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LC determination of aminoglutethimide enantiomers as dansyl and fluorescamine derivatives in tablet formulations

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Abstract

Determination of dansyl (AG-DNS) and fluorescamine (AG-F) derivatives of *rac*-aminoglutethimide in tablet formulation by HPLC has been achieved on a cellulose tris-(3,5-dimethylphenyl carbamate), known as Chiralcel OD and OD-R under normal and reversed phase columns, respectively, using a fluorescence detector (λ_{ex}, 360 nm; λ_{em}, 530 nm for AG-DNS derivatives; λ_{ex}, 395 nm, λ_{em}, 495 nm for fluorescamine derivatives (AG-F)). The best results were obtained with mobile phase ethanol:cyclohexane:methanol (95:5:2 v/v/v) for AG-DNS derivatives and acetonitrile:0.5% *ortho*-phosphoric acid (85:15 v/v) containing 0.26 mM 1-hexanesulfonic acid sodium salt (HSA) for AG-F, respectively. The lower limit of detection (signal to noise ratio of 3:1) were found to be 20 ng ml^{-1} for each enantiomer for AG-DNS and 20.5 ng ml^{-1} for each diastreoisomer for AG-F.

Keywords: Chiral chromatography; Dansyl derivatives of *rac*-aminoglutethimide and fluorescamine derivatives of *rac*-aminoglutethimide; HPLC-fluorescence detection; Enantiomeric separation; Pharmaceutical analysis

1. Introduction

Aminoglutethimide (±AG), ±-3-(4-aminophenyl)-3-ethyl-2,6-piperidinedione, was initially developed as an anticonvulsant for the treatment of epilepsy, but was subsequently withdrawn because of its inhibitory effects on adrenal function. Aminoglutethimide racemic mixture is currently used clinically as a drug of choice in the treatment of hormone-dependent metastatic breast cancer. It was reported that (+)-*R*-isomer had the most steroidogenesis inhibitory activity (two or three times more potent than the racemate), while the (−) *S*-isomer had very little activity at dose levels ten-fold higher [1].

A direct resolution of *rac*-aminoglutethimide and its acetylated metabolite has been achieved by

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HPLC on a series of Chiralcel OD and OJ columns [2,3] on a column of tris (4-methylbenzoate)cellulose covalently bonded to an aminosilica support [4] and α_1-acid glycoprotein (α_1-AGP) column [5]. Several non-chiral [6,7] and chiral assays [8] have been described for analysis of *rac*-aminoglutethimide and its enantiomers in plasma.

Fluorescamine is a well-known reagent for determination of the compounds containing primary or secondary amines [9,10]. Fluorescamine derivatives (AG-F) of the compounds can be separated by HPLC and detected sensitively by fluorescent detector on octadecyl columns [11–14]. There is no report on the separation of stereoisomers of fluorescamine-amine derivatives. Determination of AG dansyl (AG-DNS) derivatives enantiomers in plasma was reported elsewhere [15].

This paper describes two HPLC methods for separation and determination of aminoglutethimide in tablets as AG-DNS and AG-F on a cellulose tris-(3,5-dimethylphenyl carbamate) chiral stationary phase known as Chiralcel OD and Chiralcel OD-R under normal and reversed phase modes, respectively, using a fluorescence detector. The solvent effect on the resolution of aminoglutethimide enantiomers derivatives is studied. Furthermore, a comparison between the two proposed methods is presented.

2. Experimental

2.1. Materials

Rac-aminoglutethimide ($\pm$AG) and *R*-(+)-AG was kindly supplied by Ciba–Geigy (Basle, Switzerland). Dansyl chloride (DNS-Cl) and 1-hexanesulfonic acid sodium salt (HSA) were purchased from Aldrich (Milwaukee, WI, USA). Fluorescamine was purchased from Sigma (St. Louis, USA). 2-(3,4-Dimethoxyphenyl)ethylamine, fluorescein, *ortho*-phosphoric acid, HPLC grade acetonitrile, ethanol, methanol and dichloromethane were obtained from E. Merck (Darmstadt, Germany) and cyclohexane was obtained from Fischer Scientific (Fairlawn, NJ, USA).

