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*Short communication*

# High-performance liquid chromatographic assay for simultaneous estimation of aminoglutethimide and acethylamidoglutethimide in biological fluids

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**Summary.** A simple rapid high-performance liquid chromatographic assay for simultaneous estimation of aminoglutethimide and its acetylated metabolite acetylamidoglutethimide in plasma, saliva, and urine is described. This assay is suitable for pharmacokinetic studies in normal subjects and patients receiving other medication in addition to aminoglutethimide.

## Introduction

Aminoglutethimide (AG) (3-[4-aminophenyl]-3-ethyl-piperidine-2,6-dione: Orimeten, Ciba-Geigy) is being used increasingly often for palliative endocrine therapy in postmenopausal women with metastatic breast carcinoma [7]. It inhibits both adrenal steroid synthesis [2, 5] and the peripheral aromatisation of adrenal androgens [8]. When given with replacement hydrocortisone, AG blocks oestrogen synthesis in the adrenal cortex, and aromatase in extraglandular peripheral tissues and the breast carcinoma itself. Whilst much is now known of its biochemical effects, details of its pharmacokinetics remain relatively obscure. This is due at least in part to the lack of a sufficiently sensitive and simple assay for its estimation in biological fluids. This short communication presents an assay for rapid simultaneous estimation of aminoglutethimide and its acetylated metabolite in plasma, saliva and urine.

## Materials and methods

### *1. Biological materials*

Samples of plasma and unstimulated mixed saliva were obtained at various times following oral administration of aminoglutethimide to patients and normal subjects. Blood was taken into heparinised tubes and plasma separated by centrifugation and stored at −20 °C. Saliva was taken directly into plain plastic tubes and frozen immediately. Urine collections were made over a 24-h period after a single oral dose of aminoglutethimide and an aliquot stored at −20 °C. No special precautions were found necessary for collection or storage.

### *2. Analytical methods*

*i) Drug extraction.* To 0.5 ml of plasma or saliva or 20 µl urine in a 15-ml ground-glass-stoppered extraction tube, 20 µl 100 µl/ml phenacetin in methanol was added as internal standard, followed by 0.5 ml of 0.1 *M*, pH 5.8 acetate buffer and 3 ml dichloromethane. After stoppering, the tube was shaken on a Rolamix (Luckhams Ltd, Burgess Hill, Sussex) for 15 min. The tubes were then centrifuged at 800 *g* for 10 min and the dichloromethane transferred to a clean dry conical tube and evaporated to dryness in a stream of air at 40 °C.

*ii) Chromatography.* The dry extract in the conical tube was reconstituted with 100 µl of the mobile phase used for chromatography. After vortex mixing for 10 s, 40 µl of this solution was injected into the injection valve of the chromatograph. The high-performance liquid chromatograph consisted of an LC-XPS pump (Pye Unicam, Cambridge) with a variable wavelength detector LC-UV detector (Pye Unicam, Cambridge) set at 245 nm, and a Rheodyne 7125 (Berkeley, Calif) injector fitted with a 20 µl injection loop. The column was a stainless steel tube, 30 cm × 4.6 mm i.d., packed with reverse-phase $C_8$ with 5 µm particle size (Lichrosorb RP8, Micron, Middlesex). The solvent system was methanol/distilled water mixture in the ratio 42: 58 (v/v) delivered at a flow rate of 1 ml/min.

*iii) Quantitation.* Calibration curves were prepared over the concentration ranges of 1–16 µg/ml aminoglutethimide and 0.5–10 µg/ml acetylamidoglutethimide in blank plasma and saliva. Similar curves were prepared by spiking blank urine with aminoglutethimide over the range 10–160 µg/ml and acetylamidoglutethimide over the range 5–80 µg/ml. The ratios of the peak height of aminoglutethimide and acetylamidoglutethimide to that of the internal standard were used to construct calibration curves from which the concentrations of these compounds in unknown samples could be determined by linear interpolation.

