Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Enantioselective Separation of Several Piperidine-2,6-dione Drugs on Chirose C-1 Chiral Stationary Phase

Hassan Y. Aboul-Enein[a]; Ibrahim A. Al-Duraibi[a]
[a] BIOANALYTICAL AND DRUG DEVELOPMENT LABORATORY, BIOLOGICAL AND MEDICAL RESEARCH DEPARTMENT (MBC 03), KING FAISAL SPECIALIST HOSPITAL AND RESEARCH CENTRE, RIYADH, SAUDI ARABIA

Online publication date: 20 October 1999

To cite this Article Aboul-Enein, Hassan Y. and Al-Duraibi, Ibrahim A.(1999) 'Enantioselective Separation of Several Piperidine-2,6-dione Drugs on Chirose C-1 Chiral Stationary Phase', Separation Science and Technology, 34: 15, 2973 — 2979
To link to this Article: DOI: 10.1081/SS-100100816
URL: http://dx.doi.org/10.1081/SS-100100816

SEPARATION SCIENCE AND TECHNOLOGY, **34**(15), pp. 2973–2979, 1999

Enantioselective Separation of Several Piperidine-2,6-dione Drugs on Chirose C-1 Chiral Stationary Phase

HASSAN Y. ABOUL-ENEIN* and IBRAHIM A. AL-DURAIBI
BIOANALYTICAL AND DRUG DEVELOPMENT LABORATORY
BIOLOGICAL AND MEDICAL RESEARCH DEPARTMENT (MBC 03)
KING FAISAL SPECIALIST HOSPITAL AND RESEARCH CENTRE
P.O. BOX 3354, RIYADH 11211, SAUDI ARABIA

ABSTRACT

A newly developed Chirose C-1 chiral stationary phase, a highly chiral polymer, has been used for direct and isocratic enantiomeric separation of 12 piperidine-2,6-dione compounds under normal phase conditions. Baseline separation has been achieved for eight compounds, three compounds were partially separated, while one compound did not resolve.

Key Words. Piperidine-2,6-dione; High-performance liquid chromatography; Chiral separation; Chiral polymer; Chirose C-1 chiral stationary phase; Normal phase mode

INTRODUCTION

Enantiomers should be treated as separate substances because they often differ in potency, pharmacological action, or plasma disposition. For instance, the (+)-*R*-aminoglutethimide had the greatest steroidogenesis inhibitory activity (two to three times more potent than the racemate) while the (−)-*S*-isomer had very little activity at dose levels 10-fold higher (1). *N*-Acetylaminoglutethimide is the major mammalian metabolite of aminoglutethimide (2)

* To whom correspondence should be addressed. FAX: +(966)-1-442-7858. E-mail: enein@kfshrc.edu.sa

and it does not have any pharmacological activity (3, 4). Also, cyclohexylaminoglutethimide, known as 3-cyclohexyl-3-(4-aminophenyl)-2,6-piperidinedione, is a specific aromatase inhibitor for the treatment of estrogen-dependent breast cancer. (+)-(*S*)-Enantiomer is 30 times more active than the (−)-(*R*)-enantiomer (5). Since most of the pharmacokinetics, metabolism, and pharmacological activity published deals with the racemic mixtures of piperidinediones and not the pure enantiomers (6), it is therefore necessary to determine the proportion of each optical isomer present in a pharmaceutical preparation and to find an efficient method for its separation. The drug series investigated in this study has a piperidine-2,6-dione moiety in common but belongs to different pharmacological classes, such as anticancer, hypnotic, and anticholinergic agents (6–12). Several chiral methods have been described for the separation of enantiomers of some piperidine-2-6-dione compounds in this laboratory and others (13–25).

This study describes the resolution of a series of piperidine-2,6-dione racemic compounds by a direct isocratic high-performance liquid chromatography (HPLC) method on a newly developed chiral stationary phase (CSP) known as Chirose C-1.

