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Analysis of sulfonated compounds by ion-exchange high-performance liquid chromatography–mass spectrometry

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Abstract

Ion-exchange high-performance liquid chromatography (HPIEC)–mass spectrometry (MS) was used for the analysis of different sulfonated compounds. HPIEC was performed on an aminopropyl column applying a gradient with increasing concentration of a buffer consisting of ammonium acetate–acetic acid and acetonitrile as the organic modifier. HPIEC is well suited to highly efficient separation of sulfonated compounds and furthermore, due to the volatility of ammonium acetate, the method is also appropriate for LC–MS coupling by the means of either atmospheric pressure chemical ionization or electrospray ionization. The applicability range of HPIEC–MS is demonstrated on the basis of a complex mixture of model substances consisting of sulfonated aromatics and textile dyes largely differing from each other in their structural properties.

Keywords: Sulfonic acids; Organosulfur compounds; Dyes; Polynuclear aromatic hydrocarbons, sulfonated; Benzenesulfonic acid

1. Introduction

Ion-pair gradient reversed-phase high-performance liquid chromatography (RP-HPLC) is the most common tool for the analysis of sulfonated compounds. Due to the ion-pairing effect, even aromatic and aliphatic sulfonic acids exhibiting pronounced hydrophilicity can be sufficiently retained on standard RP-HPLC columns, e.g., C_{18} materials. When paired with a suitable ion such as tetraalkylammonium, they behave in a substantially hydrophobic fashion and thus a highly efficient separation by gradient RP-HPLC is achievable. Due to their low background absorption, tetrabutylammonium salts are particularly well suited for UV detection and therefore, ion-pair chromatography proves to be the method of choice for both in-process and quality control of sulfonic acids. Furthermore, owing to the fact that aromatic and aliphatic sulfonic acids, the latter including the ionic surfactant family, are often not easily degradable in environmental biological systems, they accumulate in ground and surface water. As a logical consequence, they give rise to environmental problems calling for efficient separation procedures covering a broad range of these components for subsequent precise and reliable quantitative determination [1–4].

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However, UV detection, even when effected in the diode array detection mode, is not suitable for the analysis of unknown sulfonic acid derivatives and, as a logical consequence, the use of a highly selective or even really specific detection principle is required, such as mass spectrometry (MS) as experienced in the case of LC–MS coupling. Unfortunately, an electrospray ionization (ESI) unit, where volatility of the HPLC effluent is an ultimate prerequisite, is rapidly encrusted by non-volatile ion-pairing agents. Likewise, an atmospheric pressure chemical ionization (APCI) unit is similarly contaminated and thus precludes the use of LC–MS as a standard analytical tool for this purpose. However, structural elucidation without MS is a tedious and time-consuming procedure because preparative isolation of the target components and often removal of ion-pairing agents has to be performed, prior to the subsequent use of other analytical techniques, such as nuclear magnetic resonance, infrared and/or ''off-line'' mass spectrometry.

Nevertheless, many investigations have been done on alternatives to non-volatile tetraalkylammonium salts as ion-pairing agents. In this respect, ammonium acetate is well suited in many cases: the use of aqueous ammonium acetate buffers as ion-pairing electrolytes for thermospray LC–MS is demonstrated by Escott and Chandler [5] for the analysis of phenyl ethoxysulfonates as well as by Wilder et al. [6] for the separation of sulfonated aromatics on β-cyclodextrin-bonded phase. It is also used for preparative HPLC [7], reversed-phase HPLC–MS [8,9], and analysis of sulfonated azo dyes [10–13]. Although excellently suited to both ESI and APCI mass spectrometric applications, ammonium acetate is too weak an ion-pairing agent for RP-HPLC and its use therefore has been restricted only to a limited number of compounds.

In order to provide compatibility of the column effluent with a mass-selective system a micro-membrane suppressor for trapping non-volatile ion-pairing agents used in LC–MS with a moving-belt interface is described by Escott et al. [14]. Either cationic or anionic membrane suppressors for LC–MS with an ion-spray interface were reported by Conboy et al. [15], whereas Forngren et al. [16] removed non-volatile mobile phase ingredients with an ion exchanger placed between separation column and MS interface.

