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Analysis of Sulfonates in Aqueous Samples by Ion-Pair LC/ESI-MS/MS with In-Source CID for Adduct Peak Elimination

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Determination of low-molecular-weight organic sulfonates (e.g. taurine and cysteic acid) in aqueous solutions is important in many applications of biological, environmental and pharmaceutical sciences. These compounds are difficult to be determined by commonly used reversed-phase liquid chromatographic separation combined with UV-Visible detection because of their high solubility and the lack chromophoric moieties. Here we report a method combining ion-pair liquid chromatography and electrospray ionization tandem mass spectrometry (IPLC/ESI-MS/MS) for determining sulfonates. The ability of low-molecular-weight sulfonates to form ion-pairs with quaternary ammonium cations in aqueous solutions allowed LC separation with a C_{18} column. Detection of the sulfonates was accomplished with ESI-MS that lends a universal mode of mass detection for polar, water soluble compounds. An in-source collision induced dissociation (CID) was applied to eliminate the adduct peaks in mass spectra. Characteristic marker ions showed in the second stage mass spectra lent a method for identifying sulfonates.

The LC separation was carried out by using a 5 µm C_{18} column (150 mm x 2 mm id, *Phenomenex*). The mobile phase was a 1:1 methanol-water solution with 25 mM of ammonium acetate and 10 mM of cetylpyridinium acetate which was prepared from its chloride salt by passing through a column of anion exchange resin in the acetate form. The IPLC flow rate was 0.30 mL/min. Negative-ion mass spectra were collected by using a Finnigan LCQ quadrupole ion trap mass spectrometer scanned from 50 to 500 m/z with an in-source CID of 25% relative collision energy (RCE) applied to eliminate the adduct peaks in mass spectra. The peak of m/z 80 (SO_3^-) or m/z 81(HSO_3^-) in the second stage mass spectra was selected as a marker to verify sulfonates in the samples. The RCE for MS/MS experiments was 20%.

The IPLC separation was evaluated by testing a solution containing seven sulfonates(100 µM for each). Figure 1 shows the total ion current (TIC) chromatograms obtained. These compounds (corresponding LC peaks are labeled in figure 1b) were taurine (1), 4-morpholineethanesulfonic acid (2), homocystic acid (3), isethionic acid (4), methanesulfonate (5), 2-(4-pyridyl)-ethanesulfonic acid (6) and butanesulfonate (7). As Figure 1a shows these compounds could not be separated with traditional LC, but separated satisfactorily with the IPLC (Figure 1b).

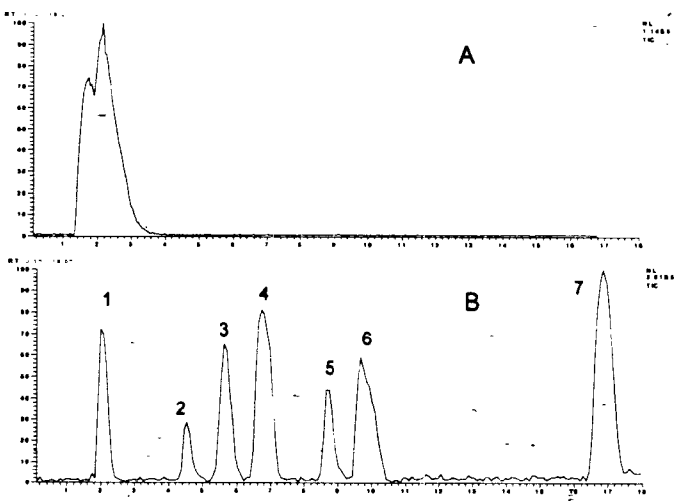


Figure 1. Total ion current (TIC) chromatograms of seven sulfonates by (A) the LC/ESI-MS and (B) the IPLC/ESI-MS

The formation of adducts can be observed in many ESI-MS applications, especially when additional salts are applied in the sample stream. Figure 2a gives the mass spectrum of isethionic acid without any in-source CID applied. The spectrum shows several peaks in addition to that corresponding to the deprotonated isethionic acid (m/z 125.1). These peaks correspond to various adducts formed by

