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Chiral separations of piperidine-2,6-dione analogues on Chiralpak IA and Chiralpak IB columns by using HPLC

Imran Ali [a], Lahoucine Naim [b], Ashraf Ghanem [c], Hassan Y. Aboul-Enein [c,*]

[a] *National Institute of Hydrology, Roorkee 247667, India*
[b] *Biomedical Analysis Department Faculty of Pharmaceutical Sciences, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands*
[c] *Centre for Clinical Research, King Faisal Specialist Hospital and Research Center, P.O. Box 3354, Riyadh 11211, Saudi Arabia*

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Abstract

Recently, two new immobilized polysaccharides based CSPs, namely tris-(3,5-dimethylphenylcarbamate) derivatives of amylose and cellulose known as Chiralpak IA and Chiralpak IB were introduced, which may be used with a wide range of solvents including standard and prohibited ones. Several racemic piperidine-2,6-dione analogues [aminoglutethimide, *p*-nitro-glutethimide, *p*-nitro-5-aminoglutethimide, cyclohexylaminoglutethimide, phenglutarimide and thalidomide] have been resolved on Chiralpak IA and Chiralpak IB columns (25 cm × 0.46 cm). The non-conventional mobile phases used were methyl-*tert*-butyl ether-THF (90:10, v/v) [I], 100% dichloromethane [II] and 100% acetonitrile [III] separately at a flow rate of 1.0 mL/min using a UV detector at 254 nm. The resolution factors for Chiralpak IA and Chiralpak IB columns were 1.00–5.33 and 0.33–0.67, respectively. Chiralpak IA column gave better results than Chiralpak IB column for the reported molecules using the developed HPLC conditions. Experimental conditions and the possible chiral recognition mechanisms have been discussed.

Keywords: Immobilized polysaccharides CSPs; Chiralpak IA; Chiralpak IB; Chiral separation; Piperidine-2,6-dione analogues

1. Introduction

Chiral resolution of enantiomers by liquid chromatography is one of the emerging areas as one of the enantiomers may be inactive or toxic [1]. These properties of the enantiomers have created an interest to study the pharmacological and toxicological behaviors of the individual eanantiomers of drugs, pharmaceuticals and agrochemicals [2,3]. The United States Food and Drug Administration has issued guidelines to pharmaceutical and agrochemical industries to specify the enantiomeric purity of the optically active compounds prior to their marketing [4]. In view of these facts, the enantiomeric resolution of a variety of compounds is gaining importance continuously. Various CSPs have been developed to achieve chiral separation of different compounds. Among these, polysaccharide based CSPs are important due to their wide ranges of applications [5].

It is interesting to note that all available polysaccharide chiral stationary phases (commercial columns) are coated on silica support, which restricts the uses of some prohibited solvents such as tetrahydrofuran (THF), chlorofom, dichloromethane, ethylacetate, pyridine, acetone, and certain ethers as eluents [5,6]. Due to these facts, coated CSPs are not capable to resolve certain drugs and pharmaceuticals, especially in the reaction mixtures having prohibited solvents. Besides, NMR and other spectroscopic techniques are supposed to have the capacity for ascertaining the chiral recognition mechanisms, which use high polar solvents (THF, acetone, pyridine, etc.) for this purpose and these solvents cannot be used in the coated CSPs [5,7]. Therefore, the need of immobilization was felt and some attempts have been made to immobilize chiral polysaccharide phases on silica gel [8–15]. Recently, Chiralpak IA and Chiralpak IB columns having tris-(3,5-dimethylphenylcarbamate) derivatives of amylose and cellulose, respectively immobilized on silica gel, were launched that can be used with a wide range of solvents [16]. Chiralpak IA column has been used for the chiral resolution of calcium sensitizing drugs [17], cyclopropanes [18], β-blockers [19], anti-inflammatory drugs [20] and several other racemates [21]. However no report is available on the chiral separation

* Corresponding author.
E-mail addresses: drimran_ali@yahoo.com (I. Ali), hyaboulenein@yahoo.com (H.Y. Aboul-Enein).

