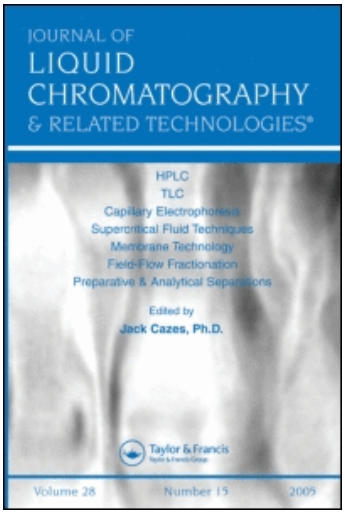

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Price, Kathi , Clausen, Andrew M. and Helmy, Roy(2008) 'Effect of Injection Diluent on a Chiral Separation on an Amylose S-α-Methylbenzylcarbamate Chiral Stationary Phase (Short Communication)', Journal of Liquid Chromatography & Related Technologies, 31: 15, 2286 — 2295
To link to this Article: DOI: 10.1080/10826070802281612
URL: http://dx.doi.org/10.1080/10826070802281612

Journal of Liquid Chromatography & Related Technologies®, 31: 2286–2295, 2008

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802281612

Effect of Injection Diluent on a Chiral Separation on an Amylose S-α-Methylbenzylcarbamate Chiral Stationary Phase (Short Communication)

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Abstract: A method to separate the four stereoisomers of a chiral thiazolidine-2, 4-dione containing two stereocenters was developed. This thiazolidine-2,4-dione is susceptible to epimerization through an enol-type intermediate, predominantly in protic solvents. While acetonitrile would provide both necessary sample solubility and sample stability for chromatographic analysis, significant peak fronting was observed when it was used as the diluent with a concomitant loss in resolution. Similar fronting was not observed when preparing sample solutions in ethanol, 2-propanol, methanol, or a 1:1 mixture of methanol and ethanol. The source of this fronting was explored by performing two sample loading studies: constant sample loading with varying volume and constant volume with varying loading. Peak asymmetry was used as a quantitative measure of the resulting peak fronting. These analyses indicate that the fronting observed when using acetonitrile as a diluent could arise due to a strong-solvent like effect of this solvent and or the solubility of the solute in the microenvironment with this combination of column packing and eluent.

Keywords: Amylose, Chiral stationary phase, Diluent, Peak asymmetry, Resolution and enantiomeric separation

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INTRODUCTION

The need for more enabling information through chromatographic analyses has continued to increase during synthetic process development of compounds being studied as drug candidates. Many of these analyses include assessment of chiral purity of synthetic intermediates and the compound itself to guide decisions on synthetic routes and process conditions.

As compounds being studied have increased in complexity, the complexity of chromatographic method development has also increased. Complicating chiral specific method development can be the presence of multiple stereocenters necessitating quantitation and separation of all isomers whose numbers increase as 2^n (where n is the number of chiral centers). Layered upon this is any intrinsic instability that the intermediate or the compound might have upon sample preparation and storage or during chromatographic analysis.

These two complications were encountered recently during method development of a compound containing a thiazolidine-2,4-dione moiety shown in Figure 1. A method to simultaneously determine enantio- and diastereomeric excess was desired. Additionally, the carbon alpha to the sulfur and carbonyl is susceptible to enolization rendering epimerization possible. Protic solvents would promote enolization and thus epimerization at this stereocenter. While achieving baseline separation in normal phase on a polysaccharide chiral stationary phase of the four isomers was relatively facile, significant band distortion was observed during method optimization.

A striking effect on peak shape was observed upon investigating the use of different solvents for sample dilution to increase both the ability to load sample and alleviate any epimerization due to sample preparation.

