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A THIRD DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR THE *IN VITRO* DETERMINATION OF AMINOGLUTETHIMIDE AND ITS N-ACETYL METABOLITE IN HUMAN PLASMA

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ABSTRACT: *Rapid third derivative (D_3) spectrophotometric analysis procedures are described for the determination of aminoglutethimide and its N-acetyl metabolite in human plasma. The developed procedures minimize the mutual interference between these compounds and allow their determination without a previous extraction step. Aminoglutethimide and N-acetylaminoglutethimide are determined through the measurement of D_3 amplitudes at 266 and 256 nm, respectively. Relative standard deviations for the assay of both compounds were less than 1.24%. Recoveries ranged from 97.2 to 99.8% for aminoglutethimide and from 97.0 to 99.4% for N-acetylaminoglutethimide.*

INTRODUCTION

Aminoglutethimide (AG), 3-(4-aminophenyl)-3-ethyl-2,6-piperidinedione, is used in the treatment of hormone-dependent breast cancer in postmenopausal women[1]. After oral administration of AG in man, the drug is absorbed and excreted unchanged in the urine within 48 h[2,3], along with N-acetylaminoglutethimide (N-AAG) and N-hydroxyaminoglutethimide as major metabolite[4,5]. Other minor metabolites have been identified in the urine of rats and human[6,7]. Only one metabolite, N-AAG, has been detected in human plasma[8,9].

Analysis of AG and N-AAG in biological fluids, such as plasma and urine, is important for the pharmacokinetic studies and to assess the bioavailability of the drug formulations. Several analytical procedures have been described for the simultaneous determination of AG and N-AAG in plasma. These include GLC[10] and HPLC[11-13] methods. To our knowledge there is only a nonspecific spectrophotometric method for the determination of AG and N-AAG in urine[4,14].

For this reason, a simple, quick and reproducible method for the assay of AG and N-AAG in plasma is needed. Study of the spectroscopic characteristics of these two substances show that their corresponding zero order absorption maxima closely overlap (Fig. 1). The technique of derivative spectrophotometry has proved useful in overcoming interferences due to spectral overlap[15,16].

This paper presents a D_3 spectrophotometric method, based on the "zero-crossing technique"[17], which is capable of the *in vitro* analysis of AG and N-AAG in human plasma.

EXPERIMENTAL

Materials

Aminoglutethimide (Ciba, Zwitzerland).
N-Acetylaminoglutethimide. This was prepared according to reported procedure[18].
0.02 N Methanolic HCl.

Apparatus

UV-VIS spectrophotometer (Shimadzu, Model 160A), was used for spectroscopic analysis. quartz cells of 1-cm path length were used. The operating conditions were as follows: wavelength wnge, 400-200 nm; scan speed, fast; cycle time T= 60 sec; $d\lambda(N)$, dT(N) were set at N= 9, the highest repetition number (to damp the noise and resolve the signals); ordinate maximum and minimum settings ± 0.125.

Procedures

Preparation of Calibration Graphs

a- AG in plasma: Place 2 ml of plasma in a centrifuge tube and spike with 8 ml methanol containing variable amounts of AG (10-50 μg). Centrifuge the tube content at 3000 rpm for 30 min. Transfer 5 ml of the deproteinized plasma into a 10 ml volumetric flask and dilute to volume with methanol. Record the D_3 spectra against the corresponding blank. Measure the D_3 values at 266 nm.

b- N-AAG in plasma: Place 2 ml of plasma in a centrifuge tube and spike with 8 ml methanol containing variable amounts of N-AAG (5-25 μg). Centrifuge the tube content at 3000 rpm for 30 min. Transfer 5 ml of the deproteinized plasma into a 10 ml volumetric flask and dilute to volume with 0.02 N methanolic HCl. Record the D_3 spectra against the corresponding blank. Measure the D_3 values at 256 nm.

In Vitro Analysis of Mixtures in Plasma

a- For AG content: Place 2 ml of plasma in a centrifuge tube and spike with 8 ml methanol containing 10 μg N-AAG and variable amounts of AG a(10-50 μg). Proceed according to directions given for AG in plasma, starting with "Centrifuge the tube ...".

b- For N-AAG content: Place 2 ml of plasma in a centrifuge tube and spike with 8 ml methanol containing 30 μg AG and variable amounts of N-AAG (5-25 μg). Proceed according to directions given for N-AAG in plasma, starting with "Centrifuge the tube...".

