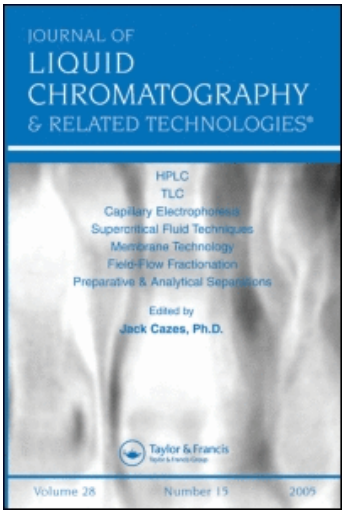

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ENANTIOMERIC SEPARATION OF AMINOGLUTETHIMIDE, ACETYL AMINOGLUTETHIMIDE, AND DANSYL AMINOGLUTETHIMIDE BY TLC WITH β-CYCLODEXTRIN AND DERIVATIVES AS MOBILE PHASE ADDITIVES

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ABSTRACT

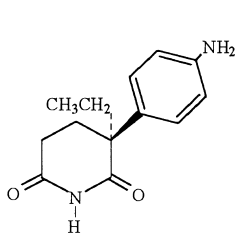
Racemic aminoglutethimide and acetylaminoglutethimide were resolved on a fluorescent Silica gel 60A TLC plate using 30% hydroxy trimethylpropylammonium-β-cyclodextrin with methanol (50:50v/v) as the mobile phase. Also, the resolution of the enantiomers of aminoglutethimide as dansyl derivatives were investigated on reversed phase TLC using a mobile phase consist-

ing of a β-cyclodextrin solution (0.05 M saturated with urea), or 15% (carboxymethyl-β-cyclodextrin) solution with methanol (65:35v/v). Enantiomeric resolution was found to be highly dependent on mobile phase composition. The effect of the type and amount of organic modifier as well as the concentration of β-CD derivative, and the pH of the mobile phase on resolution were studied. Three types of maltodextrins failed to show resolution for any of the racemic compounds studied.

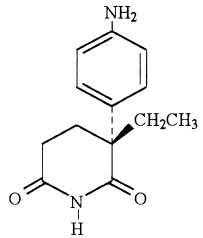
INTRODUCTION

Aminoglutethimide (AG) is chemically known as ±-3-(4-aminophenyl)-3-ethyl-2,6-piperidinedione), and the stereochemical formulae of AG enantiomers are shown in Figure 1. Aminoglutethimide was initially developed as an anticonvulsant for the treatment of epilepsy, but was subsequently withdrawn because of its inhibitory effects on adrenal function. However, rac-aminoglutethimide is currently used clinically in the treatment of hormone-dependent metastatic breast cancer.[1,2] 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, even at dose levels 10-fold higher.[3] N-acetylaminoglutethimide is the major mammalian metabolite of aminoglutethimide and is pharmacologically inactive.

During drug development of it, it is important to be able to isolate the enantiomers in order to assess which is responsible for the potency, the toxicity, and for the side effects. Resolution of the racemic aminoglutethimide by high performance liquid chromatography has been reported in earlier works.[4-14] Thin-layer chromatography (TLC) has advantages relative to other analytical techniques, such as low cost and simplicity of the method. In particular, chiral



R-(+)-Aminoglutethimide



S-(-)-Aminoglutethimide

Figure 1. The absolute configuration of Aminoglutethimide.

TLC eliminates the need for expensive machinery and columns. Reports on the use of chiral mobile phase additives (CMAs) for the separation of enantiomers by TLC have increased substantially in the last few years, and cyclodextrins (CDs) have been the subject of several reports for this purpose.[15-19]

CDs were first used as mobile phase additives for chromatography in 1980 to separate a series of structural isomers.[20-21] Derivatized β-CD has shown more promise as chiral mobile phase additive than native β-CD, as derivatization results in enhanced solubilities which, in turn, provides improved enantioselectivities relative to the native β-CD.[22-23] However, modification of the β-CD was shown to change the chromatographic behaviour as well as chiral selectivity.[15]

This paper investigates the use of chiral TLC for the direct resolution of aminoglutethimide, acetylaminoglutethimide, and dansylaminoglutethimide using chiral CMA selectors**,** including native CD (α, β, and γ), β-CD derivatives, namely hydroxytri- methylpropylammonuim-β-cyclodextrin, and carboxymethyl-β-cyclodextrin. Three type of maltodextrins namely, maltodextrin 4-7, maltodextrin 16.5-19.5, and maltodextrin 13-17, were also investigated. Furthermore, the effect of the type and concentration of the organic modifiers used in the mobile phase, its pH, as well as the β-CD concentration on the resolution of these compounds, were studied.

