

Effect of Trace Amounts of Water in the Mobile Phase of Normal-Phase Enantioselective High-Performance Liquid Chromatography on Selectivity and Resolution of Optical Isomers

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Abstract

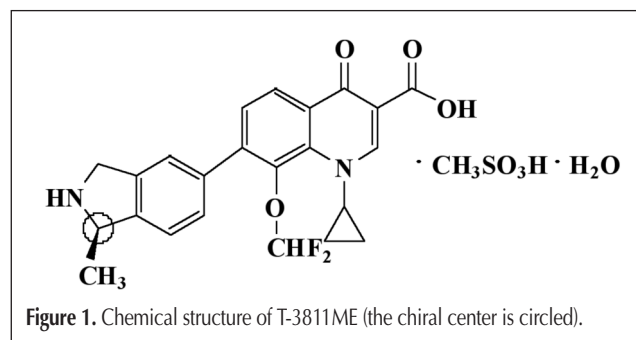
The irreproducibility of normal-phase enantioselective high-performance liquid chromatography (HPLC) could be attributed to the presence or absence of trace amounts of water in one or more components of the mobile phase. The effect of trace amounts of water on chromatographic characteristics in normal-phase enantioselective HPLC was investigated by deliberate addition of controlled, trace amounts of water into the mobile phase for the separation of T-3811ME and its undesired enantiomer. Commercial 2-propanol was pre-mixed with 2% (v/v) water and then used for preparation of the mobile phase in combination with such organic modifiers as ethanol and methanol at different ratios. The results showed up to 30% improvement in the resolution (R_s), 4% in selectivity (α), and 39% in efficiency (plate number N) compared to using a mobile phase prepared from neat commercial 2-propanol. Thus, the effect of trace amounts of water in the mobile phase of normal-phase enantioselective HPLC was demonstrated.

Introduction

In the pharmaceutical industry, enantioselective high-performance liquid chromatography (HPLC) is now essential for the research and development of chiral drugs (1–3). Derivatives of naturally occurring polysaccharides, especially cellulose and amylose, exhibit excellent properties as stationary phases for HPLC. Commercially available columns of these materials are now used extensively for both analytical and preparative separations of a wide range of enantiomers (4). Separation of enantiomers on chiral stationary phases based on cellulose/amylose derivatives is usually conducted under normal-phase

chromatography conditions (4). Mixtures of hexanes and 2-propanol are commonly used as the mobile phase for normal-phase enantioselective HPLC (3–9).

It is well known by chiral chromatographers that certain enantioselective HPLC methods are extremely difficult to reproduce on a day-to-day, analyst-to-analyst, and lab-to-lab basis, an example of which was presented by Ning (10). One of the potential reasons for such irreproducibility could be the presence or absence of random, trace amounts of water in one or more components for preparing the mobile phase(s). It is critical to investigate the effect of trace amounts of water in the mobile phase on normal-phase enantioselective HPLC chromatography in order to eliminate such irreproducibility. The effect of water in the mobile phase of normal-phase HPLC has been studied by Paanakker (11) and other researchers. However, to our best knowledge, only a few papers (12–19) have included observations with small amounts of water present in the mobile phase of normal-phase enantioselective HPLC. The objective of the research presented in this paper is to systematically study the effect of trace amounts of water in the mobile phase on selectivity and resolution of normal-phase enantioselective HPLC through monitoring the separation of an active pharmaceutical ingredient (API), T-3811ME (20), and its undesired enantiomer. The chemical structure of T-3811ME is shown in Figure 1.



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Experimental

Column

The column used in the study was a Chiralpak AD-H (150 × 4.6 mm) column from Chiral Technologies (West Chester, PA).