RESULTS AND DISCUSSION

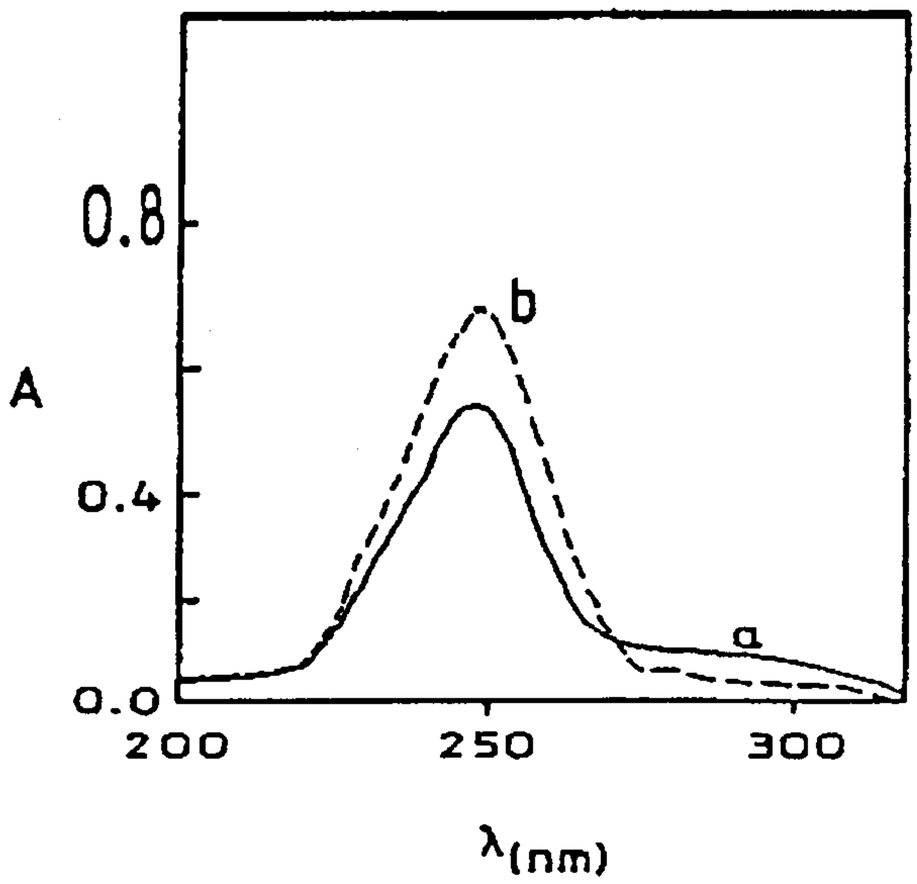
Methanolic solutions of either AG or N-AAG in plasma exhibited maximum absorbance at 248 nm and 249 nm, respectively (Fig. 1). On performing the zero order spectra of deproteinized plasma samples of each compound in 0.02 N methanolic HCl (Fig. 2), the peak of AG at 248 nm was considerably broadened and showed hypochromic effect, which suggested the protonation of the aromatic amino moiety in AG. On the other hand, N-AAG showed a slight hypochromic effect together with blue shift. Since the spectral overlapping between AG and N-AAG is not completely eliminated, simple spectrophotometric measurements cannot be utilized for individual estimation of each compound. Therefore, different derivative techniques were manipulated.

The D_3 spectra (Fig. 3) of plasma solutions of AG and N-AAG in methanol showed that AG exhibits a characteristic peak at 266 nm, whereas N-AAG displays zero-crossing. The D_3 values at 266 nm (base-line measurements) were found all proportional to AG concentration irrespective of N-AAG concentration. Fig. 4 presents the D_3 spectra of plasma solutions of AG and N-AAG in 0.02 N methanolic HCl. N-AAG displays a characteristic peak at 256 nm, at which AG gives zero D_3 values. Analogously, the D_3 values at 256 nm were proportional to N-AAG concentration and independent from the concentration of AG.

