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Electrospray interfacing for the coupling of ion-exchange and ion-pairing chromatography to mass spectrometry[a]

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ABSTRACT

Electrospray was evaluated as an interface for coupling ion-exchange and ion-pairing chromatography to mass spectrometry. Effective separation and good sensitivity were demonstrated for a liquid flow-rate as high as 50 μl/min and a matrix ion concentration as high as 10 m*M*. Minimum detectable amounts were estimated to be five to ten times poorer than those obtained in the absence of ion-exchange buffers or ion-pairing reagents. The minimum detectable amount for arsenobetaine after a dynamic ion-exchange separation was estimated to be about 20 pg.

INTRODUCTION

Almost all published work on liquid chromatography–mass spectrometry (LC–MS) using electrospray (ES) or ionspray (IS) interfacing has concentrated on reversed-phase separation [1] despite the fact that both ES and IS are only effective for ions that are pre-formed in solution. The popularity of reversed-phase separation stems partially from its effectiveness in separating relatively large analytes, such as some azodyes [2], peptides and proteins [1,3–5], and toxins [6], usually after the ionization of these species is suppressed in solution; another reason is the use of low-ionic-strength eluents, which are more compatible with ES and IS. The fact that ES and IS interfacing have rapidly become accepted techniques in LC–MS for biotechnology and environmental research demonstrates the effectiveness of this approach.

We have been interested in applying ES tandem MS to the determination of small, environmentally significant ions in complex matrices [7–9]. For these ions, reversed-phase chromatography is ineffective because they lack large hydrophobic side-chains, and ion exchange or ion pairing must be employed.

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In this paper, we report our experience in coupling ion-exchange and ion-pairing chromatography to tandem MS via an electrospray interface. The examples that we have chosen are a dynamic cation-exchange separation of several environmentally important organoarsenic ions in admixture and an ion-pairing separation of a mixture of six permissible food dyes.

EXPERIMENTAL

Instrumentation

Experiments were performed on a SCIEX TAGA, Model 6000E atmospheric pressure ionization, triple quadrupole mass spectrometer with an upper mass range of about m/z 1400. For ES interfacing, the corona discharge assembly was removed. The ES probe was mounted on a small movable platform to facilitate reproducible positioning. The optimum probe tip position was established from time to time but was generally found to be about 1–2 cm from the interface plate with the spray off-axis from the orifice. Ions desorbed from solution were heavily solvated. Under the high electric field established between the probe tip and the interface plate, they drifted towards the latter, and a small fraction was sampled via the orifice. Declustering of these sampled ions was accomplished by means of a mild collision-induced dissociation with nitrogen in the "lens" region. The declustered ions were then mass-analyzed by one or both quadrupoles.

Three types of probes were used over a span of several months during which this study was carried out: stainless steel (Hamilton, 33 gauge, *ca.* 100 μm I.D.), polyimide-clad fused silica (J & W, 50 μm I.D.), and aluminium-clad fused silica (SGE, 50 μm I.D.). Their performance was comparable. To facilitate coupling of the probe to standard LC coupling, it was mounted concentrically inside a 1/16 in. O.D. stainless-steel tube (used typically in gas chromatography) by means of epoxy glue. For IS experiments, the probe was fitted concentrically with a slightly wider stainless-steel tube (Hamilton, 22 gauge, *ca.* 400 μm I.D.) for the passage of the nitrogen nebulizer gas (20 p.s.i.) [10]. The stainless-steel tubes were electropolished before use. For the polarization of the spray probe, a Tennelec Model TC950 power supply (maximum voltage, 5 kV) was used in series with a 50-MΩ current-limiting resistor. The ES current was monitored by a laboratory-made digital microammeter that may be floated above ground. Electrical isolation of the probe was achieved by the use of fused silica or PEEK (polyether ether ketone, Upchurch) tubing for connection.

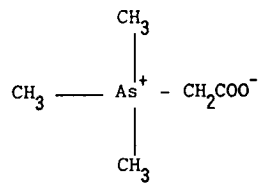
The lens and quadrupole voltages were optimized for ES. Full-scan mass spectra were usually acquired with a measurement time of 10 ms per step (typically one m/z unit) whereas selected ion or reaction monitoring was performed with a typical measurement time of 1 s per cycle. For MS–MS runs, argon was used as the target gas at a thickness of about $1.3 \cdot 10^{14}$ atoms per cm^2, which allowed on average fewer than one collision event per ion. The collision energy used was typically about 33 eV, laboratory frame.