### *3. Reagents*

All solvents were Analar grade and were used as purchased from BDH Chemicals Ltd (Poole, Dorset).

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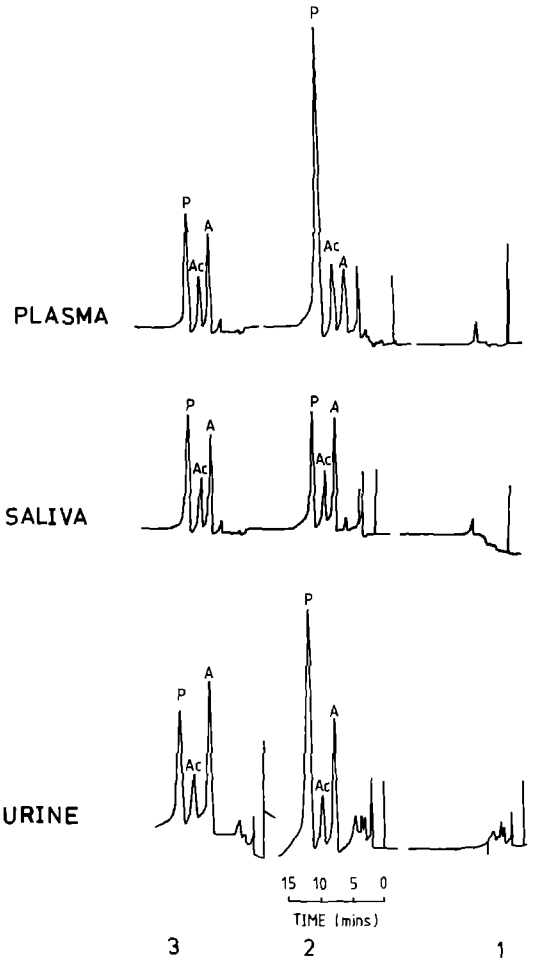


**Fig. 1.** Representative chromatograms for plasma, saliva and urine samples. In all cases chromatogram 1 was from a sample containing neither drugs nor internal standard. Peaks identified are: P – phenacetin (internal standard); Ac – acetyl-amidoglutethimide; A – aminoglutethimide. Plasma samples were from a subject of slow acetylator phenotype following a single oral dose of 500 mg A at 14 h (chromatogram 3) when the concentrations were 3.9 μg/ml A and 2.1 μg/ml Ac and at 24 h (chromatogram 2) when they were 2.1 and 1.1 μg/ml respectively. Saliva samples were replicates of a spiked salivary sample containing A 3.75 μg/ml and Ac 1.7 μg/ml. Urine samples contained 23 μg/ml A and 8 μg/ml Ac. The detector was set at 0.08 aufs and 245 nm for all recordings

mide and to Dr V. A. John of Ciba-Geigy Ltd (Horsham, Sussex) for the gift of pure aminoglutethimide.

## Results

Figure 1 shows representative chromatograms for plasma, saliva, and urine samples containing aminoglutethimide and acetylamidoglutethimide. The retention times were 7.3, 9.25, and 11.25 min for aminoglutethimide, acetylamidoglutethimide, and phenacetin, respectively. In the tests referred to in Fig. 1 the saliva samples only were spiked with AG to demonstrate retention time; AG was not detected in saliva by this technique following therapeutic AG doses.

Table 1 shows the interassay coefficients of variation for the determination of aminoglutethimide and acetylamidoglutethimide in plasma, saliva, and urine. To construct this table, six assays were carried out for each fluid and drug concentration over a period of approximately 6–8 months.

The calibration curves were linear for both compounds in all three biological fluids over the concentration ranges studied. The slope of these curves in plasma and saliva was greater for aminoglutethimide than for acetylamidoglutethimide, but the reverse applied to assays from urine. The mean ($n=6$) recoveries of aminoglutethimide and acetylamidoglutethimide from plasma were 74% and 87%, respectively; those from saliva and urine were similar, with 88% and 96% respectively. The minimum levels of detection (peak height ratio significantly different from that of a lower concentration as determined by analysis of variance) were 250 ng/ml for aminoglutethimide in plasma and saliva and 5 μg/ml for this compound in urine. For acetylamidoglutethimide the minimum levels of detection were 100 ng/ml for plasma and saliva and 2 μg/ml for urine.