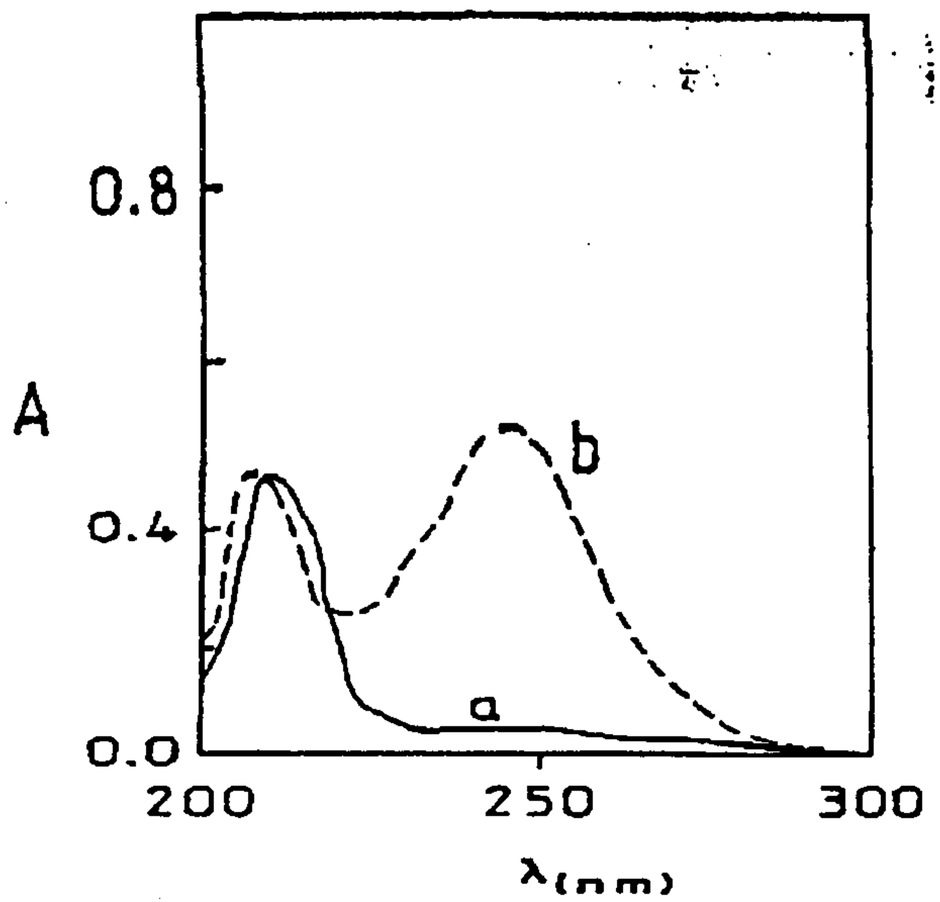
Under the described experimental conditions, all the amplitudes in the D_3 spectra of AG and N-AAG at the selected wavelengths show a linear relationship with concentration ranges given in Table 1. Least square regression analysis was carried out for the slope (b), the intercept (a) and the correlation coefficient (r). The results are presented in Table 1. The coefficient of variation (RSD,%) calculated for 5 replicate determinations at different concentration levels of each compound ranged from 1.26 to 1.49%; indicating satisfactory precision and reproducibility of the D_3 measurements.

The effect of the amount of sample was investigated for human plasma. A 8-ml volume of methanol was found to be sufficient for precipitation of plasma proteins in 2 ml of plasma.

In order to assess the validity and applicability of the developed procedures, plasma samples were spiked with known variable amounts of AG and N-AAG. Recoveries obtained are presented in Table 2. Satisfactory results obtained indicate the validity of th

