Journal of Chromatography, 305 (1984) 465—469
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1942

Note

Method for the determination of oltipraz, a new antischistosomal agent, in blood

H.M. ALI*

Department of Pharmacology, Faculty of Pharmacy, University of Khartoum, P.O. Box 1996, Khartoum (Sudan)

J.L. BENNETT*

Department of Pharmacology and Toxicology, Michigan State University, B420 Life Sciences Building, East Lansing, MI 48824 (U.S.A.)

S.M. SULAIMAN

Bilharzia Research Unit, National Health Laboratories, Ministry of Health, Khartoum (Sudan)

and

J. GAILLOT

Department of Biodynamics, Institute de Biopharmacie Rhone-Poulenc, 182/184, Avenue Aristide Briand, 92160 Antony (France)

(First received August 4th, 1982; revised manuscript received September 19th, 1983)

Oltipraz, methyl-4-(pyrazinyl-2)-5-dithiole-1,2-thione, is a highly lipid soluble compound which has significant antischistosomal activity when given orally to patients infected with *S. mansoni* or *S. heamatobium* [1—3]. The recommended dose for patients with *S. mansoni* is 20—25 mg/kg while patients with *S. haematobium* infections receive 25—30 mg/kg.

Assay methods based on thin-layer chromatography [1], gas—liquid chromatography (GLC) [1] and high-performance liquid chromatography (HPLC) [3] were previously used for the determination of oltipraz in biological fluids, in animals and in humans. Prior to chromatographic analysis, oltipraz was

* Address for reprint requests.

0378-4347/84/$03.00

extracted from the biological fluids by multiple extraction procedures using relatively large volumes of organic solvents, e.g. diethyl ether [3]. After performing the above procedures we felt that the multiple extraction procedure was too expensive, i.e. in countries like Sudan, the cost of solvents and glassware is very high. For this reason we attempted to employ a single-extraction technique, prior to chromatographic analysis, using small volumes of chloroform for analyzing oltipraz in urine, serum and plasma. Similar methods involving the single-extraction technique have been previously reported [4—7]. In this paper we describe an assay method for the analysis of oltipraz based on a single-extraction step followed by GLC analysis.

EXPERIMENTAL

Compounds and materials

Oltipraz (RP-35972) and its butyl derivative (RP-35919), Fig. 1, were kindly supplied by Rhone-Poulenc Sante (Paris, France). Thiodiglycol (lot 25c-0026-2) was purchased from Sigma (St. Louis, MO, U.S.A.).

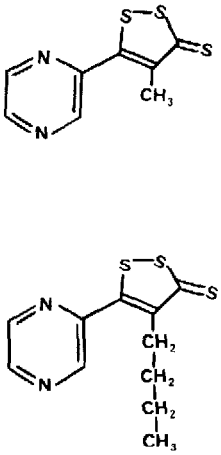

Fig. 1. Structure of oltipraz (upper) and the internal standard RP-35919 (lower).

Gas—liquid chromatography

A Perkin-Elmer F33 gas chromatograph with a flame ionization detector and linked to a Hitachi Perkin-Elmer 1.0-mV Model 56 recorder was used. A glass column (1.0 m × 5.0 mm O.D. and 3.0 mm I.D.) packed with Chromosorb Q (100—120 mesh) and coated with 3% OV-17 (Perkin-Elmer) was used under the following operating conditions: nitrogen, 100 kN/m^2; hydrogen, 100 kN/m^2; air, 180 kN/m^2; oven temperature, 235°C; and injection port temperature, 250°C. The column was conditioned at 260°C for 24 h before use. The retention times of oltipraz and its butyl derivative were 8.5 and 13.5 min, respectively.

