in the urine and serum samples and it is below the expected drug concentration [MID (minimum inhibitory dose) 15 μg ml^{-1}] in biological specimens from patients [8,9]. The determination coefficients (R^2) were at least 0.986–0.99, correlation coefficients were 0.99 for both media, the standard errors were 0.087–0.094 and p values were 0.00009–0.00027.

The intercepts did not differ significantly from zero.

The within-day C.V.s for G1 in the two media are presented in Table 1.

An early method [2] for extracting and quantifying G1 in standard and urine samples involved a solid–liquid extraction procedure with a solid-phase extraction column of C_{18}, the reagent of extraction was ethyl acetate and the analysis was done by UV spectrophotometry. The recovery was about 85% and the limit of quantitation was 10 μg ml^{-1}. Ramírez et al. [3] used a liquid–liquid extraction procedure for G1 in standard and serum samples, with chloroform used for solvent extraction, then the samples were evaporated to dryness. The residue was dissolved in 1 ml of n-heptane and was then injected into a gas chromatograph that was equipped with a packed column SE-30% and ECD but the method had not been validated.

3.1. Application of the method

Plasma from two volunteers who received 150 mg of G1 in tablet form (50 mg of G1) was analysed by this method. Fig. 3 shows chromatograms of plasma extracts from a volunteer before and after oral administration of 150 mg of G1.

The plasma levels of the parent drug after oral administration of G1 are presented in Table 2. These data demonstrate that this method is sufficiently sensitive to measure G1 levels in plasma after a single oral dose.

This study shows an alternative extraction method for G1 from biological fluids.

The present extraction and analytical method can thus be applied to pharmacokinetic studies and also to the therapeutic control of patients receiving this drug.

Table 2
G1 concentration in plasma extracts from two volunteers at various times after a dose of G1 ($n=2$)

Volunteer	Dose		G1 concentration				
	mg	mg kg^{-1}	30 min	1 h	2 h	3 h	3.5 h
30 377	150	2.87	–	6.1 μg ml^{-1}	19.3 μg ml^{-1}	8.2 μg ml^{-1}	–
260 644	150	2.12	–	4.8 μg ml^{-1}	20.6 μg ml^{-1}	9.5 μg ml^{-1}	–

Acknowledgements

We thank Cynthia de la Mora who was an excellent volunteer. The study was supported by grants from the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases.

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