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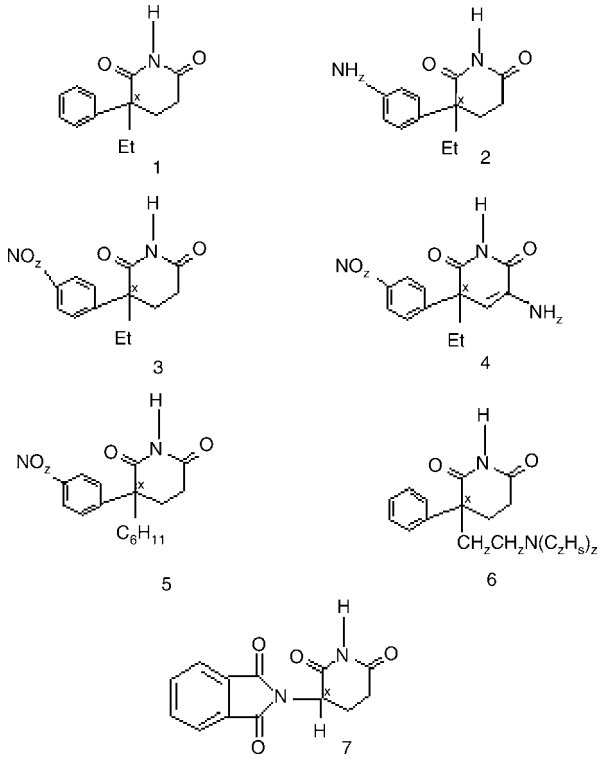

Fig. 1. Chemical structures of piperidine-2,6-dione analogues: (1) glutethimide; (2) aminoglutethimide; (3) *p*-nitro-glutethimide; (4) *p*-nitro-5-aminoglutethimide; (5) cyclohexylaminoglutethimide; (6) phenglutarimide; and (7) thalidomide; (*) denotes the position of chiral center.

capacity of Chiralpak IB except some preliminary report from the manufacturing company [22]. The aim of this work is to explore the suitability of immobilized amylose and cellulose tris-(3,5-dimethylphenylcarbamate) chiral stationary phases for the enantiomeric separation of piperidine-2,6-dione analogues (Fig. 1). Several papers are available on the chiral separations of these analogues using different coated polysaccharides based chiral columns [23–29]. This article describes the chiral recognition capabilities of Chiralpak IA Chiralpak IB columns for some piperidine-2,6-dione analogues using standard and prohibited solvents.

2. Experimental

2.1. Chemicals and reagents

The racemic mixtures of glutethimide and aminoglutethimide were obtained from Ciba-Geigy, Basle, Switzerland. Phenglutarimide was kindly supplied by Dr. P.J. Nicholls (University of Wales, School of Pharmacy, Cardiff, UK). Cyclohexylglutethimide was received from Dr. R.W. Hartmann (University of Saarland, Saarbrücken, Germany). Thalidomide was obtained from Dr. J.C. Reepmeyer (Food and Drug Administration, Division of Drug Analysis, St. Louis, MO, USA). The other piperidine-2,6-dione analogues, i.e. *p*-nitroglutethimide and *p*-nitro-5-aminoglutethimide were synthesized in our laboratory. The solutions of these derivatives (1.0 mg/mL) were prepared in methanol. Methanol, THF, methyl *tert*-butyl ether, dichloromethane and acetonitrile of HPLC grade were purchased from Fisher Scientific (Fairlawn, New Jersey, USA).

2.2. Chromatographic conditions

An aliquot of 10 μL of each of the solutions were injected on to a Waters Breeze HPLC system consisting of a binary solvent delivery pump (model 1525), an autosampler (model 717), a dual wavelength absorbance detector (model 2487), and a computer having breeze software. The order of elution of the enantiomers was confirmed by using confirmed an optical rotation detector (Chiralyzer), which was obtained from J.M. Science Inc., Grand Island, New York, USA. The columns used were Chiralpak IA and Chiralpak IB (25 cm × 0.46 cm ID) obtained from Chiral Technologies Inc., West Chester, PA, USA. The mobile phases used were methyl-*tert*-butyl ether-THF (90:10, v/v) [I], 100% dichloromethane [II] and 100% acetonitrile [III] separately. The mobile phases were filtered and degassed before use. The flow rates of the mobile phase were 1.0 mL/min. All the experiments were carried out at 23 ± 1 °C. The detection was achieved at 254 nm. The chromatographic parameters such as capacity factor (k), separation factor (α) and resolution factor (R_s) were calculated.

