Journal of Chromatography A, 1355 (2014) 291–295

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

High-performance liquid chromatographic enantioseparation of 3,5-disubstituted hydantoins analogs and temperature-induced reversals of elution orders on a polysaccharide-based chiral stationary phase

Xinying Yang[a], Li Su[a], Xuben Hou[a], Shengyong Ding[a], Wenfang Xu[a], Binghe Wang[a,b], Hao Fang[a,*]

[a] School of Pharmacy, Shandong University, Ji'nan 250012, China
[b] Department of Chemistry and Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, GA 30303, USA

ARTICLE INFO

Article history:
Received 13 February 2014
Received in revised form 4 June 2014
Accepted 6 June 2014
Available online 12 June 2014

Keywords:
Enantiomer separation
3,5-Disubstituted hydantoins
Chrialpak IA column
Reversal of elution order

ABSTRACT

Enantioseparations were achieved for eleven 3,5-disubstituted hydantoins in HPLC under the normal phase mode using Chiralpak IA. The effects of polar alcoholic modifier and column temperature on retention and enantioseparation were determined. Importantly, we found two kinds of enantiomer elution order (EEO) reversals, which include solvent-induced EEO reversal for compound **9** and temperature-induced EEO reversals for compound **3** and compound **6**. The phenomena of these EEO reversals were described for the first time in present work, which is helpful to elucidate the chiral separation mechanism of these hydantoins.

1. Introduction

Imidazolidine-2,4-dione, also known as hydantoin, is an organic heterocyclic compound and widely used in medicinal chemistry and organic chemistry. Many drugs or drug candidates, such as phenytoin, nilutamide [1], and azimilide [2,3], contain the hydantoin moiety. In 2003, Zhai et al. prepared chiral 3,5-disubstituted hydantoins to catalyze asymmetric hydrocyanation of 3-phenoxybenzaldehyde on polymeric resin [4]. More recently, 3,5-disubstituted hydantoins were reported to be chiral auxiliaries for asymmetric aldol reaction [5] and Mannich reactions [6]. It is well known that the optical purity of a chiral auxiliary has a significant effect on the quality of the product obtained by asymmetric synthesis. Therefore, there is a need to develop a facile method to determine enantiomeric purities of chiral 3,5-disubstituted hydantoins.

Currently, chiral high-performance liquid chromatography (HPLC) is an effective tool for determining optical purity and has been used in hydantoin enantiomeric separations. For example, Tic-hydantoin derivatives [7], and 3-alkyl-5-mono and disubstituted imidazolidinedione derivatives could be resolved using coated polysaccharide chiral stationary phases (CSPs) such as Chiracel OD-H, Chiracel OJ and Chiralpak AD [8,9,10].

In recent decades, there is an increased level of interest in the phenomena of enantiomer elution order (EEO) reversal in chiral chromatography because of their implications in chiral separation mechanisms. Heretofore at least three types of enantiomer elution order (EEO) reversals have been reported, including solvent-induced, temperature-induced [11–13] and sample load-induced reversal [14]. For example, Grinberg's group reported the EEO reversal of *N*-substituted alpha-methyl phenylalanine esters on the ADMPC CSP when the alcohol concentration was increased [15]. Chankvetadze' group found several kinds of EEO reversals observed depending on the chemistry of the chiral selector, temperature, major component, as well as the minor additive in the mobile phase [16]. However, EEO reversal has not been observed in most of the chiral hydantoin derivatives.

In this paper, eleven 3,5-disubstituted hydantoin chiral auxiliaries (**1**–**11**, Fig. 1) were successfully resolved using Chiralpak IA column, and solvent-induced and temperature-induced EEO reversals were also observed.

* Corresponding author. Tel.: +86 531 88382731; fax: +86 531 88382548.
E-mail address: haofangcn@sdu.edu.cn (H. Fang).