Chromatography

Chromatography was carried out by using Agilent 1100 HPLC systems equipped with DAD detectors (Agilent, Santa Clara, CA). The essential components of the mobile phase were hexanes and 2-propanol with methanesulfonic acid. Organic modifiers, such as ethanol and methanol, were also evaluated at different concentrations. The flow rate was 1.0 mL/min and the column temperature was ambient temperature. The injection volume was 5 μ L and the detection wavelength was 280 nm. A 1:1 mixture of T-3811ME and its enantiomer (approximately 0.3 mg/mL each) in 2-propanol was prepared as the sample solution for analysis. Duplicate injections were made and highly similar results were obtained for the two injections under each mobile phase condition. One representative set of results is presented in the Results section.

Chromatographic performance

Retention time (t_R), resolution factor (Rs), selectivity factor (α), and plate number (N) calculations were performed by Chem Station on Agilent systems.

Water content determination

Determination of water content in freshly opened/acquired alcohols was performed on a Brinkmann 831-Coulometer (Brinkmann, Westbury, NY) or 758-KFD Titrino Karl Fisher titrator (Brinkmann). Results showed that 2-propanol has approximately 0.01% (v/v) water. Methanol, 200-proof ethanol, and 3A ethanol have approximately 0.01%, 0.04%, and 6% (v/v) of water, respectively.

Solvents and chemicals

All solvents and reagents were purchased from commercial sources and freshly opened before use without further purification. Hexanes, 2-propanol, and methanol were of HPLC grade and from Fisher Scientific (Hampton, NH). 3A ethanol was obtained from Equistar Chemical (Houston, TX). Two-hundred-proof ethanol was purchased from Pharmaco (Bayonne, NJ). Methanesulfonic acid was from Acros (Fair Lawn, NJ). Water was obtained from an in-house Millipore apparatus (Milli-Q system; Millipore, Bellerica, MA).

Reference standards of T-3811ME and undesired enantiomer were provided by the API vendor for Schering-Plough.

Results

To study the effect of controlled, trace amounts of water present in the mobile phase on the separation of T-3811ME and its undesired enantiomer, 2% (v/v) water was pre-mixed with commercial 2-propanol. The 2% (v/v) water containing 2-propanol was then used in preparation of the mobile phase.

When more than 2% (v/v) water was mixed with 2-propanol, the water became separated from other mobile phase components when the 2-propanol–water mixture was added to hexanes at the concentration of 2-propanol ranging from 15–30% (v/v). 2-Isopropanol without pre-mixed water was also used to prepare mobile phases for comparison of the separation.

Next to water, alcohols such as methanol and ethanol are among the most polar common solvents that are used in mobile phases (21). Therefore, small amounts of methanol or ethanol were also used to investigate the chromatographic characteristics including Rs, α , and efficiency (plate number N) of the two enantiomers of T-3811ME.

Mobile phase: hexane–2-propanol–methanesulfonic acid at 70:30:0.1 (v/v/v)

Commercial and 2% (v/v) water-modified 2-propanol gave similar α values. The respective retention times of the two enantiomers is also close when 2-propanol with or without water was used. However, the resolution factor between the two enantiomers was significantly improved when using 2% (v/v) water-modified 2-propanol compared to when using commercial 2-propanol. The improvement of separation (resolution factor) mainly resulted from the improvement of peak shapes of both enantiomers, as reflected by the approximately 30% increase of the plate number (N) for both enantiomers.

Mobile phase: hexane–2-propanol–ethanol–methanesulfonic acid at 73:25:2:0.1 (v/v/v/v)

Ethanol of 2% (v/v) was added in the mobile phase. When 200 proof ethanol was added, the selectivity factor was similar. However, a significant change in resolution factor due to