3. Results and discussion

Chromatographic parameters, capacity factor (k), separation factor (α) and resolution factor (R_s) for the resolved enantiomers of aminoglutethimide, *p*-nitroglutethimide, *p*-nitro-5-aminoglutethimide, cyclohexylglutethimide, phenglutarimide and thalidomide on Chiralpak IA and Chiralpak IB columns using methyl-*tert*-butyl ether-THF (90:10, v/v), 100% dichloromethane and 100% acetonitrile are given in Tables 1–3, respectively. The values of separation factor of the resolved enantiomers of these analogues were in the range of 1.11–3.52 for Chiralpak IA and 1.73–2.05 for Chiralpak IB columns, respectively. Resolution factors obtained were 1.00–5.33 for Chiralpak IA and 0.33–0.67 for Chiralpak IB, respectively. A typical chromatogram of the resolved enantiomers of aminoglutethimide on Chiralpak IA column is shown in Fig. 2. It has been observed that R-(+)-enantiomer eluted first, followed by S-(−)-enantiomer of all the studied piperidine-2,6-dione derivatives. A variation in the chromatographic parameters was carried out to obtain the best resolution. To optimize the chromatographic conditions, ethanol, methanol, and several other solvents were tested but no good resolution could be achieved. As a result of extensive experimentation the optimized chromatographic conditions were developed and reported herein.

Since both Chiralpak IA and Chiralpak IB columns are new, we, therefore, tried to compare the chiral separations of these columns for several piperidine 2,6-dione derivatives. It has been observed that Chiralpak IA is capable to resolve all compounds except glutethimide using mobile phase I (Table 1). Mobile phase II could not resolve glutethimide and phenglutaimide racemates on Chiralpak IA (Table 2) while mobile phase III was also not capable to separate the enantiomers of glutethimide

Table 1
Capacity (k), separation (α) and resolution (R_s) factors for enantiomeric resolution of piperidine-2,6-dione analogues on Chiralpak IA and Chiralpak IB columns using methyl *tert*-butyl ether–THF (90:10, v/v) as the mobile phase with 1.0 mL/min flow rate and UV detection at 254 nm

Sl. no.	Piperidine-2,6-dione analogues	k_1	k_2	α	R_s
1.	Glutethimide	nr [nr]	nr [nr]	nr [nr]	nr [nr]
2.	Aminoglutethimide	R-(+)-1.19 [nr]	S-(−)-4.19 [nr]	3.52 [nr]	4.19 [nr]
3.	*p*-Nitroglutethimide	R-(+)-1.17 [nr]	S-(−)-1.30 [nr]	1.11 [nr]	1.30 [nr]
4.	*p*-Nitro-5-amino-glutethimide	R-(+)-0.45 [nr]	S-(−)-0.75 [nr]	1.67 [nr]	1.00 [nr]
5.	Cyclohexylamino-glutethimide	R-(+)-2.41 [nr]	S-(−)-5.33 [nr]	2.21 [nr]	5.33 [nr]
6.	Phenglutarimide	R-(+)-0.84 [nr]	S-(−)-1.93 [nr]	2.29 [nr]	1.93 [nr]
7.	Thalidomide	nr [nr]	nr [nr]	nr [nr]	nr [nr]

$k1$ and $k2$: retention factors for (+)- and (−)-enantiomers; values in square brackets are for Chiralpak IB column; nr: not resolved.

Table 2
Capacity (k), separation (α) and resolution (R_s) factors for enantiomeric resolution of piperidine-2,6-dione analogues on Chiralpak IA and Chiralpak IB columns using dichloromethane as the mobile phase with 1.0 mL/min flow rate and UV detection at 254 nm

Sl. no.	Piperidine-2,6-dione analogues	k_1	k_2	α	R_s
1.	Glutethimide	nr [nr]	nr [nr]	nr [nr]	nr [nr]
2.	Aminoglutethimide	R-(+)-2.86 [nr]	S-(−)-6.64 [nr]	2.32 [nr]	2.27 [nr]
3.	*p*-Nitroglutethimide	R-(+)-1.37 [S-(−)1.53]	S-(−)-3.31 [R-(+)2.77]	2.42 [1.92]	1.44 [0.67]
4.	*p*-Nitro-5-amino-glutethimide	R-(+)-2.95 [nr]	S-(−)-4.36 [nr]	1.48 [nr]	2.07 [nr]
5.	Cyclohexylamino-glutethimide	R-(+)-3.25 [nr]	S-(−)-7.39 [nr]	2.27 [nr]	4.14 [nr]
6.	Phenglutarimide	nr [nr]	nr [nr]	nr [nr]	nr [nr]
7.	Thalidomide	R-(+)-1.18 [nr]	S-(−)-2.13 [nr]	1.81 [nr]	2.00 [nr]

