DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 6(2), 177-185 (1980)

GAS-LIQUID CHROMATOGRAPHIC DETERMINATION OF 2-(o-CHLOROPHENYL)-2-(p-CHLOROPHENYL)-ACETIC ACID IN BIOLOGICAL FLUIDS

James Guilford, Eugene Hichman, Edward Eugere
Kirit A. Shah and Debabrata Ghosh
Texas Southern University School of Pharmacy
Houston, Texas 77004

ABSTRACT

A gas-liquid chromatographic method for the determination of 2-(o-chlorophenyl)-2-(p-chlorophenyl)-acetic acid in human blood and urine has been developed. The procedure is based on ether extraction of acidified whole blood or urine, derivatization with boron trifluoride-methanol, and GLC analysis. This method can detect 0.1 µg/ml of compound in blood. Analysis of blood and urine from human volunteers indicated that the procedure is suitable for pharmacokinetics studies and clinical monitoring of patients.

INTRODUCTION

Mitotane, 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane is used in the treatment of in-

operable adrenal cortical carcinoma of both functional and non-functional types (1). Mitotane can best be described as an adrenal cytotoxic agent. This drug has been shown to inhibit adrenal glucose-6-phosphate dehydrogenase in the dog and corticotropin stimulation of steroidogenesis in vivo but not in vitro (2,3). Previous papers have shown that there is no correlation between blood levels of mitotane and the patient response. Martz el. al. (7) suggested that a metabolite of mitotane is responsible for its activity rather than the parent compound. Sinsheimer, et. al. (6) identified 2-(o-chlorophenyl)-2-(p-chlorophenyl)acetic acid (I) as a major urinary metabolite of mitotane. Although there are several methods (8-10) available which can be used for the determination of I, they lack specificity. The present paper describes a simple and sensitive method for the determination of I in biological fluids.

EXPERIMENTAL

Materials: 2-Bis(p-chlorophenyl)acetic acid[1] (II) and boron trifluoride[2] were purchased. Compound I

1. Aldrich Chemical Company, Milwaukee, WI.
2. Chromatographic Technology, Houston, TX.

was synthesized by the method of Guilford et. al. (11). The methyl ester of I was prepared by the method of Cristol and Haller (12). The methyl ester of II was prepared by the method of Grummit and Marsh (13).

Sample Collection: A 500 mg tablet[3] dosage form of mitotane was administered to two male human volunteers. The cummulative urine were collected at 1 and 24 hrs. initially and then every day for 12 days. The samples were stored at -22° until analysis. The blood samples were collected at 30 minutes for the first three hours and then at 4, 6, 8, and 24 hours and were stored at -22° until analysis.

Extraction Procedure: One ml of whole heparinated blood or 5.0 ml of urine, 4.0 ml of water, 0.5 ml of internal standard (1 mg of II in 100 ml of methanol), 0.1 ml of 0.1 HCL and 20 ml of ether was pipetted into a 60 ml separator funnel. The mixture was shaken for 20 minutes using a mechanical shaker. The ether portion (top) was separated and then was extracted twice with 10 ml of saturated sodium bicarbonate. The ether portion (top) was separated and evaporated at room temperature under a nitrogen stream.

---

3. Calbio Pharmaceuticals, San Diego, CA.

The residue was treated with boron trifluoride (14) and then was allowed to dissolve in 0.1 ml of methanol. Three-five microliter samples were injected into the gas chromatograph.

Parameters of GLC Analysis: A gas chromatograph[4] equipped with dual hydrogen flame ionization detectors was used. The column consisted of 3% Silicone (25% 3-cyanopropyl-25% phenyl)[5] coated on 100-120 mesh acid-washed and silanized diatomaceous earth[6]. The column was operated isothermally at 240°, with the injection part at 260° and the detector at 300°. Helium was used at 40 ml/min. The flow rates of hydrogen and air were optimized so as to obtain maximum peak heights.

Linearity Experiments: Solutions of I in 1 ml of blank whole blood or 10 ml of blank urine were made containing 2,4,6,10,12, and 20 µg/ml. To each solution 5.0 µg of internal standard was added and extracted as described in the extraction procedure.

---

4. Varian Aerograph Model 2700, Palo Alto, CA.
5. OV-225
6. Varaport 30

RESULTS AND DISCUSSIONS

The discribed procedure is sufficient to detect compound I as low as 0.1 µg/ml in urine and 0.5 µq/ml in whole blood. The retention time for the methyl ester of I is 8 min. and the retention time for the internal standard, the methyl ester of II is 10 min. Even though metabolites of I were never tested during this investigation, the GLC peak for the methyl ester of I from several patients were examined on a gas chromatograph-mass spectrometer. The mass spectra corresponded to that of the methyl ester for I, indicating that there is no interference from other compounds (metabolites or drugs). During pharmacokinetics profiles of 2 patients, the peak height of methyl ester of II remained essentially constant with each run suggesting that the other medications or metabolites of I are not interfering with the internal standard peak. Therefore, it is concluded that this method is specific for I in the presence of metabolites. The calibration curve for blood was linear over the concentration range 2.0 - 20.0 µg-ml. A mean slope value of 0.617 $\pm$ 0.005 (r^2 = 0.994) was obtained. The calibration