EXPERIMENTAL

Materials

Rac-aminoglutethimide (±AG), (-)-S, and (+)-R-AG enantiomers were kindly supplied by Ciba-Geigy (Basle, Switzerland). Dansyl chloride was purchased from Aldrich (Milwaukee, Wisconsin, USA).

TLC-sheets, silica gel 60F 254, 0.2 mm layer thickness, 5 × 10 cm were obtained from Riedel-de Haën (Germany). TLC plates silica gel 60 F 254 precoated of 0.25 mm layer thickness, (5 × 10 cm, 5 × 20 cm, 10 × 20 cm, and 20 × 20 cm), were obtained from E. Merck (Darmstadt,Germany). TLC silica gel 60A, fluorescent at 254 nm (LK 6F), 0.25 mm layer thickness, 5 × 20, 20 × 20 cm were obtained from Whatman International Ltd., Maidstone, UK.

Cyclodextrins (α-,β-, and γ-) and maltodextrins were obtained from Fluka (Buchs, Switzerland). Carboxymethyl-β-cyclodextrinsodium, 2-hydroxy-3-trimethylammoniumpropyl-β-cyclodextrin (HTMA-β-CD), were obtained from Wacker-Chemie GmbH, (Munich, Germany). Synthesis of the dansyl derivative is described by Aboul-Enein.[14]

HPLC-grade methanol, acetonitrile, and other reagents of analytical grade or equivalent were used. All chemicals were used as received. All developments were run at room temperature in cylindrical glass chambers.

Spot visualization was achieved using a fixed-wavelength (254 nm) UV lamp (Upland, CA, USA). The chiral separation factor of the two spots of a resolved racemate was calculated as the ratio of the higher Rf value and the lower Rf value for the two enantiomers, using spot centres.

Three determination were made for each experiment. Several mobile phase compositions were tested in order to optimize the enantiomeric resolution as shown in (Table 1).

Preparation of Sample

Approximately 4 mg of R- or S-aminoglutethimide, 8 mg of rac-aminoglutethimide was placed in a 5 mL volumetric flask, diluted to volume with methanol, and placed in an ultrasonic bath to aid dissolution. Dansyl aminoglutethimide (8 mg) and acetylaminoglutethimide (8 mg), were prepared in the same way as the parent drug.

Preparation of Mobile Phase

The solubility of β-CD in water is $1.67.10^{-2}$ M at 25°C, although this figure can be increased by the addition of urea. In this study, saturated solutions of urea were used when necessary. 0.6 M sodium chloride was also added to the mobile phase to stabilize the binder of the plates. Without this salt, mobile phases containing more than 50% water tend to dissolve the binder of Whatman plates, there by resulting in the separation of the stationary phase from the glass support during development.

Cyclodextrin was dissolved in deionised water to give concentrations of 0.05 M, 0.1 M, and 0.15 M for α and γ, and 0.01 M, 0.03 M, and 0.05 M for β-CD with the aid of urea. Solutions of maltodextrin 4-7, maltodextrin 16.5-19.5, maltodextrin 13-17, (hydroxytrimethylpropylammonium-β-cyclodextrin), and carboxymethyl-β-cyclodextrin sodium were prepared in water to give a concentration of 5, 10, 15, 20, 25, 30, and 40% w/v. Mobile phases were prepared with methanol, or acetonitrile as the organic modifier, each at various concentrations added to the aqueous solution of cyclodextrin, cyclodextrin derivatives, or maltodextrins, to determine their influence on retention and resolution.

RESULTS AND DISCUSSION

Table 1 shows the optimized conditions for the separation of racemic aminoglutethimide and its acetylated and dansyl derivatives determined by varying the type and composition of the mobile phase. The best results in term of resolution, analysis time, and separation factor were obtained with mobile phase consisting of aqueous 30% w/v (2-hydroxy-3-trimethylpropylammonium-β-cyclodextrin):methanol (50:50) for both racemic aminoglutethimide and acetylaminoglutethimide.