As a powerful alternative to ammonium acetate–formate volatile ion-pairing agents, trialkylamines proposed by Storm et al. [17], seem to be gaining more and more importance. From the series of volatile trialkylamine homologues, tributylamine proved to be the most efficient one, as even trisulfonated compounds are satisfactorily retained on reversed-phase materials. Although tributylamine seems to be suitable for negative ESI-MS, it exhibits marked carry-over in the positive ion mode, in spite of careful cleaning of the whole HPLC–MS apparatus resulting in an intensive $[M+H]^+$ background ion at m/z 186.

Despite not being volatile ion-pairing agents, satisfactory separation is often also achievable by the use of sodium perchlorate as applied by González-Mazo et al. [18] or sodium sulfate reported by Chaytor and Heal [19].

In this study, the usefulness of ion-exchange high-performance liquid chromatography (HPIEC) coupled to MS is investigated. Due to electrostatic interaction, retention of sulfonated compounds is easily achieved, whereas elution is performed by increasing concentrations of both ammonium acetate–acetic acid and acetonitrile in the final hydro–organic eluent system. The potential of this method is demonstrated by its application to the analysis of a complex mixture of different sulfonated compounds.

2. Experimental

2.1. Chemicals

Ammonium acetate (analytical grade), acetic acid (analytical grade), water (HPLC quality) and the following sulfonated compounds were purchased from Fluka (Buchs, Switzerland): benzenesulfonic acid sodium salt (puriss, recryst., CAS No. 515-42-4), 2-naphthalenesulfonic acid sodium salt (techn., CAS No. 130-14-3), 2-anthraquinonesulfonic acid sodium salt (purum, CAS No. 131-08-8), sulforhodamine B sodium salt (C.I. No. 45100, CAS No. 3520-42-1), Crocein Orange G (C.I. No. 15970, CAS No. 1934-20-9), Eriochrome Black T (C.I. No. 14645, CAS No. 1787-61-7), 2,6-anthraquinonedisulfonic acid disodium salt (purum, CAS No. 853-68-9), 1,5-naphthalenedisulfonic acid disodium salt (techn., CAS No. 1655-29-4), Azophloxine (C.I. No.

18050, CAS No. 3734-67-6), 1,2-benzenedisulfonic acid dipotassium salt (purum, CAS No. 5710-54-3).

Acetonitrile (HPLC grade) was obtained from J.T. Baker (Deventer, The Netherlands).

For the experiments a solution of 50 ng each/μl in water was prepared.

2.2. Column liquid chromatography

The HPLC system consisted of a degassing unit (SCM 1000), a quaternary pump (P 4000), an autosampler (AS 3000) equipped with a 20-μl loop allowing injections of various sample volumes, a photodiode array detector (UV 6000LP) and a ChromQuest data acquisition unit, all obtained from ThermoQuest (San Jose, CA, USA). Separation of the sulfonated compounds was performed on a Nucleosil aminopropyl column (125×4 mm I.D., 5 μm particle size, 100 Å pore diameter) from Macherey–Nagel (Oensingen, Switzerland) at ambient temperature (ca. 21°C) and a flow-rate of 1.5 ml/min. Sample volumes of 5 μl were injected in the ''push loop'' mode. The HPLC method consists of a linear gradient followed by an isocratic section. The gradient profile is shown in Table 1 and was used throughout the whole experiment. An equilibration time of 10 min was chosen before injection. The buffer solution was prepared by mixing 38.5 g ammonium acetate (0.5 mol), 183 g acetic acid and 980 g water, resulting in a pH value of 3.80. UV detection was carried out at 270 nm (band width 5 nm).

2.3. Mass spectrometry

The MS experiments were conducted by means of a LCQ-Duo ion trap mass spectrometer from Finnigan (San Jose, CA, USA). Nitrogen (>99.999%) was used as nebulizer and sheath gas and helium (>99.9999%) served as quenching agent.