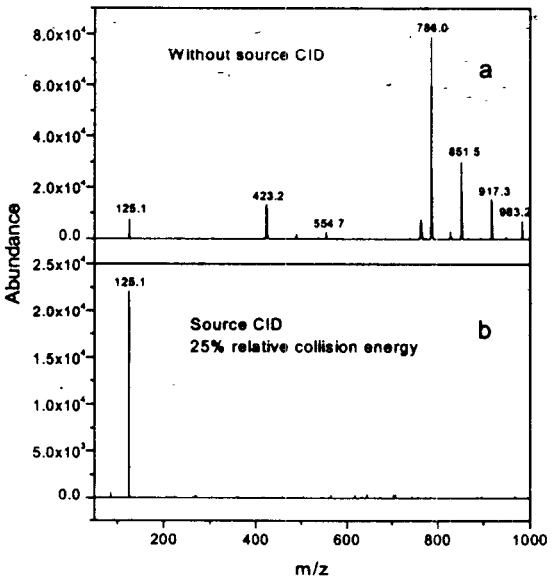
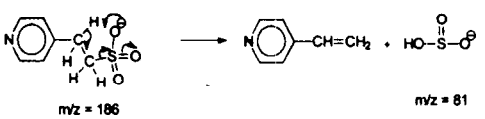


Figure 2. Mass spectra of isethionic acid (a)without source CID and (b) with a 25% relative collision energy source CID

It is known that the m/z 80 peak in CID spectra is a marker for sulfonates because it corresponds to the negatively charged SO_3^- radical. Our results on IPLC/ESI-MS/MS for many sulfonated compounds support this fact; for example, the second stage mass spectrum of butanesulfonate (Figure 3a) shows the m/z 80 peak, representing the radical ions cleaved from the precursor (m/z 137.1). However, we observed a peak with m/z 81 in the second stage mass spectrum of 2-(4-pyridyl)-ethanesulfonic acid (Figure 3b). The m/z 81 peak probably suggests that it was formed by a mechanism different from that of m/z 80. For this ion, we suggest a hydrogen transfer mechanism during the fragmentation of the molecular ion in the CID as shown below:

The formation of a resonance



stabilized π electron system in 4-vinylpyridine, as a co-product may facilitate the hydrogen transfer mechanism for forming the HSO_3^- ion.

cetylpyridinium cation (L), isethionic acid anion(M), and acetate ion (Ac) with m/z 423.2 [$(LAc_2)^+$], 489 [$(LMAc)^+$], 554.7 [$(LM_2)^+$], 786.0 [$(L_2Ac_3)^+$], 851.5 [$(L_2MAc_2)^+$], 917.3 [$(L_2M_2Ac)^+$] and 983.2 [$(L_2M_3)^+$]. The formation of adducts complicates the identification of unknown molecular ions, reduces the target compound ion intensities, and increases the TIC base line and noise levels. To eliminate the additional peaks, an in-source CID was applied to dissociate the adducts. Because the adduct formation occurs mainly due to weak interactions such as electrostatic attraction and van der Waals force, a low energy in-source CID (i.e., 20%-30% RCE) is sufficient to dissociate many adduct ions. The use of a higher collision energy may break the covalent bonds of target compounds, and therefore, should be avoided for the purpose of eliminating adducts. Figure 2b shows the use of a 25% RCE in-source CID to eliminate adduct peaks of isethionic acid in the IPLC/ESI-MS. Due to adduct dissociation, the peak intensity for isethionic anion increased 2.5-fold, resulting in an unambiguous identification of the target compound molecular ion, a much improved TIC base line, and noise levels (Figure 1).

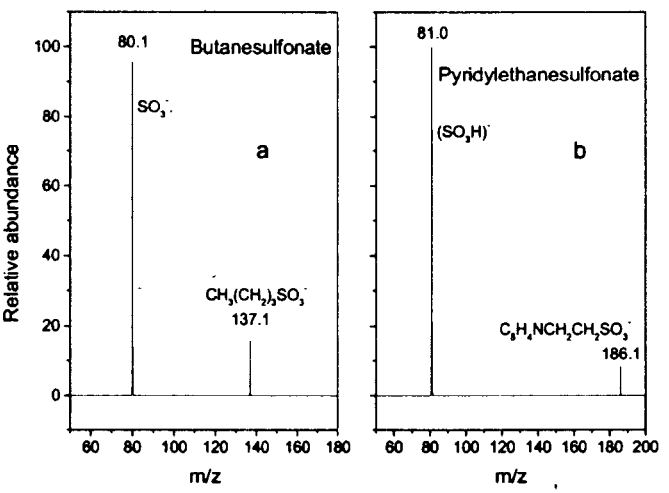


Figure 3. Second stage MS of two sulfonates in the IPLC/ESI-MS/MS

The present study has demonstrated that the separation of low-molecular-weight sulfonates can be achieved by IPLC, the adduct peaks in IPLC/ESI-MS can be eliminated by applying a low energy in-source CID, and an m/z 81 peak in CID mass spectrum may also be a marker for some sulfonates. All these characteristics result in the IPLC/ESI-MS/MS method as a suitable approach for analysis of water soluble sulfonated compounds.