A mobile phase consisting of 0.05 M native β-CD:methanol (65:35), and 30% w/v (carboxymethyl-β-cyclodextrin):methanol (65:35) were used, successfully, for the resolution of racemic dansylaminoglutethimide(DAG). These conditions possibly reflect complexation with the dansyl moiety, rather than the ring structure of AG. Although the Rf value of racemic aminoglutethimide was largest when 35% β-CD was in the mobile phase, the separation between the two spots was poor due to tailing.

The three types of maltodextrins failed to show resolution for any of the racemic compounds studied. Although different concentrations ranging from 5% to 20%, with up to 60% of each concentration for each type of maltodextrin in the mobile phase, were used, no resolution was achieved. It is probable that the lack of the resolution of racemic AG and its derivatives using maltodextrins CMAs was due to the lack of enantioselective complexation. Maltodextrins have linear configurations, unlike the chiral toroid shape of CDs, and the chiral recognition between the maltodextrin as a chiral selector and the analyte will be minimum, thus, resulting in lack of resolution.

Figures 2 and 3 show the influence of varying the concentration of 2-hydroxy-3-trimethylpropylammonium-β-cyclodextrin) in mobile phase on the chiral separation. The separation occurred over a range of 10% to 40% of 2-hydroxy-3-trimethylpropylammonium-β-cyclodextrin in the mobile phase for the racemic aminoglutethimide. An optimum resolution of $\alpha = 1.36$ was achieved when 30% of TMA was used. However, increasing the percentage of TMA led to decrease the resolution of the racemic aminoglutethimide and its derivatives.

Influence of the Nature and Concentration of the Organic Modifier

Methanol and acetonitrile were used as organic modifiers in the mobile phases. Despite increasing the percentage of acetonitrile in the mobile phase, up to 65%, on both reversed phase TLC and normal TLC, with native, β-cyclodextrin and its derivatives, no enantiomeric separation was observed

Table 1

Enantiomers Separated Using β-CD and its Derivatives as Chiral Mobile Phase Additives with Silica Gel Plate

Subject	Stationary Phase	Type and Conc. of Cyclodextrin (A)	Mobile Phase	$Rf_{1in\ mm}$	$Rf_{2in\ mm}$	α	Run Time	Spot(s) Shape
±AG*	RPHPTLC	β-CD (0.05M)	[A] + methanol 65:35	80	92	1.15	4 hr	Two spots
± AG	RPHPTLC	β-CD (0.05M)	[A] + methanol 50:50	88	98	1.11	4 hr	Two spots
± AG	RPHPTLC	β-CD (0.05M)	[A] + methanol 40:60	96	115	1.2	4 hr	Two spots
± AG	RPHPTLC	β-CD (0.05M)	[A] + methanol 35:65	135	160	1.9	4 hr	Two spots
± AG	RPHPTLC	β-CD (0.05M)	[A] + methanol 20:80	165	170	1.03	4 hr	Two spots
± AG	RPHPTLC	Maltodextrin 4-7 (20%)	[A] + methanol 60:40	–	–	1	1 hr	One spot
± AG	RPHPTLC	Maltodextrin 16.5-19.5 (20%)	[A] + methanol 60:40	–	–	1	1 hr	One spot
± AG	RPHPTLC	Maltodextrin 7-13 (20%)	[A] + methanol 35:65	–	–	1	1 hr	One spot
± AG	RPHPTLC	Maltodextrin 4-7 (5%)	[A] + methanol 20:80	–	–	1	1 hr	One spot
± AG	RPHPTLC	Maltodextrin 7-13 (5%)	[A] + methanol 10:90	–	–	1	1 hr	One spot
± AG	RPHPTLC	Maltodextrin 16.5-19.5 (5%)	[A] + methanol 5:95	–	–	1	1 hr	One spot
± AG	RPTLC	Carboxymethyl-β-cyclodextrin sodium (15%)	[A] + methanol 40:60	–	–	1	1 hr	One spot
± AG	RPTLC	Carboxymethyl-β-cyclodextrin sodium (15%)	[A] + methanol 35:65	–	–	1	1 hr	One spot