Determination of oltipraz in biological fluids

To 1.0 ml of urine, serum, plasma or aqueous solution containing oltipraz in a glass-stoppered conical centrifuge tube were added oltipraz butyl derivative

(RP-35919, 50 μm/ml, 1.0 ml) as the internal standard, sodium bicarbonate (saturated solution, 1.0 ml), water (1.0 ml), thiodiglycol (0.1 ml) and chloroform (120 μl). Aqueous solutions of oltipraz and its butyl derivative were prepared in an absolute alcohol—distilled water mixture (1:4). The tube was shaken thoroughly on a Whirlmixer (Vortex Genie) for 1.0 min and then centrifuged at 3800 *g* for 2.0—3.0 h until a clear chloroform layer was obtained. A 10-μl syringe was inserged through the aqueous phase into the chloroform layer and 5—8 μl of the organic phase were carefully withdrawn and injected onto the GLC column.

The peak height ratio (PHR: oltipraz/oltipraz butyl derivative) was calculated and the concentration of oltipraz in the biological fluid sample was determined by reference to the appropriate calibration curve. Calibration curves in water, urine, serum or plasma were separately prepared by repeating the above procedure. The calibration curve(s) data were statistically analyzed using the regression analysis method.

Recovery of compounds from biological fluids

The recovery of oltipraz from various volumes (1, 5 or 10.0 ml) of water, urine, serum and plasma was tested by spiking these media with 10 μg of the drug (in 1.0 ml of water) and extracted as described above. A reference sample (non-extracted) was prepared in chloroform (10 μg/ml) and was considered as the 100% recovery level. The recovery of oltipraz butyl derivative was examined similarly using 50 μg of this compound. A 10-μl aliquot of the chloroform extract was withdrawn and injected onto the GLC column and the corresponding peaks were measured. Duplicate injections were made in each case and the recovery of the compounds was tested on six separate replicates for each media. The peak height obtained for each of the reference samples (non-extracted chloroform solutions) was considered equivalent to 10 and 50 μg of oltipraz and its butyl derivative, respectively. The amount extracted for each compound from water, urine, serum and plasma was then determined by comparison to the reference samples. The mean values were obtained and expressed as percentages of the original amount of compound added.

The data obtained for oltipraz recovery from the six replicate runs in each media were further used to test the repeatability of the assay method.

RESULTS AND DISCUSSION

Using the GLC conditions described in the experimental section we were able to obtain sharp, symmetrical and separated peaks for oltipraz and its butyl derivative (Fig. 2). Extracts of various volumes (1, 5, or 10.0 ml) of blank serum and plasma of humans and/or animals receiving oltipraz, gave no interfering peaks, when chromatographed. Since the butyl derivative of oltipraz is almost identical to oltipraz in its physicochemical properties (Fig. 1), we selected the butyl derivative as an internal standard for the assay of oltipraz. We observed that the pH in the aqueous phase for optimal extraction of oltipraz into chloroform ranged between 5.0 and 7.0. More importantly, we were able to perform this extraction in a small volume of chloroform. In previous methods, for GLC and HPLC, 20.0 ml of diethyl ether were used per

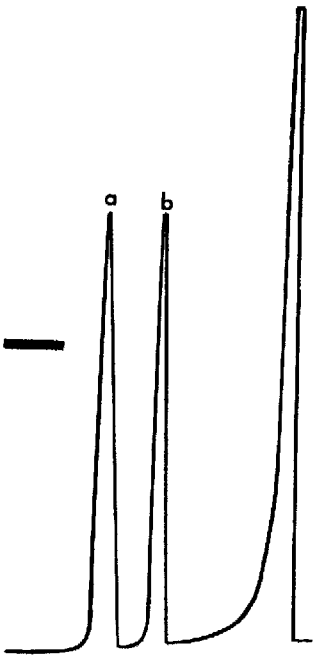

Fig. 2. A representative gas—liquid chromatogram of (a) oltipraz and (b) oltipraz butyl derivative (RP-35919). The retention times (min) of a and b were 8.5 and 13.5, respectively. Time scale equals 5 min. (Blank serum and plasma gave no interfering peaks.)

assay [1, 3]. (This reduction in solvent use becomes extremely important in countries like Sudan where the cost of solvents is 5 to 10 times higher than in the U.K. or U.S.A.) We made no attempt to identify other suitable organic solvents for this method. We did study the time required to extract oltipraz from various aqueous phases (from 20 to 0.5 min) and found that 1 min was optimal.