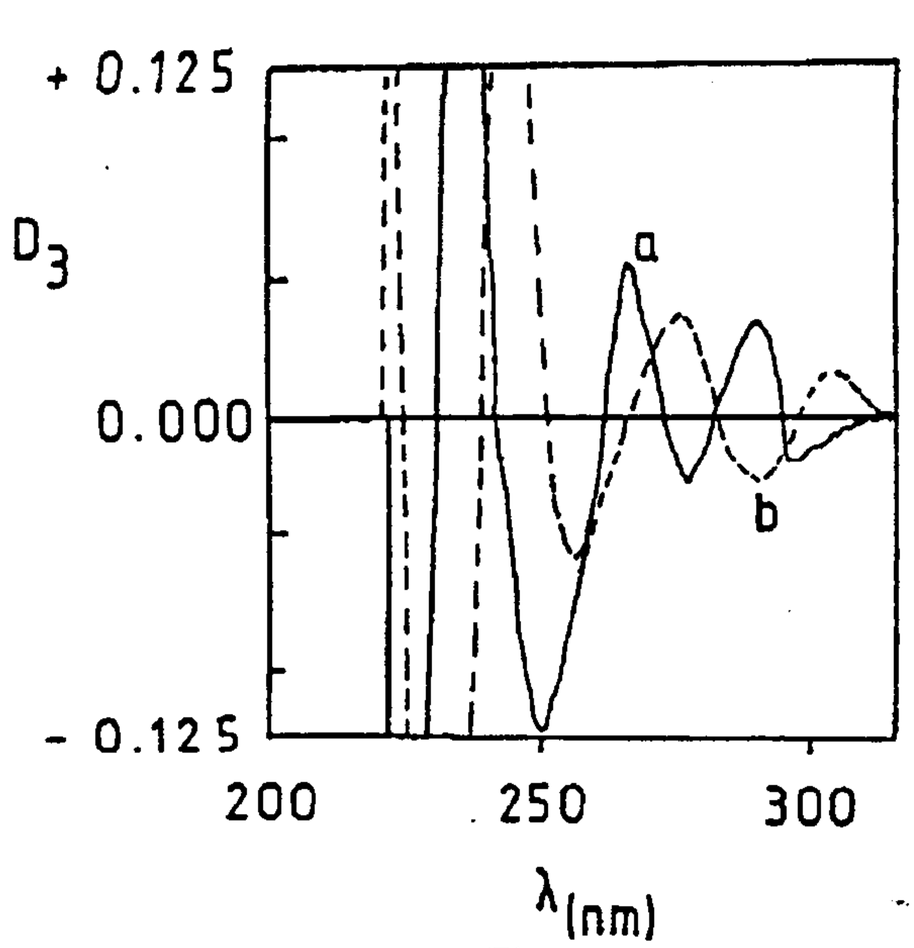


(Fig. 1)