± AG	RPTLC	Carboxymethyl-β-cyclodextrin sodium (15%)	[A] + methanol 20:80	–	–	1	1 hr	One spot
± AG	normal TLC	HTMA-β- CD (10%)	[A] + methanol 50:50	–	–	1	30 min	One spot
± AG	normal TLC	HTMA-β-CD (20%)	[A] + methanol 50:50	54	55	1.05	30 min	Two spots
± AG	normal TLC	HTMA-β-CD (30%)	[A] + methanol 50:50	55	75	1.36	30 min	Two spots
± AG	normal TLC	HTMA-β-CD (40%)	[A] + methanol 50:50	56	65	1.16	30 min	Two spots
±AAG**	normal TLC	HTMA-β-CD (30%)	[A] + methanol 50:50	55	60	1.1	30 min	Two spots
±DAG***	RPTLC	β-CD (0.05 M)	A + Methanol 65:35	–	–	1.0	1 hr	One spot
± DAG	RPTLC	β-CD (0.05 M)	A+ Methanol 65:35	70	80	1.15	3 hr	Two spots
± DAG	RPHPTLC	β-CD (0.05 M)	A + Methanol 65:35	48	80	1.7	3 hr	Two spots
± DAG	RPTLC	Carboxymethyl-β-cyclodextrin (15%)	A + Methanol 65:35	65	73	1.12	1 hr	Two spots (long wavelength)

* Aminoglutethimide. ** Acetylated aminoglutethimide. *** Dansyl aminoglutethimide.

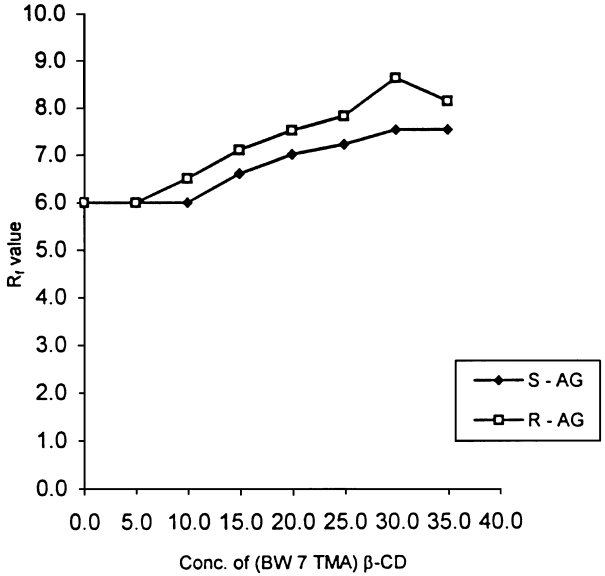


Figure 2. Plot showing the effect of HTMA-β-cyclodextrin concentration in the mobile phase on the R_f value of S-aminoglutethimide and R-aminoglutethimide.

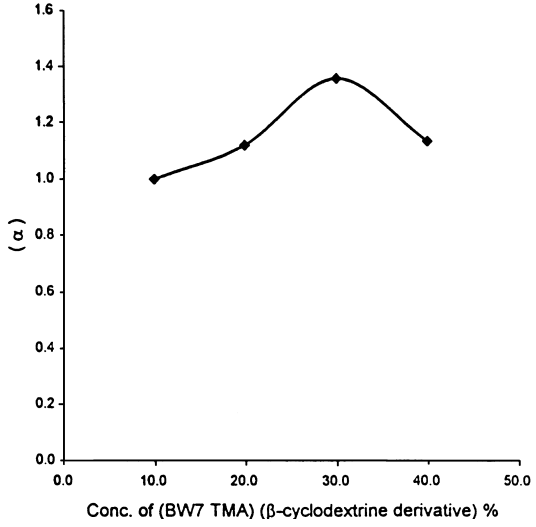


Figure 3. Influence of (HTMA-β-cyclodextrin) conc. on (α) of the racemic AG.