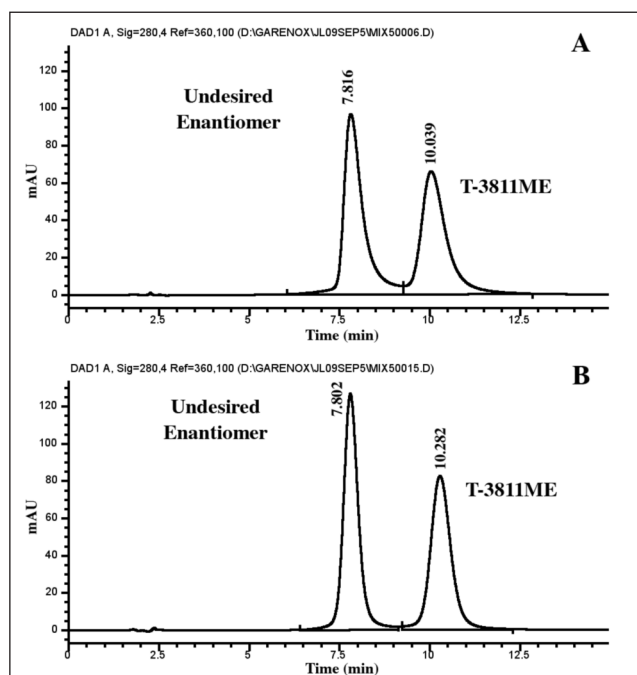


Figure 2. Typical HPLC chromatograms of a 1:1 mixture of T-3811ME and its enantiomer (approximately 0.3 mg/mL each in 2-propanol). Mobile phase is hexane–2-propanol–methanol–methanesulfonic acid (74:25:1:0.1, v/v/v/v) with flow rate of 1.0 mL/min at ambient temperature. HPLC column is Chiralpak AD-H (150 × 4.6 mm) and detector (DAD) is at UV 280 nm. Neat commercial 2-propanol (A); 2% water-modified 2-propanol (B).

improvement of peak shape was obtained when 200 proof ethanol was used with 2% (v/v) water-modified 2-propanol.

No significant improvement was achieved in resolution and selectivity of the two enantiomers when 3A ethanol was added in the mobile phase with neat commercial 2-propanol or with commercial 2-propanol pre-mixed with 2% (v/v) water. This is because 2% (v/v) 3A ethanol in the mobile phase contributes approximately 0.12% (v/v) of water to the total volume of the mobile phase, which could already be adequate for achieving most of the impact that trace amounts of water can have.

Improvement of column efficiency in terms of plate number (N) was also observed when 2-propanol with pre-mixed trace amounts of water were used with either 3A or 200-proof ethanol.

Mobile phase: hexane–2-propanol–methanol–methanesulfonic acid at 74:25:1:0.1, or at 73:25:2:0.1 (v/v/v/v)

When 1% (v/v) or 2% (v/v) methanol was used in the mobile phase with 2% (v/v) water modified 2-propanol versus neat commercial 2-propanol, there was no significant change in the selectivity factors, but the resolution factors were much improved, especially for 1% (v/v) methanol used, as shown in Figure 2. Almost baseline separation of the two enantiomers was achieved when either 1% or 2% (v/v) methanol was used with 2% (v/v) water-modified 2-propanol for mobile phase preparation.

Again, improvement of column efficiency in terms of plate number (N) was observed when 2-propanol with pre-mixed trace amounts of water was used with either 1% or 2% (v/v) methanol.

Summary of results

From the previously mentioned results, as summarized in Table I, it can be seen that the 2% (v/v) water pre-mixed 2-propanol in mobile phase preparation has significant impact on the improvement of either the peak shape of both enantiomers or α , or both.

Discussion

Studies in this paper clearly demonstrated that the presence of trace amounts of water in the mobile phase of normal-phase chiral chromatography can significantly impact the resolution and/or chiral selectivity of the method. Typically, the secondary interactions between the analytes and the column stationary phase, which are mainly caused by the residual trace metals and free silanols on the silica backbone, result in peak broadening and decrease in resolution (22). Trace amounts of water present in the mobile phase could interact with and/or solvate the residual metal ions and free silanols (or even the analytes), and these sites would not be readily available to the analytes for interactions. In this way, the secondary interactions between analytes and the stationary phase could be minimized or even completely suppressed, which would result in improved resolution and/or selectivity, as well as improved column efficiency for both enantiomers.