2.2. Equipment

Chromatography was performed using a liquid chromatographic system consisting of a Waters Model 420 Fluorescence detector (λ_{ex}, 360 nm; λ_{em}, 530 nm for DNS derivatives; λ_{ex}, 395 nm; λ_{em}, 495 nm for AG-F) a U6K injector, a 510 model pump, a Hewlett–Packard 3365 Chem Station data analysis system. Chiralcel OD column (Daicel, Tokyo, Japan, 25 cm $\times$ 4.6 mm i.d., coated on Silica gel 10 μm particle size) was used for AG-DNS while Chiralcel OD-R column (Daicel, Tokyo, Japan, 25 cm $\times$ 4.6 mm i.d., coated on Slica gel, 10 μm particle size) was used for AG-F.

A series of parameters, consisting of composition and pH of mobile phase, the column packing, flow rate and detection wavelength were tested. As the final optimized method, a mobile phase consisting of ethanol:cyclohexane:methanol (95:5:2 v/v/v) was used for AG-DNS. Acetonitrile, 0.5% *ortho*-phosphoric acid (85:15 v/v) containing 0.26 mM HSA was used for AG-F. The samples were injected at room temperature.

3. Method development

3.1. Stock solutions for AG-DNS

Stock solutions of *rac*-AG (40 μg ml^{-1}) and 2-(3,4-dimethoxyphenyl)ethylamine (IS) (0.27 μg ml^{-1}) were freshly prepared in dichloromethane, working solutions were obtained by diluting stock solutions in dichloromethane.

3.2. Stock solutions for AG-F

Stock solutions of *rac*-AG (40 μg ml^{-1}) and fluorescein (IS) (200 μg ml^{-1}) were freshly prepared in acetonitrile. Working solutions were obtained by diluting stock solutions in acetonitrile.

3.3. Standard preparation for AG-DNS

Calibration standards were freshly prepared by pipetting 25–150 μl of AG standard solutions and 100 μl of IS solution. Two hundred and fifty

microliters of DNS-Cl solution (0.4 mg ml^{-1} in acetone) and 20 mg of sodium carbonate were added and the total volume was adjusted to 1 ml with dichloromethane, vortexed for 1 min and kept at room temperature in the dark for 1 h to complete dansylation. An aliquot (500 μl) was evaporated under nitrogen, reconstituted in 500 μl of mobile phase, vortexed and 20 μl of the sample was injected into the column.

3.4. Standard preparation for AG-F

Calibration standards were freshly prepared by pipetting 20–60 μl of AG standard solutions and 250 μl of IS solution. Five hundred microliters of fluorescamine solution (1.2 mg ml^{-1} in acetonitrile) and 300 μl phosphate buffer, pH of 4, were added, and the total volume was adjusted to 1225 μl with water, vortexed for 1 min and kept at room temperature in the dark for 20 min to complete derivatization, and then 50 μl of the solution injected into the column.

3.5. Pharmaceutical tablet formulation (250 mg aminoglutethimide per tablet)

Twenty tablets were accurately weighed and the average tablet weight was determined. The tablets were ground to a fine powder and a portion of the tablet powder equivalent to 200 mg of aminoglutethimide was weighed and transferred quantitatively into a 50 ml volumetric flask and then diluted to volume with acetone–water (1:1). The mixture was sonicated for 10 min and treated in the same manner as the standards, for AG-DNS and AG-F.

4. Results and discussion

Derivatization of AG and internal standard for AG-DNS proved to be optimal and reproducible under the conditions described above.

In order to improve the resolution of AG-DNS enantiomers several mobile phase composition were tested. The best results in terms of resolution, analysis time and separation factor were obtained with mobil phase consisted of ethanol–cyclohexane–methanol (95:5:2 v/v/v). The use of cyclohexane led to an improvement in stereoselectivity [15].

For the separation of AG-F diastereoisomers acidic solvent composition is essential. Higher compositions of H_3PO_4 than 0.5% resulted in peak distortion. Addition of acidic ion pair agents improved the separation and peak shape. Lower acetonitrile concentrations resulted in longer analysis time. The best results were obtained with mobile phase consisted of acetonitrile:0.5% *ortho*-phosphoric acid (85:15 v/v) containing 0.26 mM HSA for AG-F (Table 1).