MATERIAL AND METHOD

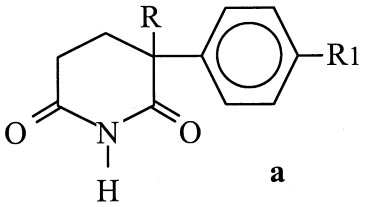
Chemicals

(±)-Glutethimide, (±)-aminoglutethimide, *R*-(+)-aminoglutethimide, and (±)-*N*-acetylaminoglutethimide were obtained from Ciba-Geigy (Basel, Switzerland); (±)-3-phenylacetylamino-2,6-piperidinedione, known as antineoplaston-A10 (A-A10), was obtained from the Burzynski Institute (Stafford, TX); (±)-thalidomide and *S*-(−)-thalidomide were gifts from Dr. J. C. Reepmeyer, Food and Drug Administration (St. Louis, MO); (±)-pyridoglutethimide was obtained from Dr. R. McCague, Chiroscience plc (Cambridge, UK); (±)-thiophenylglutethimide, (±)-cyclohexylaminoglutethimide, and *R*-(−)-cyclohexylaminoglutethimide were from Dr. R. W. Hartmann (University of Saarland, Saarbrücken, Germany); (±)-methylthalidomide, *S*-(+)-methylthalidomide, (±)-ethylthalidomide, *S*-(+)-ethylthalidomide, and (±)-*n*-propylthalidomide were kindly supplied by Professor J. Knabe (University of Saarland, Saarbrücken, Germany); and (±)-phenylgluterimide.HCl was from Paul Nicholls (University of Cardiff, UK) (see Fig. 1). HPLC grade hexane (Fisher Scientific, NJ) and ethanol 99.8% (Merck, Darmstadt, Germany) were used.

Apparatus

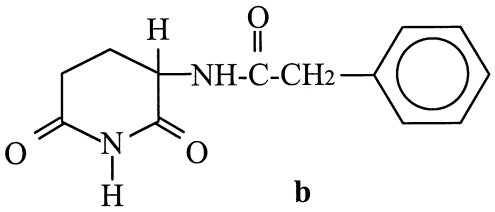
The HPLC system consisted of a Model 510 pump, Lambda-max 481 LC spectrophotometer, and Model 746 data module which were purchased from

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016



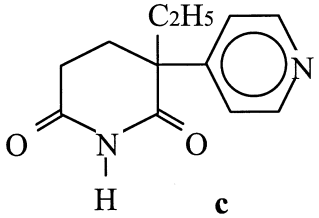
a

R	*R1*	*COMPOUND*
C_2H_5	H	Glutethimide
C_2H_5	NH_2	Aminoglutethimide
C_2H_5	$NHCOCH_3$	Acetylaminoglutethimide
C_2H_5	$CH_2CH_2N(C2H5)_2$	Phenylglutethimide
C_2H_5	SH	Thiophenylglutethimide
C_6H_{11}	NH_2	Cyclohexylaminoglutethimide



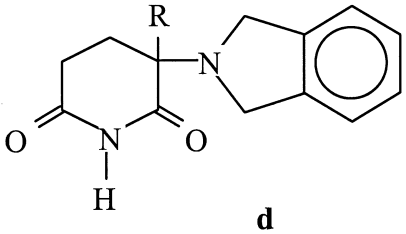
b

3-Phenylacetylaminopiperidine-2,6-dione



c

Pyridoglutethimide



d

R	*COMPOUND*
H	Thalidomide
CH_3	Methylthalidomide
C_2H_5	Ethylthalidomide
C_3H_7	n-Propylthalidomide

FIG. 1 Chemical structures of the piperidinedione compounds used in this study.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Waters Corp. (Milford, MA), and a manual injector Model 7125 was obtained from Rheodyne (Cotati, CA). The Chirose C-1 column (250 × 4.6 mm ID, 5 μm particle size) was from Chiralsep (La Frenaye, France). Shodex OR-1 optical rotation detector was purchased from JM Sciences (Buffalo, NY). The mobile phase was a mixture of hexane and ethanol (80:20, v/v) at a flow rate of 1.0 mL/min; the detection wavelength was 254 nm.

Chromatographic Parameters

Capacity factors (k') were calculated using the equation $k' = (t_R - t_0)/t_0$, where t_R is the elution time at peak maximum and t_0 is the elution time of unretained solute. The separation factor (α) was calculated using the equation $\alpha = k_2'/k_1'$, where k_2' and k_1' are the capacity factors for the second and first eluted peaks, respectively. The resolution factor (Rs) was calculated using the equation $Rs = 2[(t_{R2} - t_{R1})/(W_{b1} + W_{b2})]$, where t_{R2} and t_{R1} are the elution times of second and first peaks, respectively, and W_{b1} and W_{b2} are the peak width at the base of the first and second peaks, respectively. Kaiser's peak separation index (Rp) was calculated using the equation $Rp = a/b$, where a is the ratio of the mean valley height between the two peaks and b is the mean peak height. The value of Rp is considered to be zero when the two peaks overlap completely, and is equal to one if the peaks are resolved at the baseline (26).

