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GAS—LIQUID CHROMATOGRAPHIC ASSAY OF AMINOGLUTETHIMIDE AND A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR ITS ACETYL METABOLITE IN BIOLOGICAL FLUIDS

A.M. ADAM and H.J. ROGERS*

Department of Clinical Pharmacology, Guy's Hospital Medical School, London Bridge, London SE1 9RT (U.K.)

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SUMMARY

A rapid, sensitive and selective gas—liquid chromatographic assay for aminoglutethimide is described. The same extraction procedure may be employed prior to a high-performance liquid chromatographic assay for acetamidoglutethimide which is also detailed. Both assays are suitable for the study of the pharmacokinetics of aminoglutethimide and acetamidoglutethimide in biological fluids in man.

INTRODUCTION

Aminoglutethimide [3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione] was initially developed as an anticonvulsant in the 1950s but was later withdrawn from clinical use after reports of adrenal insufficiency [1]. It was subsequently shown to suppress adrenal steroid synthesis by inhibiting the enzymic conversion of cholesterol to pregnenolone [2]. It has therefore been used in the treatment of adrenocortical tumours, Cushing's syndrome (in combination with metyrapone [3]), and to perform "medical adrenalectomy" in patients with metastatic breast cancer [4, 5]. In post-menopausal women the major oestrogen source is the aromatisation of androstenedione to oestrone in fat, muscle and liver. Aminoglutethimide also blocks this enzyme: an additional and perhaps more important action [6] since this is the rate-limiting step in oestrogen production.

A spectrophotometric method for estimation of aminoglutethimide in biological fluids has been previously described [7]. These authors, however, found cross-reactions with Ehrlich's aldehyde reagent by a number of endogenous and exogenous compounds to restrict the applicability of this assay es-

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pecially for aminoglutethimide concentrations of less than 1 μg/ml. The concentrations of aminoglutethimide expected in saliva after a single dose of aminoglutethimide are thus too low to be measured by this method [8]. A high-performance liquid chromatographic (HPLC) assay has been briefly described utilising an isocratic acetonitrile—water (containing 0.05% perchloric acid) system [9] but the authors experienced difficulty in measuring plasma levels with this system and the isocratic system was replaced with a methanol gradient [10]. Under these conditions the retention times were very long: 15 min for aminoglutethimide and 17 min for acetamidoglutethimide [11]. No internal standard was used to correct for variable extraction of these compounds from biological fluids.

In the present study a gas—liquid chromatographic (GLC) method for aminoglutethimide was developed. Acetamidoglutethimide gave an asymmetric peak shape on this system. A simple and rapid HPLC assay was therefore developed for the acetyl metabolite. Although the extraction process was carried out separately for the two assays, this could easily be simplified to a single extraction process since the two internal standards used do not interfere with either of the chromatographic techniques.

MATERIALS AND METHODS

Chemicals and reagents

Aminoglutethimide was a gift of Ciba-Geigy (Basle, Switzerland). Acetamidoglutethimide was kindly donated by Dr. P.J. Nicholls (Welsh National School of Pharmacy, Cardiff, U.K.) and Dr. M. Jarman (Ludwig Institute, Sutton, U.K.). Heptabarbitone was obtained from Ciba-Geigy (Horsham, U.K.) and primidone from ICI (Macclesfield, U.K.). All other reagents and solvents were AnalaR grade from BDH (Poole, U.K.), and were used without further purification.

Apparatus

The gas chromatograph used was a Pye Unicam Series 104 model fitted with a nitrogen-selective detector. The glass column (1.5 m × 0.4 mm I.D.) was packed with 2% CDMS (cyclohexanedimethanol succinate on Chromosorb W, 80—100 mesh, acid-washed, dichloromethylsalisilane treated).

For the HPLC assay an Altex Model 110A solvent pump was used with a fixed-wavelength (254 nm) detector (Applied Chromatography Systems, Luton, U.K.). The column was a stainless-steel tube (15 cm × 4.6 mm I.D.) packed with reversed-phase Magnusphere C_{18}, 7-μm particle size (Magnus Scientific, Sandbach, U.K.). Injections were made via a Rheodyne type 7125 valve and 20-μl injection loop.