$k1$ and $k2$: retention factors for (+)- and (−)-enantiomers; values in square brackets are for Chiralpak IB column; nr: not resolved.

and thalidomide (Table 3). On the overall, it may be concluded that Chiralpak IA column is capable of resolving most of the reported racemates by using the three mobile phases reported. In brief, the suitability of the reported mobile phases with Chiralpak IA column was in the order of I > II > III, respectively. Contrarily, Chiralpak IB column could not separate any of the studied racemates by using mobile phase I (Table 1) while *p*-nitroglutethimide resolved only partially with mobile phase II (Table 2). Glutethimide and aminoglutethimide racemates could be resolved partially using mobile phase III (Table 3). Therefore, chiral recognition capacity of Chiralpak IA is quite high in comparison to Chiralpak IB towards these piperidine 2,6-dione derivatives.

Better resolution capacity of amylose may be due to the helical nature of amylose CSPs possessing well defined grooves, making it different from the corresponding cellulose analogues, which appeared to be more linear and rigid in nature with shallow grooves [30]. Therefore, amylose provides better chiral grooves than these racemates. It is very interesting to mention here that the order of elution was reversed on Chiralpak IB column in comparison to Chiralpak IA column under the identical chromatographic conditions, which indicates the effect of the different configurations of two CSPs. Sometimes, the phenomenon of reversal order of elution occurs by just changing mobile phase on the same CSP [31]. Contrarily, reverse order of elution was obtained using the same mobile phase but by changing the CSPs [32]. Briefly, the reverse order of elution is a result of the change in supramolecular configuration of the CSP. Attempts have been made to explain the chiral recognition mechanisms on the reported CSPs but, unfortunately, the exact mechanisms of the chiral resolution on polysaccharides CSPs are not known. However, it is known that the interactive forces such as hydrogen bondings, π–π interactions, van der Waal forces and steric effects are responsible for the chiral resolution using these CSPs. However, native cellulose and amylose are not efficient chiral selectors because of the insufficient optical resolving power. On the other hand, polysaccharides are easily converted to a variety of derivatives such as tris-esters and

Table 3
Capacity (k), separation (α) and resolution (R_s) factors for enantiomeric resolution of piperidine-2,6-dione analogues on Chiralpak IA and Chiralpak IB columns using acetonitrile as the mobile phase with 1.0 mL/min flow rate and UV detection at 254 nm

Sl. no.	Piperidine-2,6-dione analogues	k_1	k_2	α	R_s
1.	Glutethimide	R-(+)-0.86 [S-(−)-0.14]	S-(−)-3.71 [R-(+)-0.29]	4.32 [2.05]	4.33 [0.33]
2.	Aminoglutethimide	R-(+)-2.80 [S-(−)-0.28)]	S-(−)-6.20 [R-(+)-0.49]	2.21 [01.73]	1.88 [0.33]
3.	*p*-Nitroglutethimide	R-(+)-1.20 [nr]	S-(−)-2.40 [nr]	2.00 [nr]	1.71 [nr]
4.	*p*-Nitro-5-amino-glutethimide	R-(+)-2.50 [nr]	S-(−)-3.00 [nr]	1.20 [nr]	1.00 [nr]
5.	Cyclohexylamino-glutethimide	R-(+)-3.75 [nr]	S-(−)-8.66 [nr]	2.31 [nr]	1.54 [nr]
6.	Phenglutarimide	nr [nr]	nr [nr]	nr [nr]	nr [nr]
7.	Thalidomide	R-(+)-0.50 [nr]	S-(−)-1.00 [nr]	2.00 [nr]	1.00 [nr]

$k1$ and $k2$: retention factors for (+)- and (−)-enantiomers; values in square brackets are for Chiralpak IB column; nr: not resolved.

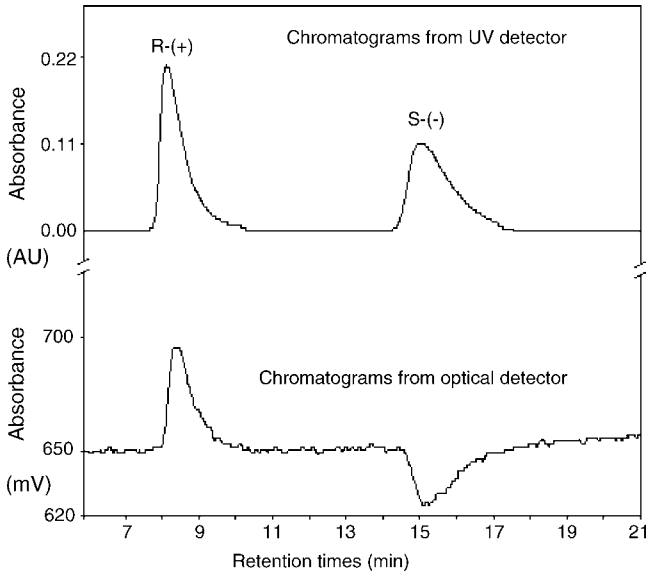

Fig. 2. A chromatogram of the resolved enantiomers of aminoglutethimide on Chiralpak IA using hundred percent acetonitrile as the mobile phase.