Therefore, in normal-phase enantioselective HPLC, if the mobile phase contains alcohols or other relatively polar water-miscible solvents, the random variation in the trace amounts of water in such polar solvent(s) tend to make the method irreproducible on a day-to-day, analyst-to-analyst, and lab-to-lab basis. Thus, it is critically important to assess the effect of trace amounts of water on the chromatographic characteristics, based on which different actions should be taken. If the assessment shows that trace amounts of water in the mobile phase of normal-phase enantioselective HPLC can improve the chiral selectivity and resolution as shown in this study, controlled, trace amounts of water are recommended to be pre-mixed with the polar solvent(s) for mobile phase preparation to suppress the variation of the amount of water. The amount of water to be pre-mixed can be determined for individual cases based on the analyte properties and mobile phase ratio. As such proper precaution

Table I. Effect of Trace Amounts of Water (2%, v/v) in 2-Propanol on Separation of 1:1 Mixture of T-3811ME and its Undesired Enantiomer

Hexane (%)	IPA* (%)	Water [†] (%)	Organic modifier	MSA [‡] (%)	RT [§] (min)		Separation		N ^{**}	
					E ^{††}	T ^{††}	Rs ^{§§}	α ^{***}	E	T
70	30	–	–	0.1	5.57	7.00	1.87	1.37	1191	1039
70	30 (m)	0.60	–	0.1	5.49	7.07	2.38	1.42	1564	1329
73	25	0.12	2% 3A EtOH	0.1	6.85	8.93	2.52	1.41	1547	1408
73	25 (m)	0.62	2% 3A EtOH	0.1	6.72	8.48	2.54	1.35	2003	1882
73	25	–	2% 200-proof EtOH	0.1	6.69	8.40	2.15	1.35	1474	1410
73	25 (m)	0.50	2% 200-proof EtOH	0.1	6.66	8.54	2.50	1.38	1730	1574
74	25	–	1% MeOH	0.1	7.82	10.04	2.26	1.37	1372	1282
74	25 (m)	0.50	1% MeOH	0.1	7.80	10.28	2.93	1.42	1912	1782
73	25	–	2% MeOH	0.1	6.92	8.98	2.58	1.40	1633	1570
73	25 (m)	0.50	2% MeOH	0.1	6.72	8.67	2.82	1.39	2081	1914

* 2-Propanol. The mark "m" in parenthesis means the 2-propanol used was pre-mixed with 2% (v/v) water.

[†] The amount of water is the total contribution of the water pre-mixed (if any) with 2-propanol and the water from the alcoholic components.

[‡] Methanesulfonic acid.

[§] Retention time.

^{**} Plate number.

^{††} Undesired enantiomer.

^{†††} T-3811ME.

^{§§} Resolution factor.

^{***} Selectivity factor.

is taken, a reproducible and rugged normal-phase enantioselective HPLC method can be developed. Moreover, other normal-phase chromatographic or separation techniques can potentially utilize and benefit from the investigation of the effect of trace amounts of water in mobile phase and the practice of using controlled trace amounts of water in the mobile phase because they follow highly similar fundamental principles as enantioselective HPLC. These techniques may include analytical chromatography such as HPLC (achiral), supercritical fluid chromatography, and thin layer chromatography, and preparative chromatography such as preparative HPLC and simulated moving bed chromatography, though future studies are yet to be carried out to confirm the effect.

Conclusions

Trace amounts of water can play a profound role in improving the selectivity, resolution, and efficiency of optical isomers in normal-phase enantioselective HPLC. Investigation of the effect of trace amounts of water in the mobile phase of normal-phase enantioselective HPLC should be carried out during method development. If trace amounts of water in the mobile phase impact the resolution and/or selectivity of enantiomeric analytes, controlled, trace amounts of water can be added into the polar component(s) of the mobile phase in order to suppress the irreproducibility of the amount of water presence in the mobile phase.

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