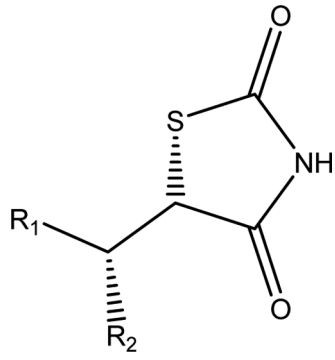

Figure 1. Structure of thiazolidine-2,4-dione.

As with standard practice, samples were initially dissolved in the mobile phase.[1,2] Changing the diluent to 100% acetonitrile caused substantial fronting. Such effects on peak shape by sample diluent have been reported before, though these reports involved either reversed-phase[3–13] or size exclusion chromatography.[14] In some cases this behavior is termed a "strong-solvent" effect whereby it is thought that the sample band is distorted at the head of a column by a plug of solvent with significantly greater eluotropic strength than the mobile phase.[2–6,9–11] Other distortions of peak shape with mobile phase-diluent mismatch have been attributed to viscous fingering.[12–16] This phenomenon results from the hydrodynamic instabilities that occur when the viscosities of the mobile phase and diluent differ. Our investigation into the fronting observed due to the use of acetonitrile as a diluent for this compound has led us to conclude that the band distortion we observed is due to a strong-solvent type effect and or the solubility of the solute (in this case the thiazolidine-2,4-dione moiety) in the microenvironment of the eluent.

EXPERIMENTAL

Equipment

Chromatographic analyses were performed on an Agilent 1100 HPLC system equipped with a photodiode array detector (Wilmington, DE, USA). A Chiralpak AS-H [amylose S-alpha-methylbenzyl carbamate) coated on silica gel] column (25 cm × 4.6 mm) was purchased from Chiral Technologies (West Chester, PA, USA). Chromatographic data was acquired and processed by a PE Nelson data system equipped with Turbochrom software (version 6.1.2.0.1:D19) (PE Nelson, San Jose, CA, USA).

Materials

HPLC grade hexane, acetonitrile, methanol, and 2-propanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The 200-proof, dehydrated ethanol was purchased from Quantum Chemical Co. (Newark, NJ, USA). A proprietary compound containing a thiazolidine-2,4-dione moiety and its enantiomer-diastereomeric mixture was provided by Process Research, Merck Research Laboratories (Rahway, NJ, USA).

Chromatographic Conditions

An eluent consisting of 85:5:10 v/v/v of HPLC grade hexane to ethanol to methanol was used for all chromatographic analyses. The mobile phase flow rate was 0.75 ml/min, and the column was held at 22°C. Ultraviolet absorbance detection was performed at 280 nm.

For each chromatographic experiment, the solution of an enantiomeric pair was injected at least twice and the reproducibility of the results was confirmed. To allow for identification of which peaks corresponded to the elution of enantiomer, solutions were prepared such that one enantiomer was more enriched than the other. All results were confirmed on multiple Agilent 1100 HPLC systems and Chiralpak AS-H columns.

RESULTS AND DISCUSSION

Upon method development to separate the enantiomer-diastereomeric mixture of the compound containing a thiazolidine-2,4-dione moiety, an interesting result was obtained when the diluent for the sample matrix was changed from acetonitrile to 2-propanol or methanol or ethanol or 50:50 v/v methanol:ethanol; the resolution and asymmetry factor were affected. It is important to note that the structure of the thiazolidine-2, 4-dione moiety possesses a stereocenter which will undergo epimerization via enol-tautomerization that is enhanced in protic solvent systems. Consequently, the sample was dissolved in acetonitrile. As can be observed in Figure 2, the resolution and peak symmetry factors for the stereoisomers appear to be affected by the sample diluent. Figure 2 illustrates the effect various diluents on the chromatography of the mixture of isomers. Acceptable chromatography is obtained with most diluents (alcohol/hexane mixtures); however, poor resolution and peak asymmetry are observed with acetonitrile as the diluent.