Typical retention times (standard deviations, S.D.) for IS, (*R*) AG-DNS and (*S*) AG-DNS were 7.250 (0.007), 8.970 (0.024) and 11.560 (0.059), respectively ($n = 20$).

The retention times (S.D.) for IS and AG-F stereoisomers were 5.506 (0.180), 11.429 (0.099), 21.151 (0.213), 24.052 (0.512), 32.999 (0.566), respectively, ($n = 7$) (Fig. 1).

Inter-day differences in mobile phase composition did not significantly alter chromatographic performance. Relative standard deviation (RSD) of AG-F for intra and inter-day precision were in the ranges 2.73–6.89 and 1.66–5.21%, respectively (Tables 2 and 3). The lower limit of detection (signal to noise ratio of 3:1) were found to be 20 ng ml^{-1} for each enantiomer for AG-DNS and 20.5 ng ml^{-1} for each diastreoisomer for AG-F.

Table 1
Effects of changing proportions of the mobile phase on the chromatographic peaks of AG-F

Solvent composition ACN:0.5% *ortho*-phosphoric acid (containing HSA 0.26 mmol)	k_1	k_2
90:10	2.14	9.77
88:12	1.41	6.69
87:13	1.14	5.41
85:15	1.44	6.72
80:20	2.44	11.25
70:30	3.45	15.78

ACN, acetonitrile; k_1, capacity factor of the (*S*)AG-F; k_2, capacity factor of the (*R*) AG-F.

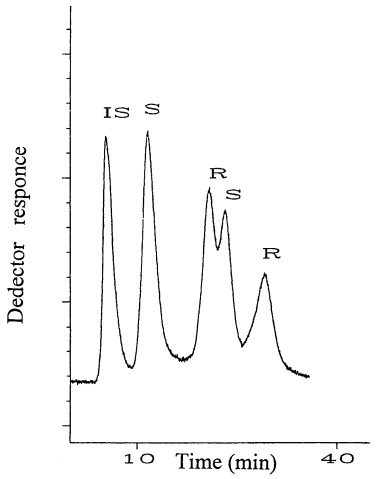


Fig. 1. Representative chromatogram of fluorescamine derivatives (AG-F) of *rac*-aminoglutethimide (IS, internal standard).

Table 2
Intra-day precision and accuracy for AG-F

Compound	Added (ng 50 μl^{-1})	Recovery (%)	R.S.D. (%)
(*S*)-AG	50	98.02	2.73
	30	100.30	2.86
	20	110.85	3.47
(*R*)-AG	50	99.53	6.89
	30	104.81	2.89
	20	112.72	4.24

The results are the mean of four determinations.

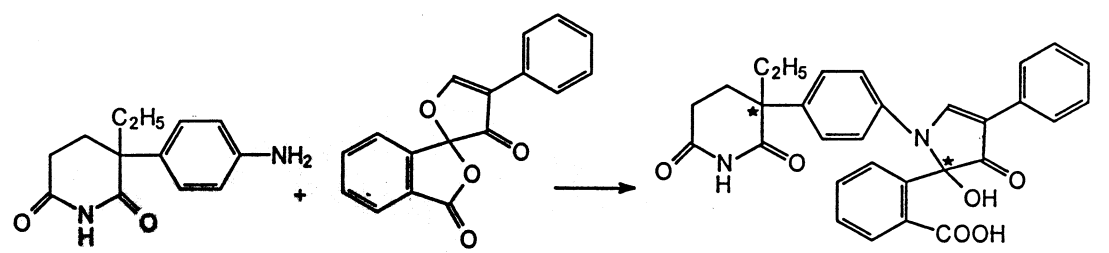
Table 3
Inter-day precision for AG-F

Compound	Added (ng 50 μl^{-1})	Found (ng 50 μl^{-1})	R.S.D. (%)
(*S*)-AG	21.37	24.38	1.66
(*R*)-AG	21.37	25.27	3.16
(*S*)-AG	29.38	28.72	4.66
(*R*)-AG	29.38	31.44	3.48
(*S*)-AG	48.25	48.36	3.17
(*R*)-AG	48.25	50.82	5.21

The results shown are the mean of four determinations.

