A Volatile Ion-Pairing Chromatography Reagent for an LC–MS Mobile Phase

Reversed-phase liquid chromatography (LC) relies upon the nonpolar portions of analyte molecules for its retention properties with C18 columns. Many dyes are highly sulfonated to increase water solubility. The consequence of this high degree of sulfonation is that virtually no retention occurs on a C18 reversed-phase column. Ion-pairing chromatography using molecules such as tetrabutylammonium salts in the mobile phase is the classic way to solve this problem. Being nonvolatile, these components are incompatible with standard LC–mass spectrometry (MS) methods that require volatile buffers and additives in their mobile phases. This article presents a volatile ion-pairing reagent that enables good retention of highly polar anionic species such as sulfonated azo dyes using reversed-phase LC–MS methodologies.

Many dyes are highly sulfonated to increase their water solubility. Some azo dyes may have as many as six sulfonate groups attached to them. This sulfonation leads to poor retention in reversed-phase liquid chromatography (LC) methods that use C18 columns. Tetrabutylammonium and other quaternary ammonium salts are used as ion-pairing chromatography reagents to increase the retention of anionic compounds (1).

I recommend that the pH of the mobile phase remains basic so that all anionic groups such as carboxylic acids are in their ionized state. The negatively charged $RCOO^-$ is strongly attracted to the adsorbed positively charged tetrabutylammonium ion, which enables increased retention. In addition to quaternary ammonium salts, Snyder and Kirkland (2) have suggested using tri-*n*-octylamine with perchloric acid as a pH adjuster for ion-pairing chromatography.

This method has been the classic approach to increasing the retention of highly polar anionic analytes on reversed-phase LC columns. Many LC–mass spectrometry (MS) instruments require volatile components in their mobile phases to eliminate contamination of the interface between the liquid chromatograph and the mass spectrometer. These volatile components have led to the replacement of sodium phosphate buffers with ammonium bicarbonate and the ammonium or triethylamine salts of formic or acetic acid (3).

New orthogonal designs of LC–MS interfaces have moderated this concern to some extent, but the use of strictly volatile components in the mobile phase still is preferred. This article deals with the use of a tertiary amine as a volatile ion-pairing reagent for the increased retention time of highly sulfonated dyes. One property of the sulfonated dyes of which this method takes advantage is that they remain soluble and ionized at slightly acidic pH values. This property allows the mobile phase to be used at pH 5, which keeps the tertiary amine protonated. This protonated tertiary amine will act as the ion-pairing reagent to extend the retention time of the sulfonated species. Wehr (3) previously suggested triethylamine's use as a volatile buffer, and I will show that it also can be an effective ion-pairing reagent when used at a 10 mmol concentration and when the mobile-phase pH is reduced to pH 5 with acetic acid.

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Experimental

Equipment: An LCQ Classic mass spectrometer was operated in the electrospray ionization negative-ion mode and connected to a P4000 SpectraSystem liquid chromatograph (both from Thermo Finnigan, San Jose, California). I used a 150 mm × 2 mm, 5-µm d_p Luna C18(2) reversed-phase column (Phenomenex Inc., Torrance, California). The flow rate was 0.3 mL/min using a gradient that went from 100% aqueous phase to 10% aqueous phase during a 10-min time span and was held at 10% aqueous phase for 5 min. The organic phase was a 50:50 (v/v) methanol–acetonitrile mixture.

In this work, I compared two aqueous phases: a standard mobile phase using 10 mmol ammonium acetate buffer at pH 7.5 and an ion-pairing reagent mobile phase using 10 mmol triethylamine adjusted to pH 5 with acetic acid.

Analyte: I tested many proprietary dyes in the course of this work, but I will use one common, commercially available dye to illustrate the effect of the triethylamine ion-pairing reagent mobile phase.

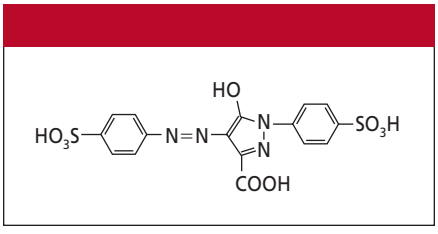

Figure 1: Acid Yellow 23 (molecular weight 468.41).

Figure 1 shows the structure of Acid Yellow 23, an azo dye with two sulfonate groups and one carboxylate group. This dye is known to have very short retention times when analyzed using reversed-phase LC methods.

Results

I injected the Acid Yellow 23 dye onto the LC–MS system under the previously described conditions. As Figure 2 shows, the standard acetate mobile phase resulted in a retention time of 1.93 min. When I used the triethylamine ion-pairing reagent mobile phase (Figure 3), the retention time of the dye was increased to 6.08 min. The associated mass spectra are shown below the chromatograms. The two main masses — 466.9 and 233.1 — correspond to the singly and doubly charged species of the dye ($[M - 1H]^{1-}$ and $[M - 2H]^{2-}$, respectively).

When using the ion-pairing reagent mobile phase, the single charge state is favored. This example, along with other samples not presented in this article, shows that multiple charge states are suppressed when the triethylamine ion-pairing reagent is used to the extent that some multiple charge states might not be observed at all. This result was unexpected, and it could cause loss of important information, because the presence of multiple charge states can be useful for interpreting unknown structures.