Extraction procedure for aminoglutethimide

Plasma and saliva. To 0.5 ml of plasma or saliva in a centrifuge tube 15 μl of an aqueous solution of primidone (100 μg/ml) were added as internal standard followed by 1 ml of 0.1 *M* citrate buffer, pH 4 (0.1 *M* citric acid—0.2 *M* disodium hydrogen phosphate 62:38 and 5 ml of dichloromethane. After stoppering, the tube was shaken on a Rolamix (Luckhams, Burgess Hill, U.K.)

for 10 min. The tubes were then centrifuged at 800 *g* for 15 min and the dichloromethane transferred to a clean, dry conical tube and evaporated to dryness in a stream of air at 35°C. The contents of the conical tube were reconstituted in 20 μl of methanol. After mixing, 1—2 μl of this solution were injected directly into the column of the gas chromatograph. The peak height ratio of aminoglutethimide to primidone was used to construct a standard curve and to estimate unknown aminoglutethimide concentrations by interpolation.

Urine. For urine, the same procedure was used except that 15 μl of 1 mg/ml primidone in methanol were used as internal standard.

The operating conditions for the chromatograph were: column temperature 240°C, detector temperature 350°C. Gas flow-rates were: carrier gas (nitrogen) 100 ml/min, hydrogen 65 ml/min and air 300 ml/min.

Calibration procedure

Blank plasma or saliva was spiked with an aqueous solution of aminoglutethimide over the range 1—10 μg/ml and carried through the whole procedure. For urine, blank urine was spiked with aminoglutethimide in methanol over the range 10—300 μg/ml.

Extraction procedure and calibration for acetamidoglutethimide

The same extraction method was used. The internal standard was 100 μl of 100 μg/ml heptabarbitone in water. Calibration curves were prepared over the concentration range 500 ng/ml—4 μg/ml for plasma or saliva and 5—100 μg/ml for urine. The amount of internal standard used for the urine assay was 100 μl of 2 mg/ml heptabarbitone in methanol.

Chromatography

The mobile phase was prepared from citrate buffer made by adding 0.1 *M* citric acid to 0.2 *M* disodium hydrogen phosphate in the ratio of 76:24. The pH of this mixture was then adjusted to 3.4 by addition of 1 *M* hydrochloric acid or 1 *M* sodium hydroxide as required. This buffer was mixed with methanol in the ratio 500:280. The mobile phase flow-rate was 1.2 ml/min (about 10 MPa).

RESULTS AND DISCUSSION

Typical chromatograms are shown in Figs. 1 and 2. The retention times were: aminoglutethimide 2.8 min and primidone 4.9 min on the GLC system; acetamidoglutethimide 3.5 min and heptabarbitone 5.6 min on the HPLC system. No interfering peaks due to endogenous substances were observed.

Aminoglutethimide

The inter-day coefficients of variation (C.V.) are shown in Table I. The within-assay coefficients of variation for the aminoglutethimide are shown in Table II.

The calibration curve was linear between 0.25 and 300 μg/ml (the mean correlation coefficient for assays shown in Table I was 0.996, S.D. 0.003) and the minimum level of detection of aminoglutethimide was 100 ng/ml. The fol-