No endogenous plasma components were found to interfere with this assay in a series of more than 120 studies in individual patients and normal individuals. Glutethimide (an impurity in commercially available aminoglutethimide) does not give rise to interference, nor do the following drugs administered to patients during our studies: salicylates, ibuprofen, flurbiprofen, morphine, dextropropoxyphene, paracetamol, dothiepin, cyclopenthiazide, hydrochlorothiazide, amiloride, methyldopa, propranolol, prednisolone, diazepam, or temazepam.

**Table 1.** Interassay coefficients of variation ($N = 6$) for determination of aminoglutethimide and acetylamidoglutethimide in plasma, saliva and urine

| Biological fluid | | Aminoglutethimide | | | | | Acetylamidogluthimide | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Plasma | Concentration (μg/ml) | 1 | 2 | 16 | 4 | 8 | 0.5 | 1 | 10 | 2 | 4 |
| | Mean peak height ratio | 0.14 | 0.35 | 3.60 | 0.84 | 1.75 | 0.12 | 0.22 | 2.00 | 0.45 | 0.84 |
| | CV (%) | 14.3 | 8.6 | 3.0 | 6.0 | 6.9 | 8.3 | 9.1 | 3.8 | 4.4 | 4.8 |
| Saliva | Concentration (μg/ml) | 1 | 2 | 4 | 8 | 16 | 0.5 | 1 | 2 | 4 | 10 |
| | Mean peak height ratio | 0.19 | 0.43 | 0.90 | 2.21 | 3.60 | 0.12 | 0.22 | 0.45 | 0.95 | 2.00 |
| | CV (%) | 10.5 | 9.3 | 7.8 | 3.6 | 3.0 | 8.3 | 9.1 | 2.2 | 9.5 | 3.8 |
| Urine | Concentration (μg/ml) | 10 | 20 | 40 | 80 | 160 | 5 | 10 | 20 | 40 | 80 |
| | Mean peak height ratio | 0.54 | 1.23 | 2.12 | 4.90 | 9.83 | 0.22 | 0.41 | 0.89 | 1.84 | 3.73 |
| | CV (%) | 3.7 | 5.7 | 3.3 | 3.7 | 1.9 | 4.5 | 2.4 | 4.5 | 2.7 | 0.8 |

## Discussion

A spectrophotometric method for estimation of aminoglutethimide in biological fluids has been previously described and used for both pharmacokinetic and bioavailability studies in plasma [6, 9] and urine [4]. The originators of the assay, however, found that cross-reactions with Ehrlich's aldehyde reagent by a number of endogenous and exogenous compounds made estimation of serum aminoglutethimide concentrations below 1.8 μg/ml impossible. In addition, this technique could not be used to estimate acetylamidoglutethimide. A high-performance liquid chromatographic assay for aminoglutethimide in urine has been briefly described; it utilises an isocratic acetonitrile/water system [1]. This method has been modified for simultaneous measurement of plasma concentrations of both aminoglutethimide and its acetyl metabolite by replacement of the isocratic system with a methanol gradient which requires complex equipment [3]. The retention times were comparable to those recorded with the present technique (aminoglutethimide 16 min and acetylamidoglutethimide 18 min), but full details and specifications of this assay and its use with biological fluids other than plasma or serum have not been published.

The assay presented above is simple, requires no special apparatus, and is rapid (some 20–25 assays can be carried out in a working day). The accuracy is suitable for determination of bioavailability, and the possibility of determining drug concentrations in biological fluids other than blood makes it applicable to the extended pharmacokinetic studies which will be necessary if lower doses or sustained-release preparations of aminoglutethimide are used in the future in management of advanced breast cancer.

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