For improvement of ionization by the means of the ESI technique, post-column addition of 2-(2-methoxyethoxy)ethanol to the HPLC effluent was effected with a type P100 isocratic pump from ThermoQuest at a flow-rate of 100 μl/min via a zero dead-volume Valco T-piece (Valco Instruments, Houston, TX, USA) positioned after the UV detector. The complete flow was split by another Valco-T piece using two fused-silica capillaries of 75 μm I.D. (Composite Metal Services, The Chase, Hallow, UK) of different length (3 cm to the waste, 90 cm to the mass spectrometer). The resulting flow fed into the ESI ion source of the mass spectrometer was approximately 50 μl/min as calculated from the split ratio resulting from the different length of the capillaries. The conditions for ESI spectra were as follows: spray voltage, 4.5 kV; capillary voltage, 46 V; capillary temperature, 250 °C; sheath gas, 50 units.

For APCI spectra no split was used and the conditions set as follows: vaporizer temperature, 450°C; sheath gas, 45 units; auxiliary gas, 45 units; discharge current, 5 μA; capillary voltage, 10 V; capillary temperature, 200°C.

The mass spectrometer was operated in negative ion full-scan mode (3 micro scans, 50 ms inlet time; mass-to-charge range 100–600 m/z).

Table 1
Mobile phase composition over time

Time (min)	Water (%)	Acetonitrile (%)	Buffer (%)
0	88	10	2
16	5	65	30
20	5	65	30

3. Results and discussion

Sulfonic acids, as either free acids or alkali salts, show widespread use in organic chemistry as well as in additional important applications for environmental and toxicological purposes. The substances chosen for the investigations reported in this paper comprise components from the family of textile dyes, optical brighteners and anionic surfactants.

Whereas in-process and quality control of sulfonic compounds is easily achievable by conventional ion-pair HPLC with UV detection, structure elucidation of by-products, other contaminants and unknowns is difficult, primarily attributable to the reasons mentioned above. However, recently a lot of effort has been made in order to replace non-volatile ion-pairing agents by volatile ones, because volatility of

the whole mobile phase is an ultimate prerequisite for reliable mass spectrometric analysis.

In this report, a HPIEC method for the analysis of sulfonated compounds is presented having both highly efficient chromatographic resolution and compatibility with either ESI or APCI ion sources. The usefulness of this approach is demonstrated with a mixture of various sulfonated compounds, consisting of small monosulfonated aromatics, mono- and disulfonated textile dyes, as well as of disulfonated aromatics with low or medium molecular masses (for structures, see Fig. 1). These commercially available substances were selected because they cover a broad range of sulfonic acid chemistry.

Separation is performed on a weakly basic aminopropyl bonded stationary phase and elution of the test substances takes place by simultaneously increasing the amounts of buffer and acetonitrile,