http://dx.doi.org/10.1016/j.chroma.2014.06.022
0021-9673/

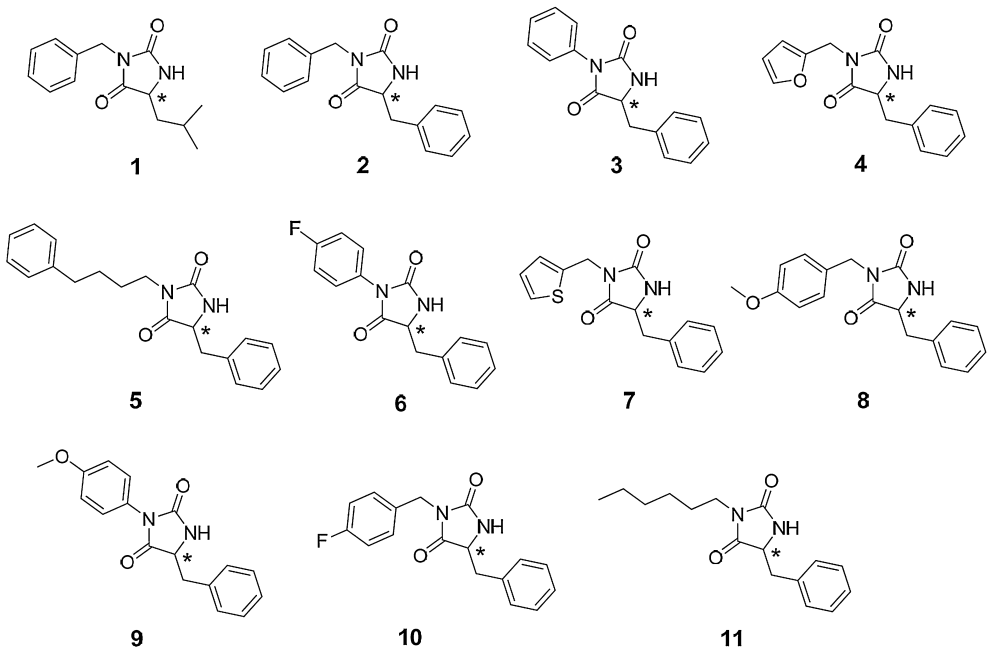

Fig. 1. Structures of the hydantoin compounds.

2. Experimental

2.1. Chemicals

Each enantiomer of hydantoin derivatives **1**–**11** was synthesized according to literature methods [17]. ^{1}H- and ^{13}C-NMR were conducted on a Bruker Avance spectrometer at 300 and 75 MHz, respectively. Mass spectrometric analyses were performed on an API 4000 spectrometer. *n*-Hexane, ethanol (EtOH), 2-propanol (IPA), and 1-butanol (BuOH) were purchased from Dikma Technologies Co. Ltd. (Tianjin, China). 1-Propanol (PrOH) and *tert*-butanol (*t*-BuOH) were obtained from Kermel Chemical Reagent Co. Ltd. (Tianjin, China). All solvents were of HPLC grade and were filtered and degassed in an ultrasonic bath before use.

Sample solutions of the analytes (0.5 mg mL^{-1}) were prepared by dissolution in the corresponding mobile phase, and filtered through a 0.45 μm filter.

2.2. Chromatography

HPLC measurements were carried out on a Shimadzu HPLC system (Shimadzu, Kyoto, Japan), consisting of two LC-10ATvp pumps, an SPD-M10Avp Photodiode array detector, a manual injector with a 20 μL sample loop and an SCL-10 Avp system controller. Chromatographic data were acquired and processed by a LC solution (Ver.1.2) workstation. The column was Chiralpak IA, 250 mm × 4.6 mm I.D. with 5 μm particle size from Daicel (Tokyo, Japan). The mobile phases were composed of *n*-hexane/alcoholic modifier. The flow rate was set at 0.5 mL min^{-1}, and the UV detection was carried out at 220 nm. The injection volume was 20 μl. The hold-up time (t_0) was determined according to the first perturbation of the base line Retention factors of the first and the second eluted enantiomer are k_1' and k_2' respectively, which could be calculated from $(t_R - t_0)/t_0$ (t_R is the retention time and t_0 is the hold-up time). Separation factor α was obtained from k_2'/k_1'.

3. Results and discussions

The enantioseparation of 11 racemic 3,5-disubstituted hydantoins was performed on Chiralpak IA column using normal phase. Baseline resolution of all compounds was obtained in *n*-hexane/EtOH (Supporting Information, Fig. S1). The elution orders in all cases were determined and *S*-enantiomer would elute firstly in most cases (Table 1). In our further studies, different polar organic modifiers were investigated and EEO reversals induced by both solvent and temperature were observed.

3.1. The effects of polar modifier and solvent-induced EEO reversals

Herein, five polar modifiers, such as EtOH, PrOH, IPA, BuOH and *t*-BuOH, were used and baseline separation was obtained for most of the chiral hydantoin derivatives with 20% alcohol (Table 1). However, compound **1**, **3** and **6** could not be enantioseparated using *n*-hexane/IPA [80/20(v/v)].

The nature and content of the polar alcoholic modifier could influence the retention factors for the first-eluting enantiomers (k_1') or separation factors (α). For example, branched alcohols, such as IPA and *t*-BuOH, give lower k_1' than linear alcohols. On the other hand, most of compounds exhibit higher retention factors and separation factors when the content of alcoholic modifier decreased. For example, when the ratio of EtOH decreased from 30% to 10% and that of IPA from 20% to 5%, all the hydantoin derivatives exhibit increased retention factors and separation factors. Further studies showed that the solvent-induced enantiomer elution order (EEO) reversal could occur in the resolution of analyte **9** with IPA. As shown in Fig. 2, the *S*-enantiomer of analyte **9** elutes first when using EtOH, PrOH, BuOH and *t*-BuOH, while the ***R*-9** eluted first using IPA as polar modifier.

According to the data in Table 1, structural variations can significantly affect retention factors. For example, the k_1' (retention factors of first-eluting enantiomers) of analytes **3**, **6** and **9** are always higher than that of other analytes. The possible reason may be due to the presence of the phenyl ring in the N3 position of these hydantoins, which could provide additional π–π interactions between the CSP and analytes. On the other hand, analyte **11** always exhibits the lowest k_1' in different alcoholic modifier, which indicates that the interaction between compound **11** and the CSP is very weak, possibly due to the long alkyl substitution at N3 position without phenyl or benzyl ring.