tris-carbamates by the reactions of active hydroxyl groups with appropriate reagents.

Fig. 3 represents the interactions between *p*-nitroglutethimide and the amylose tris-(3,5-dimethylphenylcarbamate) CSP, which indicates the various interactions between the CSP and the analyte. Different possible bondings and interactions are hydrogen bondings (between –C=O and amine groups), π–π interactions (between phenyl groups of CSP and glutethimide derivative) and van der Waal's forces (among alkyl groups of CSP and glutethimide derivative). It is worthy to mention here that glutethimide could be resolved only in mobile phases III, which may be due to sufficient bondings. Contrarily, glutethimide could not be resolved using mobile phase I and II due insufficient bonding available when using mobile phase I and II. *p*-Nitroglutethimide got separated successfully in spite of having poor π–π interactions (due to electron withdrawing

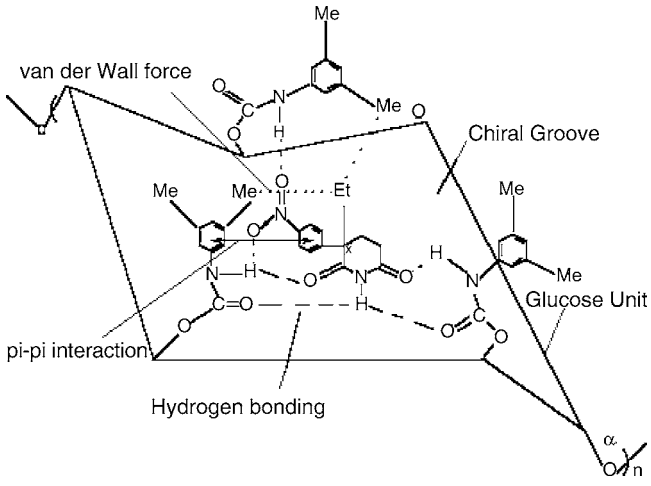

Fig. 3. Schematic diagram showing various interactions between piperidine-2,6-dione analogue (*p*-nitroglutethimide) and amylose tris-(3,5-dimethylphenylcarbamate); (*) denotes the position of chiral center.

nature of nitro group) in comparison to glutethimide. This fact may be explained on the basis of the overall bonding capacities of molecule. Of course *p*-nitro derivative of glutethimide has poor π–π interactions capacity but also provides, due to the presence of nitro group, the extra hydrogen bondings in comparison to glutethimide. Therefore, the overall bonding capacity of *p*-nitro derivative is greater than glutethimide itself and, hence, *p*-nitro derivative got resolved while glutethimide did not (in mobile phase I and II). Similarly, the chiral separations of other glutethimide derivatives can be explained on the basis of these bondings. In brief, it may be concluded from this work that the chiral resolution on these CSPs is due to the overall combination of all types of bondings. No single bonding is capable for the enantiomeric resolution of the reported molecules. We compared our results with the earlier reported papers of chiral separations of piperidine-2,6-dione on coated polysaccharide CSPs [23–29] and it was found that there is no specific trend of the chiral resolution on these two types of CSPs. In some cases coated columns have good resolution capacity while in another cases, immobilized phases seems to be better one. Therefore, the separation efficiencies of coated and immobilized CSPs are considered complimentary to each other. However, immobilized CSP has an advantage of using a wide range of solvents.

4. Conclusion

In this study, the chiral resolution abilities of Chiralpak IA and Chiralpak IB for some racemic piperidine-2,6-dione analogues are presented. Successful resolution of most of the analogues studied has been achieved on Chiralpak IA column using three mobile phases. The resolution efficiency of Chiralpak IB column was poor and hence, it is not useful for the enantiomeric resolution of these molecules using the reported chromatographic conditions. Chiralpak IA column may be useful for the enantiomeric resolution of racemic piperidine-2,6-dione analogues on a semi-preparative scale.

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