In our trials with humans we have measured the concentration of oltipraz in blood. Peak plasma concentration in 5.0-ml samples from humans after a 25 mg/kg dose is 1.07 μg per ml of blood. Recent work by the manufacturers on oltipraz in primates are unlikely to warrant use of urine as a reliable source of the drug for pharmacokinetic studies.

The average amounts (X + S.E.) of oltipraz extracted from a given aqueous media (1, 5 or 10.0 ml) spiked with 10 μg/ml drug, using our single-extraction GLC method, were almost identical. This was true with all four extraction media tested: water (9.32 + 0.18 μg/ml), urine (9.29 + 0.17 μg/ml), serum (6.20 + 0.08 μg/ml) and plasma (6.08 + 0.15 μg/ml). We have also tested the reproducibility on a given sample of human sera spiked with 10 μg/ml oltipraz (the sample was assayed daily over a period of five days) and found the mean value and standard error to be almost identical to that reported above for serum.

When oltipraz blood levels were determined, following oral administration of the drug to humans or monkeys, using this assay method, the lower limit of detection was approximately 150 ng. The lower limit of quantification using serum and plasma ranged between 1.0 and 2.0 μg/ml. At present we are using

this assay in analyzing the effects of food on the bioavailability of oltipraz in humans. Dietary components and/or endogenous compounds have not interfered with the chromatographic analysis of oltipraz in human and animal trials.

The extractability of oltipraz and/or is butyl derivative from the various media was different. This implies that the calibration curves are to be prepared using the same media in question and that extrapolation from one media to another should not be attempted. These findings were further confirmed when the absolute recoveries of oltipraz and its butyl derivative were compared when extracted from water, urine, serum and plasma samples. The correlation coefficient values obtained for the four different aqueous media ranged between 0.9989 to 0.9999 and all the curves passed through the origin, indicating a linear relationship between the concentration of oltipraz (2.0—16.0 μg/ml) and the peak height ratio.

In the light of above findings, the single-extraction GLC assay method used for the determination of oltipraz from aqueous and biological fluids has good reproducibility, sensitivity and concentration: peak height ratio linearity. Furthermore, the single-extraction GLC assay method, compared to the previously described ones for oltipraz [1, 3], has some advantages: (1) reduction in chances of losing drug since transfer has been avoided; (2) reduction in usage of glassware; and (3) a reduction in the amount of organic solvent.

ACKNOWLEDGEMENTS

Support for this research was from the Edna McConnel Clark Foundation, N.I.A.I.D. grant AI 16312-05 and Rhone Poulenc Sante.

REFERENCES

1 J.P. Leroy, M. Barreau, C. Cortel, C. Jeanmart, M. Messer and F. Benazet, Current Chemother., 14 (1978) 148
2 M. Gentilini, B. Duflo, D. Richard-Lenoble, G. Brucker, M. Danis, G Niel and Y. Neunier, Acta Tropica, 37 (1980) 271
3 R. Pieron, B. Lesobre, Y. Mafart, D. Meyniel, F. Lancastre, A. Renard, J. Simon, J. Gregoire and P. Basset, Rev. Med. Interne, 2 (1981) 231.
4 J. Ramsey and D.B. Cambell, J Chromatogr., 63 (1971) 303.
5 V. Aggarwal, R. Bath and I. Sunshine, Clin. Chem., 20 (1974) 307.
6 W.J. Serfontein, D. Boha and L.S. De Villiers, J. Pharm. Pharmacol., 27 (1975) 937.
7 H.M. Ali and A.H. Beckett, J Chromatogr., 223 (1981) 208.