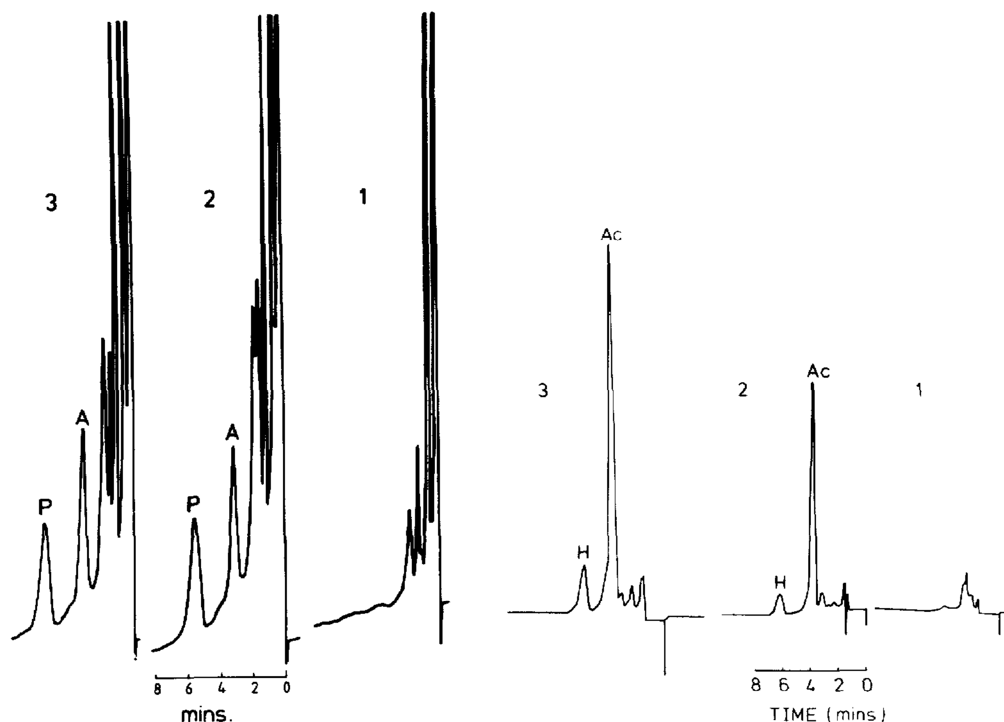

Fig. 1. Representative gas chromatograms of human plasma. 1, Blank plasma; 2, plasma containing 2 μg/ml aminoglutethimide (A), and the internal standard, primidone (P); and 3, plasma from a patient 6 h after oral administration of 500 mg of aminoglutethimide. In each chromatogram the injection artifact is shown on the right-hand side of the trace.

Fig. 2. Representative high-performance liquid chromatograms of blank human plasma. 1, Blank plasma; 2, plasma containing 2 μg/ml acetyl aminoglutethimide (Ac) and the internal standard, heptabarbitone (H); and 3, plasma from a patient 6 h after oral administration of 500 mg of aminoglutethimide.

TABLE I

INTER-DAY REPRODUCIBILITY OF THE AMINOGLUTETHIMIDE ASSAY

	Spiked aminoglutethimide concentration (μg/ml)	Mean peak height ratio (n = 6)	S.D.	C.V. (%)
Plasma	1	0.51	0.05	9.8
	3	1.49	0.01	0.7
	4	2.05	0.09	4.4
	6	3.10	0.07	2.3
	8	4.13	0.14	3.4
	10	5.17	0.17	3.3
Saliva	1	0.60	0.06	10.0
	2	1.09	0.17	15.6
	4	2.33	0.08	3.4
	6	3.58	0.12	3.4
	8	4.69	0.13	2.8
Urine	20	1.22	0.19	15.6
	100	5.6	0.52	9.3
	200	11.74	0.30	2.6
	300	17.2	0.41	2.4

TABLE II

WITHIN-ASSAY COEFFICIENT OF VARIATION (AMINOGLUTETHIMIDE ASSAY)

	Spiked aminoglutethimide concentration (μg/ml)	Mean (n = 6) (μg/ml)	S.D.	C.V. (%)
Plasma	2	1.98	0.05	2.5
	5	5.04	0.14	2.8
	10	9.98	0.25	2.5
Saliva	2	2.0	0.02	1.0
	4	4.05	0.09	2.2
	8	7.99	0.03	0.4
Urine	10	10.2	0.24	2.4
	50	49.9	0.19	0.4
	200	199.2	1.70	0.9

lowing drugs were found not to interfere with this assay: glutethimide (an impurity in the tablet), phenytoin, paracetamol, aspirin, dextropropoxyphene, diazepam and temazepam.

Acetamidoglutethimide

Tables III and IV give comparable data on assay variability over several months of investigation for acetamidoglutethimide.