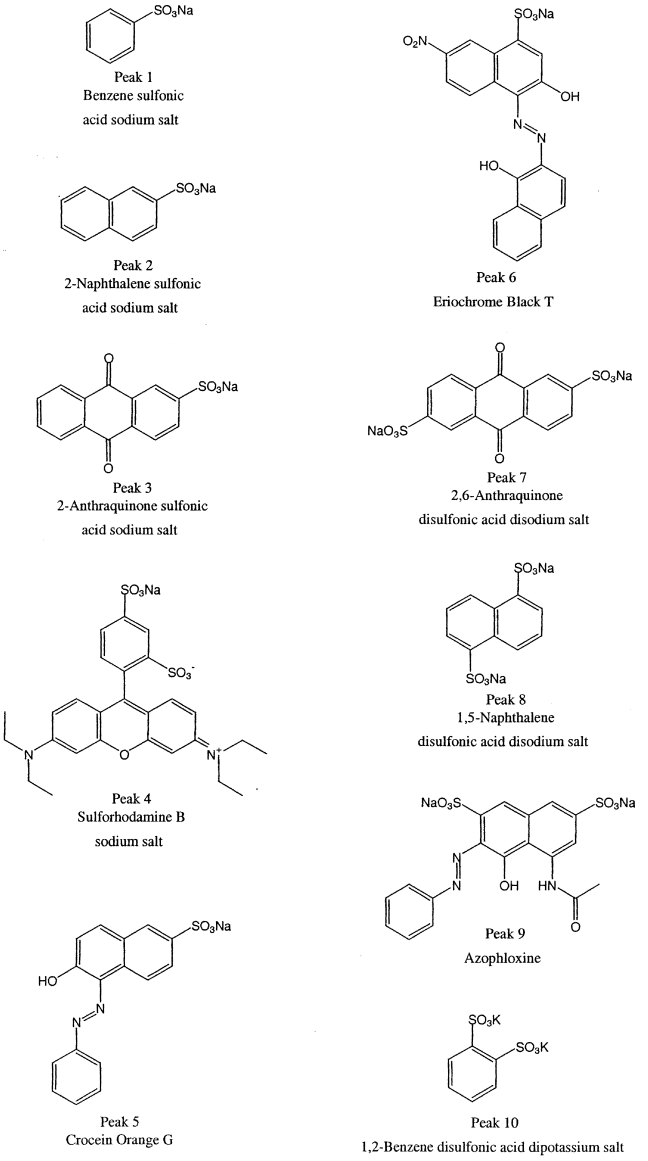

Fig. 1. Structures, names and elution order of the test substances.

respectively. As a consequence of the rising percentage of the buffer, ionic–ionic solute–stationary phase interactions are more and more weakend, whereas the increasing percentage of acetonitrile as the organic modifier efficiently counterbalances residual hydrophobic Van der Waals interactions between unpolar sites of the analytes and the short propyl chain of the stationary phase (for detailed HPLC conditions, see Table 1).

By comparing both the situation in ion-pair HPLC and ion-exchange HPLC it should be emphasized that transition from one to the other is often associated with substantial changes in selectivity and thus rank order of elution because in the former procedure retention is primarily governed by the overall hydrophobicity of the analyte–ion-pairing complex, in this case the analyte's residual hydrophobicity invoked by aromatic ring structures and/or long chain substituents cannot be neglected and thus often renders prediction of retention behaviour a difficult task.

Fig. 2 shows the HPLC chromatogram of the test substances. Peaks resulting from impurities due to some technical grade products are not assigned. The most striking characteristic of this chromatogram is the ''group-specific'' elution of the test substances: As expected from the principle of increasing ionic analyte–stationary phase interactions with increasing net charge, the monosulfonated compounds elute in the first group (peaks 1–6), followed by the disulfonated compounds (peaks 7–10).

However, it should be underlined that separation characteristics within a group are markedly dependent on the remaining functionalities of the molecules. For example Crocein Orange G (peak 5), containing one phenolic group, is eluted before Eriochrome Black T (peak 6), which has two phenolic groups. In this respect, the presence of an additional relatively acidic phenolic OH functionality significantly increases retention time.

A number of tri- and polysulfonated compounds (textile dyes, predominantly) were injected in separate runs, but with the final amount of buffer under the applied HPLC conditions, i.e., 30%, displacement cannot be accomplished. Investigations of higher buffer concentrations – up to 70% – were done, indeed resulting in elution of these polysulfonated compounds, but peak shape and resolution were very poor. Further research for high-resolution chromatography of polysulfonated compounds is currently under investigation, but at the time, was not scope of this study.