We reasoned that this poor resolution and peak asymmetry in acetonitrile could be due to the differences in solubility of the thiazolidine-2,4-dione in the various diluents. Table 1 shows the measured equilibrium solubility by HPLC of the compound in the various solvents. As can be observed in Table 1, the solubility of the analyte in these solvents ranges from 8 to >100 mg/mL. These results indicate that under the conditions of the chromatographic analysis the compound is soluble in both the mobile phase eluent and diluent.

Noting that acetonitrile is not recommended for use as a mobile phase with the Chiralpak AS-H column, we wanted to ensure that there is not an irreversible effect on the column when using acetonitrile as the injection solvent. Figure 3 illustrates the effect of using acetonitrile

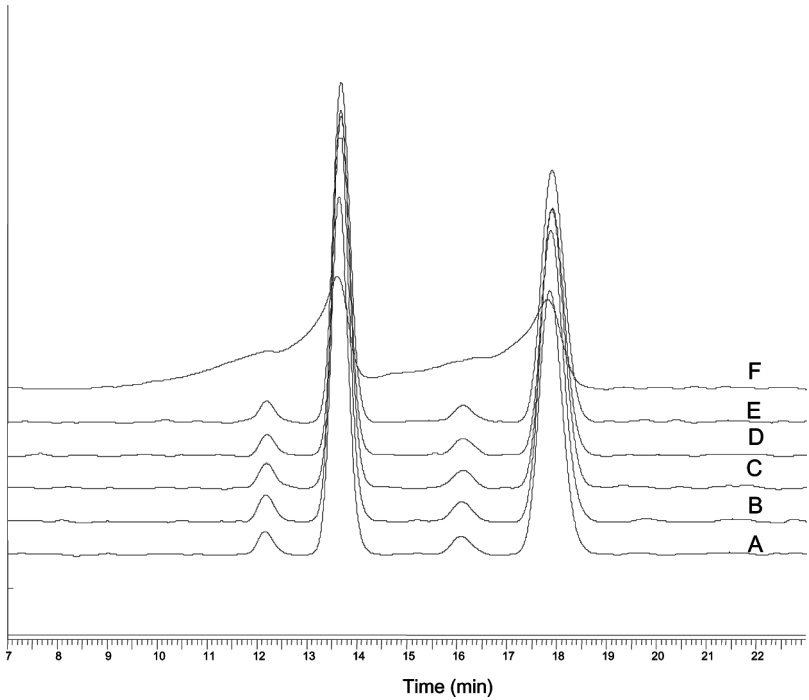

Figure 2. Effect of the diluent on thiazolidine-2,4-dione's peak asymmetry; Column, 25 × 0.46 cm I.D. Chiralpak AS-H, flow rate 0.75 mL/min, temperature 35°C, UV detection at 280 nm (A) methanol, (B) ethanol, (C) isopropanol, (D) 1:1 methanol:ethanol, (E) hexanes/methanol:ethanol at 85/5:10, (F) acetonitrile.

as the sample diluent and varying the injection volume on the chromatography of the enantio-diastereo mixture. This effect was then studied using an enantiomerically pure sample of the compound, varying the injection volume and measuring the peak asymmetry. Figure 4 shows that as the injection of the thiazolidine-2,4-dione in acetonitrile is at kept at low volumes ($0.8 - 1.0\,\mu L$), the peak is symmetrical. On the other hand, as volume of acetonitrile injected onto the column is increased to $10.0\,\mu L$ the peak asymmetry factor decreases to 0.59, indicating fronting. More importantly, the observed peak asymmetry displays no hysterisis (Figure 4 shows only one cycle for simplicity). The change from fronting to symmetric peaks as the injection volume is decreased can be reproduced exactly with a relative standard deviation of less than 0.1%. These results indicate that there is little or no memory effect of acetonitrile and thiazolidine-2,4-dione on the chiral stationary phase of the Chiralpak AS-H. Rather, the effect seems to be emerging as

Table 1. Solubility of the thiazolidine-2, 4-dione moiety containing compound in various solvents