Derivatization of aminoglutethimide with fluorescamine resulted in a derivative with two chiral centres as shown in equation. There is no report on the formation and separation of four streoisomers of AG-F of chiral amines by HPLC hitherto [11,12]. However, it is of interest to mention that the four stereoisomers of AG-F were separated on Chiralcel OD-R under the chromatographic conditions described in the experimental section as shown in Fig. 1.

The peak identification was achieved by the HPLC separation of fluorescamine derivative of pure *R*-AG enantiomer (Fig. 2). Fluorescein was chosen as IS because of its fluorescense properties and chemical structure. No reaction was observed between fluorescamine and fluorescein.



Aminoglutethimide Fluorescamine AG-F

*Asterisk denotes the position of the chiral center

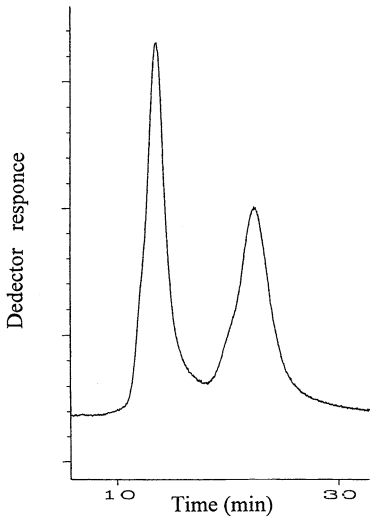


Fig. 2. Chromatogram of fluorescamine derivative of (*R*)-aminoglutethimide.

4.1. Linearity of assay

The calibration curves for *R*-AG and *S*-AG were linear in the concentration ranges 0.3–3.0 μg ml^{-1} for AG-DNS; 0.4–1.3 μg ml^{-1} for AG-F. Tablet concentrations were derived from linear regression analysis of the peak height ratios (analyte/IS) versus concentration curves (Table 4). The peaks which retention times are 11.43 (*S*-AG) and 33 (*R*-AG) were chosen to construct the calibration curves because of their complete separation.

The proposed methods were applied for the analysis of the pharmaceutical tablet formulations containing 250 mg of *rac*-aminoglutethimide (Orimeten® tablet). Results are given in Table 5. There was no interferences from the tablet excipients which consisted mainly of starch, lactose, magnesium stereate.

Table 5
Analysis of Orimeten® tablets (250 mg aminoglutethimide per tablet)

Method	Enantiomer	
	S	*R*
	Recovery (R.S.D.) (%)	Recovery (R.S.D.) (%)
AG-DNS	101.92 (1.67)	101.26 (1.51)
AG-F	102.28 (1.05)	101.17 (1.83)

These results are the mean of six determinations.

5. Conclusion

Efficient and reliable HPLC methods for the stereoselective analysis and quantitation of aminoglutethimide were developed. The AG-DNS method is the method of choice for determination of AG enantiomers in pharmaceutical formulations because of simplicity and selectivity. The AG-F method is more complicated due to the formation of four enantiomers which could be separated, although partially. The methods were validated in terms of limit of detection, quantitation, linearity, precision, accuracy and range.

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Table 4
Linearity of the assay for AG-DNS and for AG-F

Enantiomer	Correlation coefficient	Slope ($\pm$S.D.)	Intercept ($\pm$S.D.)
(*R*)-AG DNS	0.999	$0.1298 \pm 9.9 \times 10^{-6}$	$0.07 \pm 1.8 \times 10^{-5}$
(*S*)-AG DNS	0.999	$0.0895 \pm 5.0 \times 10^{-3}$	$0.089 \pm 1.2 \times 10^{-3}$
(*R*)-AG F	0.999	$0.5584 \pm 5.6 \times 10^{-3}$	$-0.1154 \pm 5.2 \times 10^{-3}$
(*S*)-AG F	0.999	$1.1412 \pm 2.7 \times 10^{-3}$	$0.0510 \pm 3.0 \times 10^{-3}$

These results are the mean of six determinations.

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