Chromatographic separation was performed using two reciprocating piston pumps (Waters, Model 6000A) controlled via a gradient former (Waters, Model 680). Samples were introduced via a valve injector (Rheodyne, Model 7125) with a 20-μl sample loop. We prefer the use of regular-bore columns because of wider column selection. Two of these were used: a Hamilton PRP-1 column (5-μm packing, 15 cm

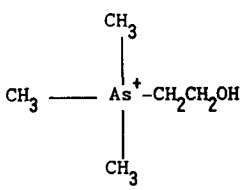
× 0.41 cm I.D.) for the separation of organoarsenic species and a Waters Novapak C_{18} column (4-μm packing, 15 cm × 0.39 cm I.D.) for the separation of food dyes. The typical flow-rate was 1 ml/min. To obtain the appropriate flow-rate for ES, the column effluent was split. About 5% (*ca.* 50 μl/min) was directed into the ES interface; the remaining 95% was routed to a UV–visible variable-wavelength detector (Waters, Model 450) or to waste. Effluent splitting was accomplished by means of a zero dead-volume tee union (SSI) and PEEK connection tubes of the appropriate length and internal diameter. Small changes in the split ratio were inconsequential as ES-MS is insensitive to small changes of flow-rate. When chromatographic separation was unnecessary, the samples were continuously infused by means of a syringe pump (Harvard Apparatus, Model 22) at typical flow-rates of 20–50 μl/min.

Reagents

Polypropylene glycol (PPG) 1000, sodium octanesulphonate and tetrabutylammonium phosphate (TBAP) were analytical grade (Aldrich). Myoglobin (horse heart) was available from Sigma. Arsenobetaine (AB), arsenocholine (AC) iodide, and te-



AB



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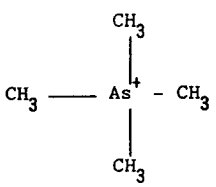
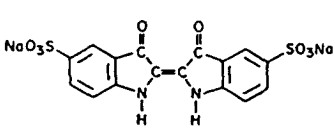


TMA

Fig. 1. Structure of organoarsenic species. AB = arsenobetaine; AC = arsenocholine; TMA = tetramethylarsonium.

tramethylarsonium (TMA) iodide (Fig. 1) were synthesized by W. R. Cullen (Department of Chemistry, University of British Columbia). The food dyes, Indigotine, Tartrazine, Sunset Yellow FCF, Amaranth, Fast Green FCF, and Brilliant Blue FCF (Fig. 2), were purified and donated by F. Lancaster (Health Protection Branch, Health and Welfare Canada). These are six out of ten synthetic food colours permitted in Canada. Organic solvents were LC grade. Water was distilled and deionized (DDW) by means of a commercial cartridge system (Cole-Palmer).

For the dynamic cation exchange of AB, AC and TMA, the PRP-1 column was pre-coated with octanesulphonate as described [11]. Optimum separation was obtained with an eluent of DDW–methanol (80:20) containing 1 m*M* sodium octanesulphonate and 10 m*M* ammonium citrate. For the determination of AB alone, an eluent of DDW–methanol (70:30) containing 10 m*M* sodium octanesulphonate and 0.5% acetic acid on the Novapak C_{18} column was found to work best. For the food dyes, an ion-pairing separation as recommended by Health and Welfare Canada [12] was used; the food dyes were separated in a linear gradient program from 50:50 to 40:60 DDW–methanol containing 5 m*M* TBAP in 12 min.



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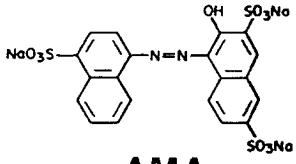
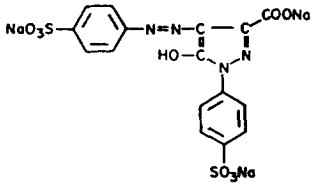
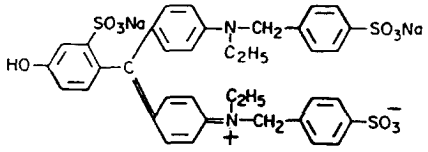


AMA



TAR



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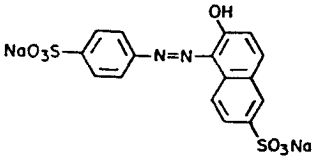
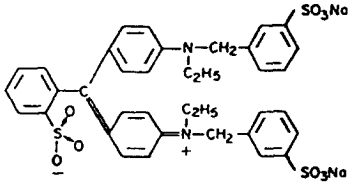


SUN



BRI

Fig. 2. Structure of permissible food dyes. IND = Indigotine; TAR = Tartrazine; SUN = Sunset Yellow FCF; AMA = Amaranth; FAS = Fast Green FCF; BRI = Brilliant Blue FCF.

RESULTS AND DISCUSSION

Before attempting to interface ion-exchange and ion-pairing separation to MS, preliminary investigations were performed to assess the compatibility of ES to the flow-rates and matrix ion concentrations that were necessary for these modes.