Ammonium acetate buffers do not significantly impair mass spectrometric analysis (see Introduction). Therefore the HPLC method presented in this study is applicable for ESI and APCI-MS. In Figs. 3 and 4, the corresponding total ion current

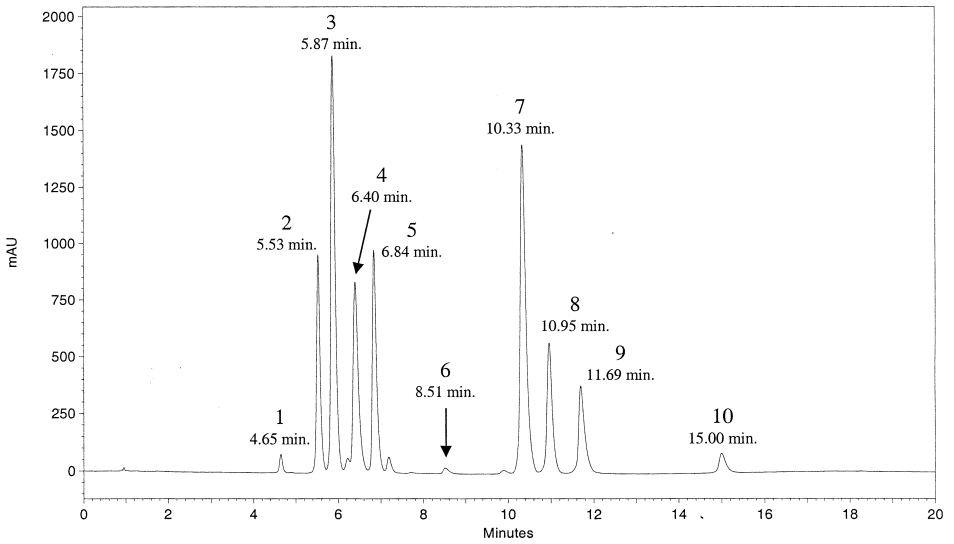

Fig. 2. HPLC chromatogram (270 nm) of the test mixture (see Fig. 1).

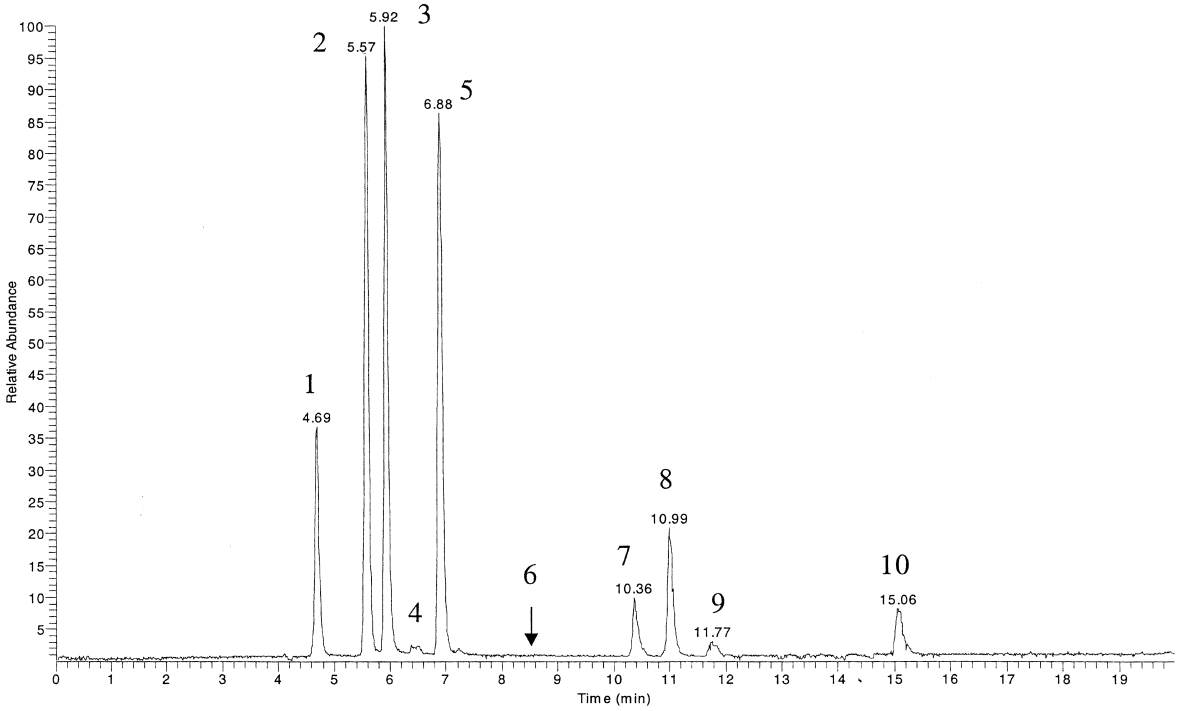

Fig. 3. APCI-MS-TIC of the test substances.