Discussion

Triethylamine is an effective ion-pairing reagent for sulfonated azo dyes when it is

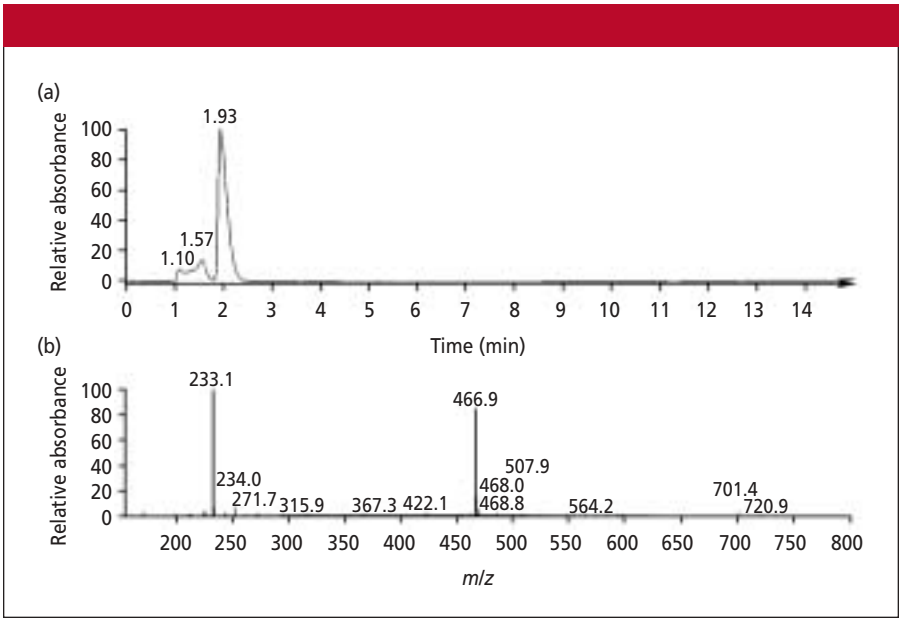

Figure 2: LC–MS analysis of Acid Yellow 23 using the standard ammonium acetate mobile phase. Shown are the (a) ion chromatogram and (b) mass spectrum.

used at slightly acidic pH levels. It extends retention times, improves peak shape, and enables the separation of otherwise coeluted analytes.

An unexpected result of the triethylamine reagent is the suppression of multiple charge states. These additional mass peaks sometimes can be useful in structural interpretation. Therefore, it is important to remember that using the standard mobile phase without the ion-pairing reagent can be useful to obtain this additional information.

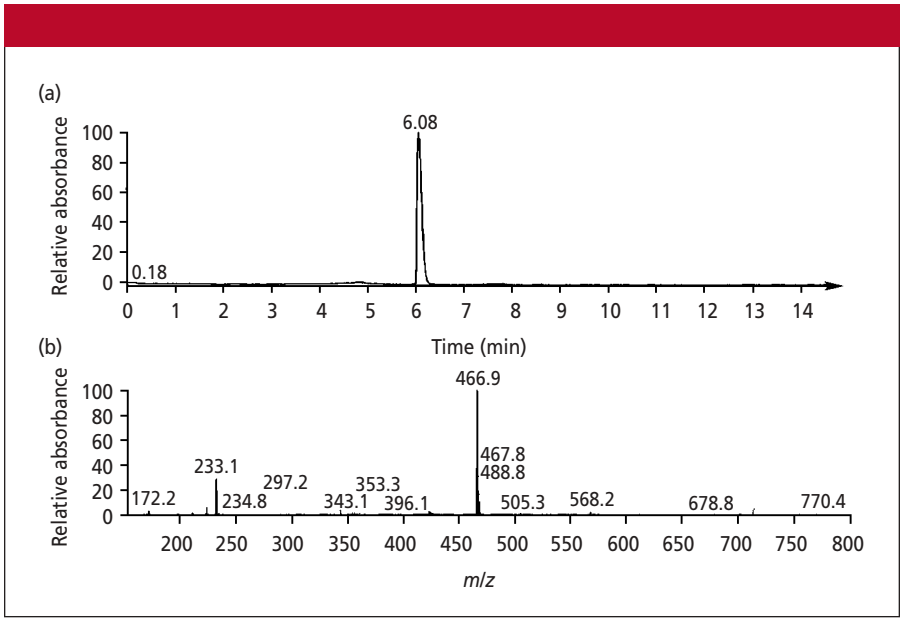

Figure 3: LC–MS analysis of Acid Yellow 23 using the triethylamine ion-pairing mobile phase. Shown are the (a) ion chromatogram and (b) mass spectrum.

It should be noted that triethylamine can be strongly adsorbed onto the surfaces of a vacuum manifold and the parts therein, which can result in memory effects (4). The consequence could be a persistent 102 mass in the electrospray ionization positive ion mode. Users should take care to clean these parts when a system is to be used in the positive ion mode for analyzing low molecular weight analytes.

References

(1) L.R. Snyder, J.J. Kirkland, and J.L. Glajch, *Practical HPLC Method Development* (John Wiley & Sons, New York, 2nd ed., 1997), p. 318.

(2) L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography* (John Wiley & Sons, New York, 2nd ed., 1979), p. 462.

(3) Tim Wehr, *LCGC* **18**(4), 415 (2000).

(4) H. Rutters, T. Mohring, J. Rullkotter, J. Griep-Raming, and J.O. Metzer, *Rapid Commun. Mass Spectrom.* **14**(2), 122–123 (2000). ■