RESULT AND DISCUSSION

The direct stereochemical separation of racemic piperidine-2,6-diones was achieved without derivatization on a newly developed Chirose C-1 CSP under normal phase mode conditions. The newly developed Chirose C-1 CSP was successful in the baseline resolution of eight compounds and a partial resolution of three compounds out of 12 racemic compounds used in this study (Table 1), using a mixture of hexane with ethanol (80:20, v/v) as an eluent.

Chirose C-1 CSP is a highly chiral polymer reticulated in a three-dimensional network. This polymer is mainly hydrophobic and bears organic functions available for hydrogen bonding with alcohols, thiols, carboxylic acids and derivatives, and amines and derivatives. The crosslinking phenomenon creates macrochiral cavities useful for the discrimination of the C-2 symmetry compounds (Dr. Raphaël Duval, personal communication, Chiralsep, La Frenaye, France). Therefore, the data obtained indicate that the alcohol acts as a modifier and competes with the analyte for the interactive sites on the CSP, mainly through hydrogen bonding. Furthermore, spatial arrangements of the Chirose C-1 CSP represent the chiral cavities. The alcohol and the analyte fit differently into these macrochiral cavities, causing the difference in the separation factor (α) and the resolution factor (Rs) observed. Furthermore, the re-

TABLE 1
Chromatographic Parameters,[a] Capacity Factor (k'), Separation Factor (α), Resolution Factor (Rs), and Kaiser's Peak Separation Index (Rp)

Compound	k'_1	k'_2	α	Rs (Rp)
Glutethimide	(−) 3.24	(+) 3.54	1.1	0.9 (0.66)
Aminoglutethimide	S (−) 13.29	R (+) 17.79	1.34	3.3
Cyclohexylaminoglutethimide	R (−) 10.29	S (+) 20.07	1.95	8.2
N-Acetylaminoglutethimide	(−) 6.56	(+) 8.65	1.32	2.6
3-Phenylacetylamino-2,6-piperidinedione	(−) 11.02	(+) 13.15	1.19	1.8
Pyridoglutethimide	(−) 8.11	(+) 9.09	1.12	1.2 (0.92)
Thiophenylglutethimide	(−) 3.22	(+) 3.97	1.23	2.1
Thalidomide	S (−) 20.0	R (+) 21.06	1.05	0.5 (0.39)
Methylthalidomide	R (−) 11.81	S (+) 13.99	1.18	2.1
Ethylthalidomide	R (−) 10.54	S (+) 12.58	1.19	2.0
n-Propylthalidomide	(−) 8.63	(+) 10.23	1.19	1.8
Phenylgluterimide.HCl		Not resolved		

[a] Chromatographic conditions are described in the Material and Method section.

sults (Table 1) show the effect of different substituents at the chiral carbon on the racemic compounds. It is of interest to mention that in the case of thalidomide and analogs (Fig. 1d), the capacity factor (k') decreases with the increase of the length of the alkyl chain in the following order: $H > CH_3 > C_2H_5 > C_3H_7$. In contrast, α and Rs increase with an increase of the alkyl chain: $H < CH_3 < C_2H_5 < C_3H_7$. In the piperidine-2,6-dione drugs shown in Fig. 1(a) it was found that the capacity factor (k') decreases according to the nature of the substituent on the phenyl ring in the following order: $NH_2 > CH_2CH_2N(C_2H_5)_2 > NHCOCH_3 > H > SH$. The separation factor α and the resolution factor Rs increase in the order $H < CH_2CH_2N(C_2H_5)_2) < SH < NH_2 < NHCOCH_3$. This reflects the effect of these substituents on the stabilities of the inclusion complexes between the solute and CSP. Accordingly, the difference in the stability of the inclusion complexes will affect, in part, chiral discrimination and, consequently, resolution.