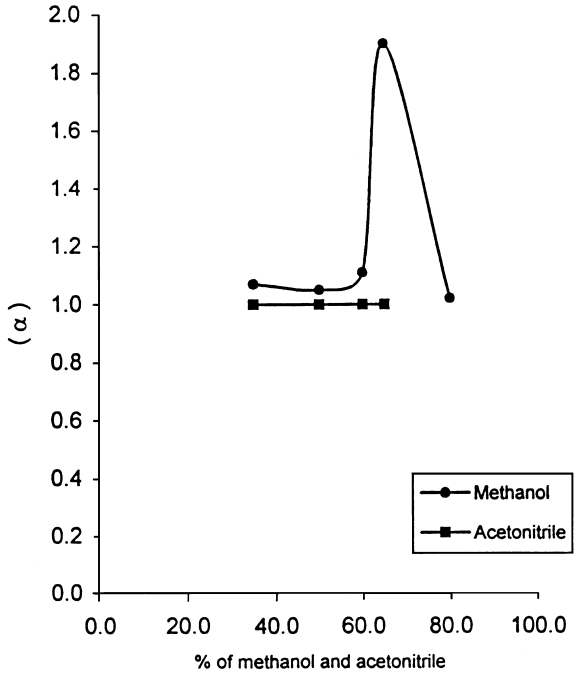


Figure 4. Influence of organic modifier (%) with native β-cyclodextrin on (α) of racemic aminoglutethimide.

(Figure 4). As the percentage of methanol increased in the mobile phase, the resolution increased, with an accompanying decrease in analysis time. An optimum chiral separation of $\alpha = 1.9$ for racemic aminoglutethimide was observed, when the methanol percentage in the mobile phase was 65%, with native β-cyclodextrin (0.05M) on reversed phase high performance TLC. When the methanol percentage increased to 80%, the enantiomeric separation drastically decreased.

For the dansylaminoglutethimide, the optimum methanol percentage in the mobile phase was 35%, with native β-cyclodextrin, which gave $\alpha = 1.15$, while no resolution was observed in the case of acetylaminoglutethimide, in spite of using different percentages of methanol with the native β-cyclodextrin in the mobile phase on different types of TLC plates. The mobile phase composed of methanol:carboxymethyl-β-cyclodextrin (35:65) showed enantiomeric separation of the racemic dansylaminoglutethimide of $\alpha = 1.12$, but no resolution for the racemic aminoglutethimide and acetylaminoglutethimide. Methanol gave maximum chiral separation of $\alpha = 1.4$ for racemic aminoglutethimide and $\alpha =$

1.1 for racemic acetylaminoglutethimide when added to 30% hydroxytrimethylammoniumpropyl-β-cyclodextrin in 50:50%v/v as mobile phase on normal TLC plates.

The aforementioned results indicate that the eluent compositions plays an important role in the enantiomeric separation (Table 1). It is of interest to mention that the (-)-S-AG eluted first, followed by (+)-R-enantiomer in all the successful resolutions obtained in this study. This indicates that the R-enantiomer is preferentially complexed by the CD.

Influence of pH

To test the influence of pH on retention and resolution, racemic aminoglutethimide was resolved on normal fluorescent TLC plates, with a mobile phase consisting of 30% hydroxytrimethylpropylammonium-β-cyclodex-

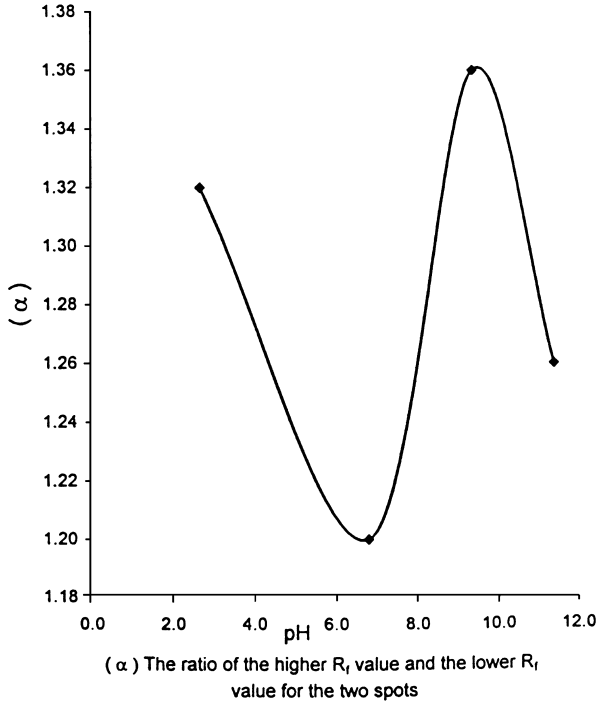


Figure 5. Influence of pH on (α) of racemic amino-glutethimide with methanol and trimethyl propyl hydroxyammonium-β-cyclodextrin.

trin:methanol at pH 2.7, 6.8, 9.4, 11.4. The pH was adjusted by addition of dilute phosphoric acid and/or dilute sodium hydroxide The retention time (an average of three runs) was about 30 min. The best resolution of racemic aminoglutethimide of $\alpha = 1.36$ was obtained at pH 9.4 as shown in Figure 5.