Diluent	Concentration (mg/mL)
Methanol	20
Ethanol	28
2-propanol	15
50:50[a] Methanol:Ethanol	10
85:5:10[a] Hexanes:Ethanol:Methanol	8
Acetonitrile	150

[a]Volume ratio.

a result of the interaction between injection solvent and eluent. The chromatograms in Figure 3 show not only a change in peak symmetry but also in resolution; however, if an injection of less than 1 μL of a 1 mg/mL solution of thiazolidine-2,4-dione in acetonitrile is made onto the Chiralpak AS-H column the symmetry and resolution are ideal.

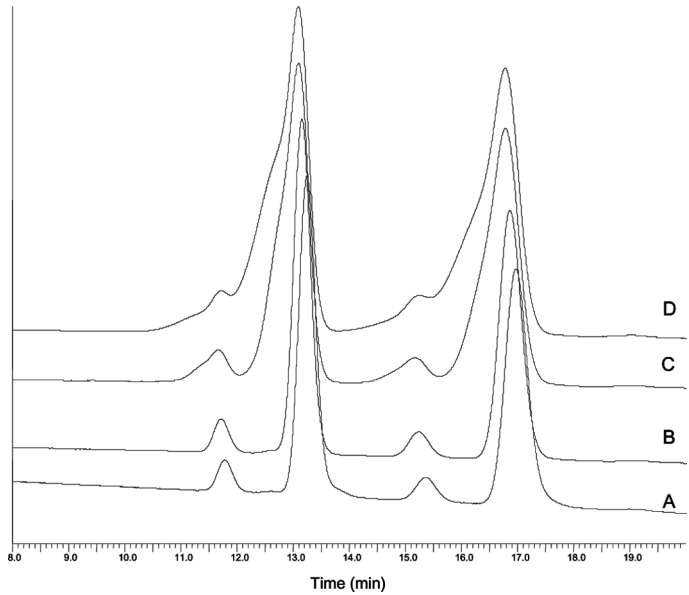

Figure 3. Effect of injection volume of acetonitrile on peak asymmetry of thiazolidine-2,4-dione; same chromatographic conditions as Figure 2 (A) 0.5 μl, (B) 1 μl, (C) 2 μl, (D) 4 μl.

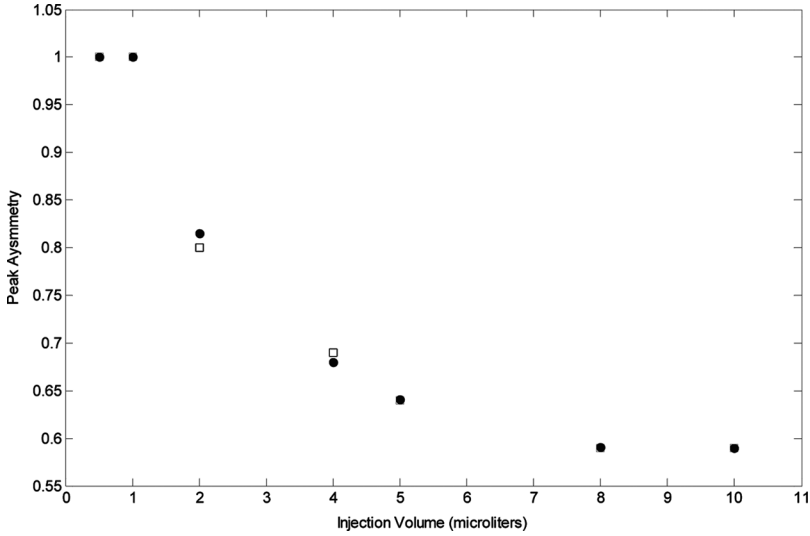

Figure 4. Effect of varying the injection volume of acetonitrile on the peak asymmetry of thiazolidine-2,4-dione; ∉ forward Γ and back analyses.