Enantiomeric identification of the chromatographic peaks was achieved by injecting the individual enantiomers, when available, on the HPLC system. Otherwise, the optical rotation sign [(+) or (−)] for each chromatographic peak was determined with the optical rotation detector. It was observed, for all racemic compounds resolved, that the enantiomers with (−) sign eluted first, regardless of their configurations (*S* or *R*). It is of interest to report that when using a cellulose derivative CSP known as Chiralcel OJ-R under the reversed phase mode, a reversal of elution order for these drugs was observed, i.e., the (+)-enantiomers eluted first (19).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Glutethimide was the least retained of all the compounds tested under the chromatographic conditions used. However, when using hexane and ethanol at a 90:10 percentage, a baseline separation was achieved for glutethimide ($\alpha = 1.3$, $Rs = 1.72$). When this mobile phase was used for all the other drugs, the second enantiomer was retained in the column for more than 120 minutes (data not shown).

ACKNOWLEDGMENT

The authors thank the administration of the King Faisal Specialist Hospital and Research Center for their support to the Bioanalytical and Drug Development Research Program.

REFERENCES

1. N. Finch, R. Dziemian, J. Cohen, and B. G. Steinetz, "The Absolute Configuration of the Enantiomers of Glutethimide and Aminoglutethimide," *Experientia, 31*, 1002–1003 (1975).
2. J. S. Douglas and P. J. Nicholls, "The Partial Fate of Aminoglutethimide in Man," *J. Pharm. Pharmacol., 24*(Suppl.), 150P (1972).
3. A. B. Foster, L. J. Griggs, I. Howe, M. Jarman, C. S. Leung, D. Manson, and M. G. Rowlands, "Metabolism of Aminoglutethimide in Humans. Identification of Four New Urinary Metabolites," *Drug Metab. Dispos., 12*, 511–516 (1984).
4. A. B. Foster, M. Jarman, C. S. Leung, M. G. Rowlands, and G. N. Taylor, "Analogues of Aminoglutethimide: Selective Inhibition of Cholesterol Side-Chain Cleavage," *J. Med. Chem., 26*, 50–54 (1983).
5. R. W. Hartmann, C. Batzl, A. Mannschreck, and T. Pongratz, "Stereoselctive Aromatase Inhibition by the Enantiomers of 3-Cyclohexyl-3-(4-aminophenyl)-2,6-piperidinedione," in *Chirality and Biological Activity* (B. Holmstedt, H. Frank, and B. Testa, Eds.), Alan R. Liss, New York, NY, 1990, Chap. 9, pp. 185–190.
6. *Physicians Desk Reference*, 52nd ed., Medical Economics Co., Oradell, NJ, 1998.
7. *The Merck Index*, 12th ed. (S. Budarari, Ed.), Merck & Co., 1996.
8. A. L. Harris, T. J. Powles, I. E. Smith, R. C. Coombes, H. T. Ford, J. C. Jazet, C. L. Harmer, M. Morgan, H. White, C. A. Parsons, and J. A. McKinna, "Aminoglutethimide for the Treatment of Advanced Postmenopausal Breast Cancer," *Eur. J. Cancer Clin. Oncol., 19*, 11–17 (1983), and references cited therein.
9. R. J. Santen, T. J. Worgul, A. Lipton, H. Harvey, A. Boucher, E. Samojlik, and S. A. Wells, "Aminoglutethimide as Treatment of Postmenopausal Women with Breast Carcinoma," *Ann. Intern. Med., 96*, 94–101 (1982).
10. D. F. Child, C. W. Burke, D. M. Burley, L. H. Rees, and T. R. Fraser, "Drug Control of Cushing's Syndrome. Combined Aminoglutethimide and Metyrapone Therapy," *Acta Endocrinol., 82*, 330–341 (1976).
11. J. A. Copland, L. B. Hendry, C. K. Chu, J. C. Wood, R. W. Wreen, C. G. Pantazis, and V. B. Mahesh, "Inhibition of Estrogen Stimulated Mitogenesis by 3-Phenylacetylamino-2,6-piperidinedione and Its Para-hydroxy Analog," *J. Steroid Biochem. Mol. Biol., 46*, 451–462 (1993).
12. J. C. Wood, J. A. Copland, T. G. Muldoon, and L. B. Hendry, "3-Phenylacetylamino-2,6-piperidinedione inhibition of rat Nb2 lymphoma cell mitogenesis," *Proc. Soc. Exp. Biol. Med., 197*, 404–408 (1991).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