The calibration curve was linear between 50 ng/ml and 80 μg/ml. The mean correlation coefficient for the data in Table III was 0.998, S.D. 0.004. The minimum level of detection was 10 ng/ml. None of the following drugs which our patients were taking was found to interfere with this assay: methyldopa, aspirin, paracetamol, nitrazepam, temazepam, dextropropoxyphene. Glute-

TABLE III

INTER-DAY REPRODUCIBILITY OF THE ACETAMIDOGLUTETHIMIDE ASSAY

	Spiked aminoglutethimide concentration (μg/ml)	Mean peak height ratio (n = 6)	S.D.	C.V. (%)
Plasma	0.5	0.95	0.10	11
	1	1.90	0.16	8.4
	2	3.90	0.31	7.9
	3	5.75	0.45	7.8
	4	7.8	0.22	2.8
Saliva	0.5	0.96	0.03	3.1
	1	1.73	0.16	9.2
	2	3.67	0.22	6.0
	3	5.62	0.10	1.8
	4	7.58	0.14	1.8
Urine	5	0.36	0.06	16.7
	10	0.82	0.03	3.7
	20	1.71	0.05	2.9
	30	2.38	0.07	2.9
	50	3.97	0.08	2.0
	80	6.18	0.09	1.5

TABLE IV

WITHIN-ASSAY COEFFICIENT OF VARIATION (ACETAMIDOGLUTETHIMIDE ASSAY)

	Spiked aminoglutethimide concentration (μg/ml)	Mean (n = 7) (μg/ml)	S.D.	C.V. (%)
Plasma	0.5	0.52	0.01	1.9
	1	1.00	0.01	1.0
	3	3.01	0.02	0.7
Saliva	1	0.99	0.02	2.0
	2	2.00	0.02	1.0
	4	4.00	0.02	0.5
Urine	10	10.0	0.29	2.9
	50	49.6	2.50	5.0
	80	80.5	2.63	3.3

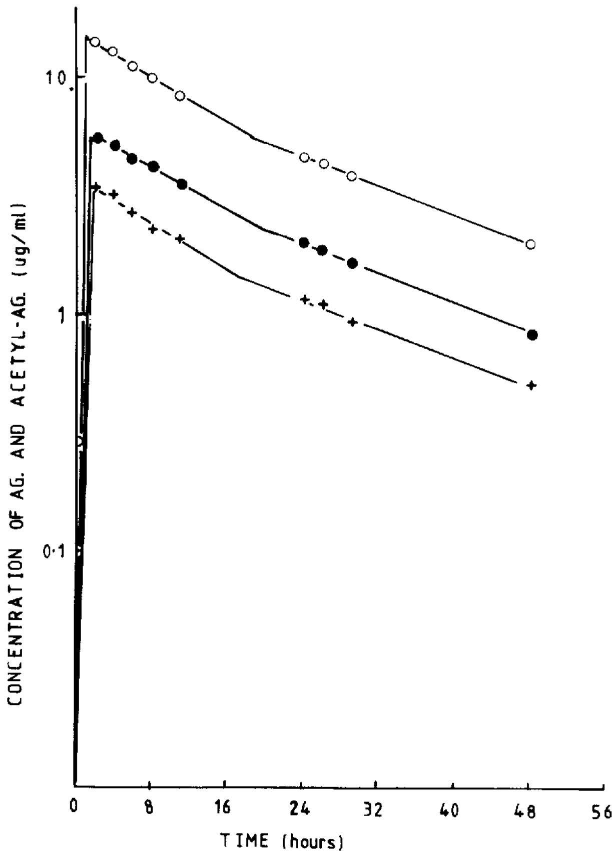

Fig. 3. Concentration—time curves for aminoglutethimide in plasma (○) and acetylaminoglutethimide in plasma (●) and saliva (+) following oral administration of 500 mg of aminoglutethimide to a patient with carcinoma of the breast.

thimide, which may be an impurity in the aminogluthethimide tablet also did not interfere with the assay.

Aminoglutethimide and acetamidoglutethimide

These two assays were used to study the pharmacokinetics of aminoglutethimide and acetamidoglutethimide in patients and volunteers and were both found to be satisfactory (Fig. 3). The salivary pharmacokinetics of acetamidoglutethimide could also be studied. Aminoglutethimide was not detected in saliva despite adequate assay sensitivity and a reported plasma protein binding for aminoglutethimide of 21—25% [8] confirmed in this laboratory to be 31—34%. This subject excreted 8.4% of the dose as aminoglutethimide in urine in 48 h.

Chromatographic assays have been developed to quantify aminoglutethimide and its acetyl metabolite in biological fluids. Adequate sensitivity and reproducibility of calibration data have been shown and these assays are suitable for the conduct of pharmacokinetic studies in normal subjects or patients undergoing treatment for breast cancer with aminogluthetimide.

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