(TIC) traces are shown. However, due to the different ionization mechanism of these two ionization techniques, peak heights of the compounds differ. The mass spectra can be interpreted easily, as with both ionization techniques only low fragmentation and low cluster formation occurs. In the range of decreasing relative ion intensity, following ions can be observed: $[M-H]^-$, $[2M-H]^-$, $[M+CH_3COO^-]^-$, $[M-SO_3-H]^-$, $[2M-SO_3-H]^-$.

As an example, Fig. 5 shows the APCI mass spectrum of 2-naphthalenesulfonic acid.

Cluster ions between analyte and sodium or potassium, e.g., $[M-2H+Na]^-$, are not observed because the small hydrophilic cations are repelled from the positively charged aminopropyl ligands and therefore pass the stationary phase unretained. Despite the use of an acidic buffer, unlike the situation with carboxylic acids, no ion-suppression effect occurs and the completely dissociated and thus strongly acidic compounds move through the column as their conjugated bases and reach the mass spectrometer as the free acids.

For ESI-MS measurements, 2-(2-methoxyethoxy)-ethanol was used as a post-column addition agent for signal intensification (for details, see Experimental section). The beneficial influence of this agent was first published by Yamaguchi et al. [20] and could be confirmed during the course of these investigations.

4. Conclusion

HPIEC–MS is well suited to structure elucidation of sulfonated compounds. Chromatographic resolution of this method is comparable with ion-pair HPLC and although high amounts of ammonium acetate (up to 500 mmol) are necessary for the elution, ionization with both an ESI and an APCI ion source is not impaired.

This study presents the method of choice for the LC–MS analysis of unknowns and by-products components in sulfonic acid chemistry.

The limit of detection was evaluated at 50 pmol [measured with 2-naphthalenesulfonic acid sodium salt in single ion monitoring (SIM) mode], whereas