13. H. Y. Aboul-Enein and V. Serignese, "Enantiomeric Separation of Several Cyclic Imides on a Macrocyclic Antibiotic (Vancomycin) Chiral Stationary Phase under Normal and Reversed Phase Conditions," *Chirality*, *10*, 358–361 (1998).
14. H. Y. Aboul-Enein, V. Serignese, C. Minguillon, and L. Oliveros, "Enantioselective Separation of Several Piperidine-2,6-diones on a Covalently Bonded Cellulose 3,5-Dimethylphenyl Carbamate/10-Undecenoate Chiral Selector," *Biomed. Chromatogr.*, *11*, 303–306 (1997).
15. H. Y. Aboul-Enein and M. R. Islam, "Direct Chromatographic Resolution of Racemic Aminoglutethimide and Its Acetylated Metabolite Using Different Cellulose Based Chiral Stationary Phases," *Chromatographia*, *30*, 223–227 (1990).
16. H. Y. Aboul-Enein and M. R. Islam, "Direct HPLC Separation of Glutethimide Enantiomers Using a Cellulose Tricinnamate Chiral Stationary Phase," *Acta Pharm. Nord*, *2*, 415–420 (1990).
17. H. Y. Aboul-Enein and S. Bakr, "Direct Chromatographic Resolution of Racemic Cyclohexylaminoglutethimide and Its Acetylated Metabolite Using Cellulose Based Chiral Stationary Phases," *Chirality*, *3*, 204–207 (1991).
18. H. Y. Aboul-Enein and M. R. Islam, "Isocratic High-Performance Liquid Chromatographic Resolution of Glutethimide Enantiomers and Their 4-Hydroxyglutethimide Metabolites Using Cellulose Tribenzoate Chiral Stationary Phases," *J. Chromatogr. Sci.*, *28*, 307–310 (1990).
19. H. Y. Aboul-Enein and S. Bakr, "Enantiomeric Separation of Some Piperidine-2,6-dione Drugs Using Chiralcel OJ-R Column," *Chirality*, *9*, 10–12 (1997).
20. A. V. Overbeke, H. Y. Aboul-Enein, W. Baeyens, G. V. Weken, and C. Dewaele, "Enantiomeric Separation of Some Piperidine-2,6-dione Drugs on Tolylcellulose by Liquid Chromatography," *Anal. Chim. Acta*, *346*, 183–189 (1997).
21. M. E. Swartz, J. R. Mazzeo, E. R. Grover, P. R. Brown, and H. Y. Aboul-Enein, "Separation of Piperidine-2,6-dione Drug Enantiomers by Micellar Electrokinetic Capillary Chromatography Using Synthetic Chiral Surfactants," *J. Chromatogr. A*, *724*, 307–316 (1996).
22. H. Y. Aboul-Enein and M. R. Islam, "Direct Enantiomeric High-Performance Liquid Chromatographic Separation of Aminoglutethimide and Its Major Metabolite on a Series of Chiralcel OD and Chiralcel OJ Columns and Its Application to Biological Fluids," *Biomed. Chromatogr.*, *5*, 74–77 (1991).
23. J. C. Reepmeyer, "Separation of *R*- and *S*-Thalidomide by Reversed-Phase HPLC with β-Cyclodextrin in the Mobile Phase," *Chirality*, *8*, 11–17 (1996).
24. Y. Tang, J. C. Reepmeyer, L. K. Revelle, and J. A. Wilson, "Enantioseparation of 3-Phenylacetylamino-2,6-piperidinedione and Related Chiral Compounds," *J. Chromatogr. A*, *752*, 93–99 (1996).
25. C. Weinz, G. Blaschke, and H. M. Schiebel, "Investigation of the Stereoselective *in vitro* Biotransformation of Glutethimide by High-Performance Liquid Chromatography and Capillary Electrophoresis," *J. Chromatogr. B, Biomed. Appl.*, *690*, 233–242 (1997).
26. A. A. L. van Overbeke, W. R. G. Baeyens, A. Beyaert, H. Y. Aboul-Enein, and H. Oda, "Chiral Resolution of Several Phenothiazine Compounds and Trimipramine, a Dibenzazepine Drug on Chiracel OJ-R," *J. Liq. Chromatogr. Related Technol.*, *20*, 693–705 (1997).

Received by editor November 30, 1998
Revision received January 1999

Request Permission or Order Reprints Instantly!