CONCLUSIONS

Resolution of racemic aminoglutethimide was achieved without the need to use a chiral TLC plate. 2-Hydroxy-3-trimethylpropylammonium-β-cyclodextrin as a mobile phase additive has a pronounced effect on the retention properties of the enantiomers of both aminoglutethimide and acetylated aminoglutethimide. Direct separation of racemic dansylaminoglutethimide was achieved using β-CD and carboxymethyl-β-CD. This technique can be used for the determination of aminoglutethimide and its major metabolite in biological fluids and is now in progress in this laboratory.

REFERENCES

1. G. Cocconi, Breast Cancer Res. Treat., **30**, 57 (1994).

2. H. V. Vander Bossche, H. Moereels, L. K. Koymans, Breast Cancer Res. Treat., **30**, 43 (1994).

3. N. Finch, R. Dziemian, J. Cohen, B. G. Steinetz, Experientia, **31**, 1002 (1975).

4. H. Y. Aboul-Enein, A. I. Al-Durabi, Sep. Sci. Technol., **34**,2973 (1999).

5. H. Y. Aboul-Enein, M. R. Islam, Chromatographia, **30**, ,223 (1990).

6. H. Y. Aboul-Enein, M. R. Islam, J. Chromatogr. Sci., **26**, 616 (1988).

7. L. Lepri, M. Del Bubba, V. Coas, A. Cincinelli, J. Liq. Chrom. & Rel. Technol., **22**, 105 (1999).

8. H. Y. Aboul-Enein, M. R. Islam, "Direct Stereochemical High Pressure Liquid Chromatographic Separation of Aminoglutethimide and Its Major Metabolite," in **Chiral Separations by Liquid Chromatography**, S. Ahuja, ed., Washington, D.C., 1991, Chapter 12, pp. 203-215.

9. H. Y. Aboul-Enein, V. Serignese, C. Minguillon, L. Oliveros, Biomed. Chromatogr., **11**, 303 (1997).

10. H. Y. Aboul-Enein, V. Serignese, Chirality, **10**, 354 (1998).

11. A. V. Overbeke, H. Y. Aboul-Enein, W. Baeyens, G. Van der Weken, C. Dewaele, Anal. Chim. Acta., **346**,183 (1997).

12. M. L. Bieganowska, A. Petruczynik, Chem. Anal. (Warsaw), **43**, 583 (1998).

13. H. Y. Aboul-Enein, S. A. Bakr, Chirality,**9,** 10 (1997).

14. H. Y. Aboul-Enein, S. Özkirimli, T. I. Apak, Arch. Pharm. Pharm. Med. Chem. **332**, 145 (1999).

15. J. C. Reepmeyer, Chirality, **8**, 11 (1996).

16. T. K. Xuan Huynh, M. Lederer, J. Chromatogr. A, **659**, 191 (1994).

17. W. L. Hinze, in **Separation and Purification Methods**, **Vol**. **10**, C. J. Van Oss, ed., New York, Marcel Dekker, 1981, pp. 159.

18. D. W. Armstong, W. DeMond, J. Chromatogr. Sci., **22**, 411 (1984).

19. D. W. Armstrong, S. M. Han, Crit. Rev. Anal. Chem.,**9**, 175 (1988).

20. D. W. Armstrong, J. Liq. Chromatogr., **3**, 896 (1980).

21. W. L. Hinze, D. W. Armstrong, Anal. Lett., **13**, 1093 (1980).

22. D. W. Armstrong, J. R. Faulkner, S. M. Han, J. Chromatogr., **542**, 323 (1988).

23. J. D. Duncan, D. W. Armstrong, J. Planar Chromatogr., **3**, 65(1990).

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