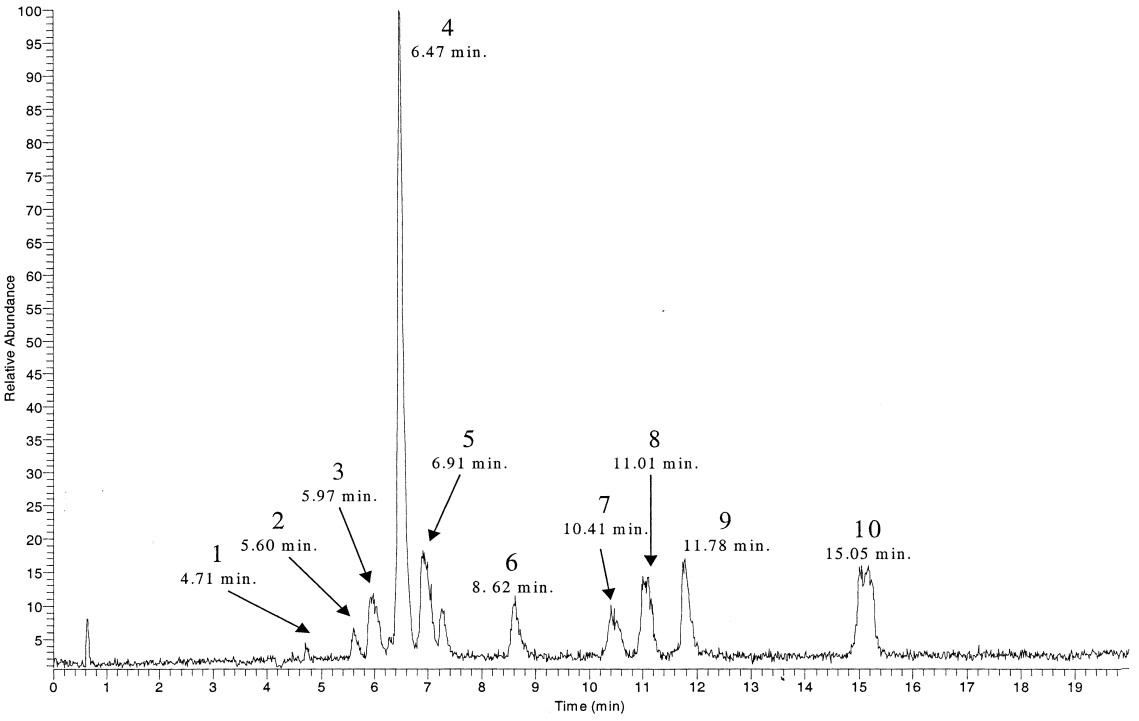

Fig. 4. ESI-MS-TIC of the test substances.

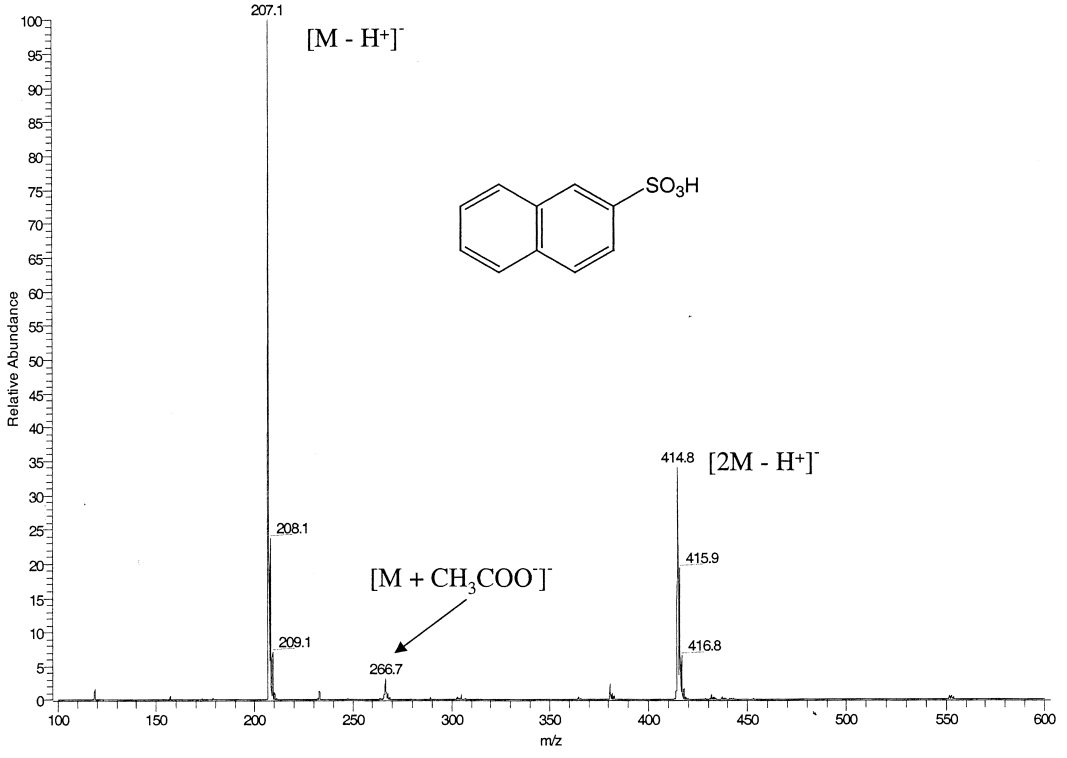

Fig. 5. APCI mass spectrum (negative ion mode) of 2-naphthalenesulfonic acid.

the reported method is applicable for structure elucidation of compounds in trace amounts.

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