Nevertheless, when an injection volume of greater than 1 μL is made, the quality of the separation begins to suffer. Such loss in performance as a result of mismatch between injection solvent and chromatographic eluent are observed; however, similar observations can also result from column overloading.

In order to further investigate the cause of the observed phenomenon two sample loading experiments were conducted: constant sample mass loading with varying volume and constant volume injection with varying mass loading of thiazolidine-2,4-dione. Figure 5 summarizes the results of these studies. Figure 5(a) demonstrates the effect of the thiazolidine-2,4-dione peak symmetry at a constant load of 30 μg per injection while changing the volume of acetonitrile that is injected. The observed peak symmetry degrades from a symmetrical peak (asymmetry factor of 1) at an injection volume of 1 μL to a fronting peak (asymmetry factor of less than 1) as injection volume is increased. Figure 5(b) shows the asymmetry as a function of sample concentration at constant injection volume. The thiazolidine-2,4-dione peak asymmetry remains constant and near unity throughout the varying concentrations at a constant injection volume of acetonitrile. These results indicate that the observed peak fronting of the thiazolidine-2,4-dione containing compound is not dependent on the concentration of the sample but more importantly on the injection volume of acetonitrile. If the

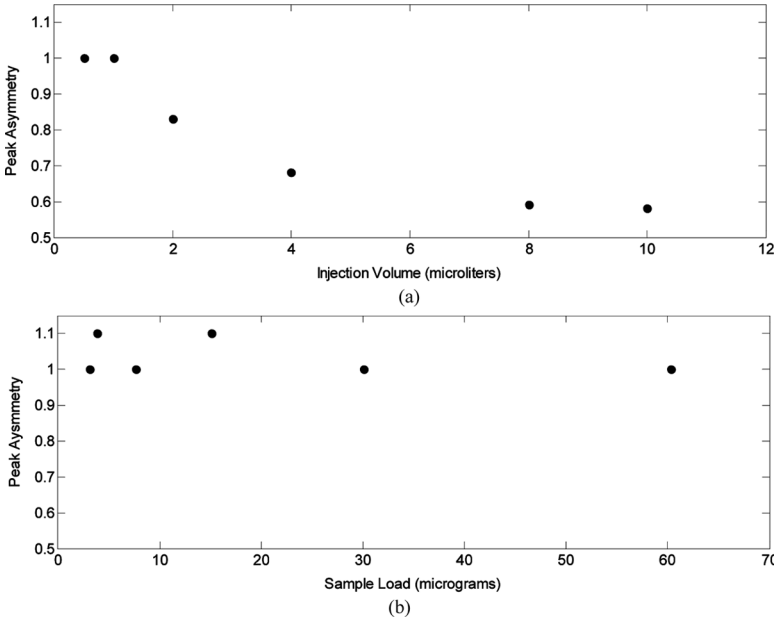

Figure 5. Effect of sample loading on peak asymmetry of thiazolidine-2,4-dione (a) constant mass load, (b) constant injection volume.

injection volume of acetonitrile is less than or equal to 1 μL, then there is no observed fronting and optimal resolution of the stereoisomers is achieved, while the converse is true when the injection volume of acetonitrile is greater than 1 μL.

CONCLUSIONS

The development of a chiral HPLC analyses usually entails varying mobile phase modifiers, temperature, and type of chiral stationary phase to optimize the resolution of a mixture of stereoisomers. Investigation of sample diluents is less frequently undertaken. As can be seen in the separation of this set of thiazolidine-2,4-dione stereoisomers, the role of injection solvent can have a profound influence on chromatographic performance. Consequently, a careful analysis of injection solvent may be warranted whenever a mismatch between eluent and injection solvent is required.

ACKNOWLEDGMENTS

The authors wish to acknowledge Drs. Chris Welch and Nelu Gringberg for many enlightening discussions and recommendations and Dr. Don Gauthier for providing the thiazolidine-2,4-dione isomers.

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