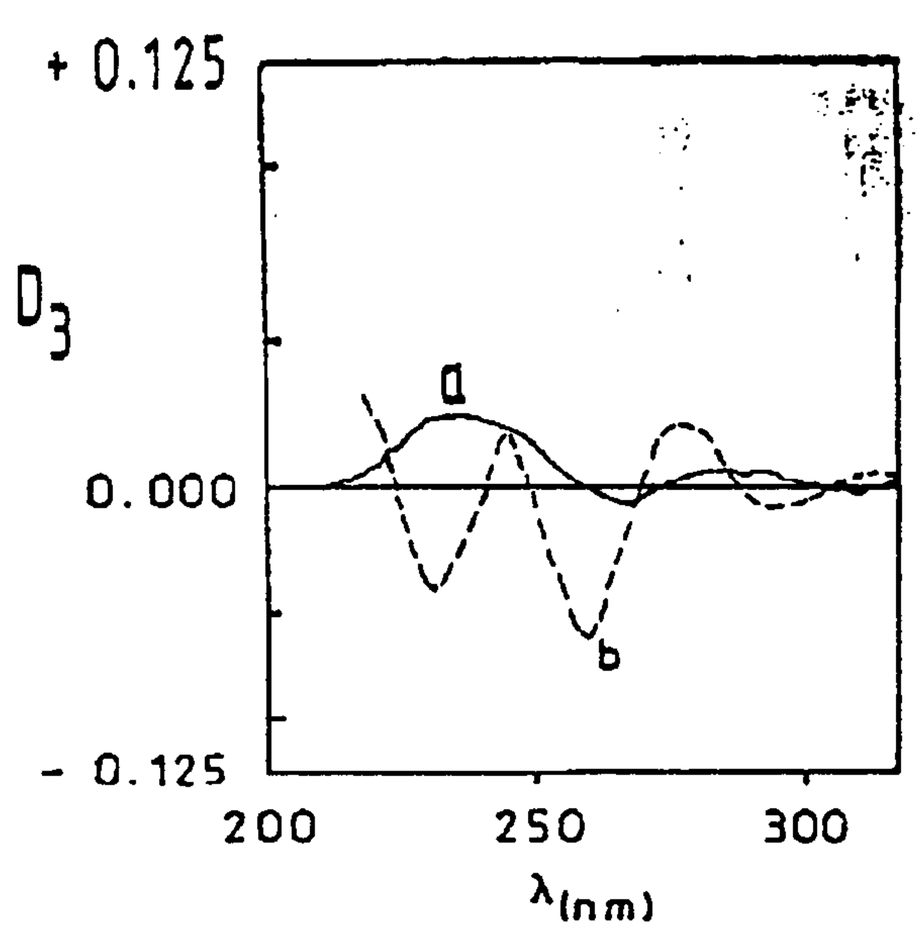


(Fig. 2)

Zero order ultraviolet spectra of plasma samples of (a) aminoglutethimide (10 μg/ml) and (b) acetylaminoglutethimide (10 μg/ml) in methanol (Fig. 1), in 0.02 N methanolic HCl (Fig. 2).



(Fig. 3)



(Fig.4)

D_3 spectra of plasma samples of (a) aminoglutethimide (1.5 μg/ml) and (b) acetylaminoglutethimide (0.75 μg/ml) in methanol (Fig. 3), in 0.02 N methanolic HCl (Fig. 4).

Table 1: Assay parameters for aminoglutethimide and N-acetylaminogluthimide using third derivative spectrophotometric method.

Compound	Sample	λ_{max} nm	Range, μg/ml	Regression equation*			RSD, %**
				(a)	(b)	(r)	
AG	Plasma	266	0.5-2.5	-0.25	9.4	0.9998	1.26
N-AAg	Plasma	256	0.25-1.25	0.75	19.2	0.9997	1.49

* $D_3 = a + bC$, where C is μg/ml.
** Relative standard deviation, five replicate determinations.

Table 2: Percentage recovery of aminoglutethimide and N-acetyl-aminoglutethimide in mixtures from spiked plasma.

AG		N-AAG	
Added* μg/ml	Recovery, %	Added** μg/ml	Recovery, %
0.5	97.2	0.25	97.0
1.0	97.8	0.50	97.3
1.5	98.6	0.75	98.9
2.0	99.8	1.00	98.3
2.5	98.5	1.25	99.4
Mean	98.4		98.2
RSD, %	1.24		1.20

* Each mixture contains 0.75 μg/ml N-AAG.
** Each mixture contains 1.50 μg/m AG.

proposed method for the simultaneous assay of AG and its N-acetyl metabolite in human plasma.

AG could be estimated in plasma at the level of 5 μg/ml, while N-AAG at the level of 2.5 μg/ml. It has been reported that the administration of 1 g daily of AG gives 11.5 μg/ml plasma of AG[19]. The AUC (area under the plasma concentration-time curve) ratios between N-AAG and AG ranged from 0.22 to 0.38[9]. These ratios give concentration levels between 2.53-4.37 μg/ml plasma of N-AAG. Accordingly, the developed D_3 method can be applied successfully to the *in vivo* determination of AG and N-AAG in human plasma.

In conclusion, the proposed D_3 spectrophotometric method is suitable for clinical analysis. The method has the advantages of simplicity, precision and accuracy.

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تعيين الأمينوجلوتيثيمايد وخلات الأمينوجلوتيثيمايد فى البلازما البشرية بإستخدام المشتق التفاضلى الثالث فى آن واحد

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نظرا للتداخل فى امتصاص الضوء بمنطقة الأشعة فوق البنفسجية لكل من الامينوجلوتيثيمايد وناتج أيضه خلات الامينوجلوتيثيمايد عند قياسها طيفيا فى البلازما البشرية، فقد تم فى هذا البحث استخدام المشتق التفاضلى الثالث لمنحنى الامتصاص الضوئى لتعيين كل مركب عند نقطة تقاطع المركب الآخر مع خط الصفر.

وقد أثبتت التجارب أن طريقة المشتق التفاضلى الثالث يمكن أن تستخدم فى تحليل كل من المركبين فى البلازما البشرية وكان معدل الاسترجاع المئوى بين ٩٧,٢ الى ٩٩,٨ لمركب الامينوجلوتيثيمايد وبين ٩٧ الى ٩٩,٤ لخلات الامينوجلوتيثيمايد.

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