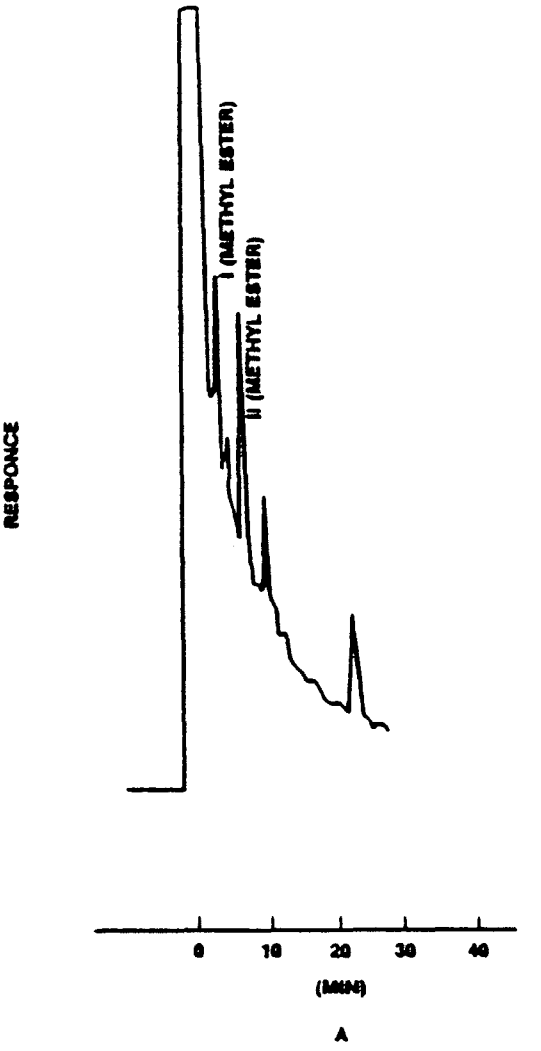

Figure 1 - Chromatograms of (A) human urine spiked with 0.500 µg/ml of 2-(o-chlorophenyl)-2-(p-choro-phenyl)acetic acid and 5.0 µg/ml of the internal standard and (B) blank urine.

Figure 1 (continued)

curve for urine was linear over the same concentration range. A mean slop value of 0.622 ± 0.004 (r^2 = 0.998) was obtained..

The within-run precision was determined by analyzing urine and blood samples spiked with I (2.0 to 20.0 µg/ml). The assay precision ranged from 5.3 to 2.4% SE and the accuracy ranged from 5.8 to 7.1% SE. The recovery of extracted standards relative to direct standards was 76.68 for urine and 52.9% for blood.

Figure I shows the chromatogram of a human urine extract spiked with 0.5 µg of 2-(o-chlorophenyl)-2-(p-chlorophenyl)acetic acid and 5.0 µg of the internal standard. No interference by the normal urine components were recorded.

REFERENCES

1. A. M. Hutter and D. E. Kayhoe, AM J. Med., 41, 581, (1966).
2. M. H. Hart and J. A. Straw, Biochem. Pharmacol., 20, 1679 (1971).
3. J. H. Grady and D. J. Azavmolf, R. Crenqer, D. H. Hutterman and J. Nichols., Proc. Soc. Exp. Bio. Med., 119, 238, (1965).

4. B. H. Moy Lab. Clin. Med., 58, 286 (1961).

5. Y. Tluitou, A. Bogdan, J. C. Legrand, and P. Desgrez. Ann. Endoc., 29, 13, (1977).

6. J. E. Sinsheimer, J. Guilford, L. J. Borbrin and D. E. Schteingant, J. Pharm. Sci., 61,314 (1972).

7. F. Martz and J. A. Straw Drug Metab. Disposition., 5, 482, (1977).

8. V. D. Reif, J. E. Sinsheimer, J. E. Wand and D. E. Schteingant J. Pharm. Sci., 63, 1730, (1974).

9. J. C. Fiel, J. H. Lamoureus and B. G. Zaylskie, J. Agric. Food Chem., 33, 363, (1961).

10. V. D. Reif and J. E. Sinsheimer, Drug Metab. Disposition., 3, 15, (1975).

11. J. Guilford, E. Hichman and D. Ghosh, Texas J. Sci. 24, 260, (1977).

12. S. Grummitt and D. J. Marsh, Amer. Chem. Soc., 71, 4156 (1949).

13. O. J. Cristol and H. L. Haller, J. Amer. Chem. Soc. 67, 2222 (1945).

14. L. D. Metclafe and A. A. Schmitz, Anal. Chem., 33, 363, (1961).