Fig. 3 shows the result of selected ion monitoring a 0.25 μM PPG 1000 solution in DDW–methanol (80:20) containing 1 mM ammonium acetate and adjusted to pH 10 with ammonia at a flow-rate of 50 μl/min. PPG 1000 is a mixture of polypropylene glycols containing different numbers of propylene units, with an average molecular weight in the vicinity of 1000 dalton. The ions monitored were two of the many singly charged ammonium adduct ions of PPG [13]. The experiment was conducted in part in IS, *i.e.*, a nitrogen nebulizer gas was added and its flow-rate was varied over a wide range (0 to 1000 and back to 0 ml/min) in a span of 10 min. The nitrogen flow-rate appeared to have little influence on response, which was relatively stable. Essentially identical results were obtained when the liquid flow-rate was lowered from 50 to 10 μl/min. Consequently, all further experiments were conducted with ES at a liquid flow-rate of 50 μl/min.

The presence of organic solvent appears to play a significant role in response stability. In our hands, it is possible to run samples of virtually 100% water as solvent at room temperature (Fig. 4) (and we do so when the presence of organic solvent is undesirable), but signal stability almost always improves when 10% or more methanol is introduced (Fig. 5). Others have reported that the electrospray of 100% aqueous solutions may be stabilized by operating at approximately 50°C [14].

Once the flow-rate compatibility question was answered, the next step was to determine the effect of the presence of significant concentrations of matrix ions as dictated by ion-exchange and ion-pairing chromatography. It was known that they would suppress analyte ion signal [15]; the question was how much. Fig. 6 shows the result of such an experiment on arsenobetaine. The variation of protonated AB response and ES current with the polarization voltage in the presence of varying

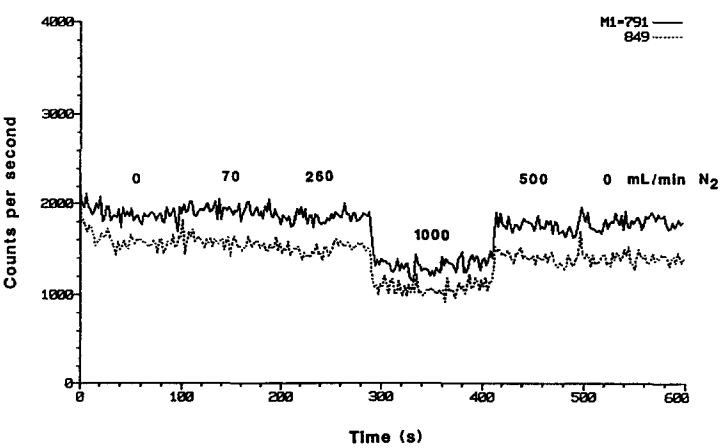


Fig. 3. PPG 1000 response *versus* time and nebulizer gas flow-rate. Conditions: 0.25 μM PPG 1000 solution in DDW–methanol (80:20) containing 1 mM ammonium acetate at pH 10 with ammonia; liquid flow-rate, 50 μl/min; nebulizer gas flow-rate as indicated.

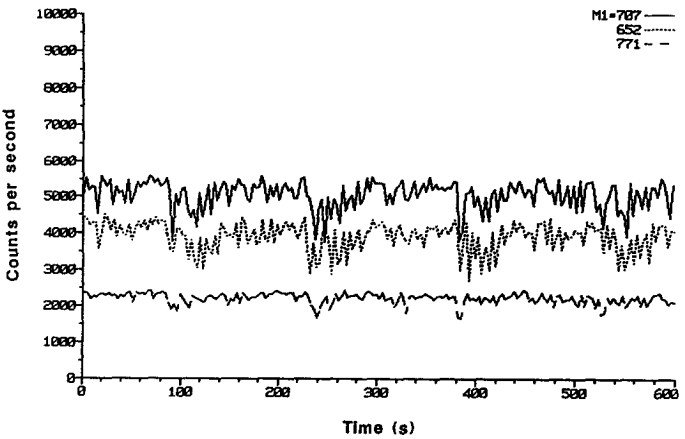


Fig. 4. ES response of myoglobin *versus* time. Conditions: 0.5 μM equine myoglobin in DDW containing 0.05% acetic acid; liquid flow-rate, 50 μl/min. The ions monitored are protonated myoglobin: m/z 652, 26 H^+; m/z 707, 24 H^+; m/z 771, 22 H^+.

amounts of matrix ions was monitored. As expected, the analyte response and ES current were functions of the applied voltage. The presence of matrix ions resulted in signal suppression, but the effect was relatively small even when a matrix ion concentration as high as 100 mM citrate was encountered. At the optimum voltage of about 3.5 kV, the AB response decreased by a factor of 8 when 100 mM ammonium citrate and 1 mM sodium octanesulphonate were added. Similar results were obtained for AC (Fig. 7); a suppression factor of about 5 at the optimum voltage was evident.

Thus it was apparent that ES is quite compatible with the flow-rates and matrices necessary for interfacing ion-exchange and ion-pairing chromatography to MS. The first real interfacing we attempted was the coupling of dynamic ion-exchange separation of AB, AC and TMA ion to tandem MS.

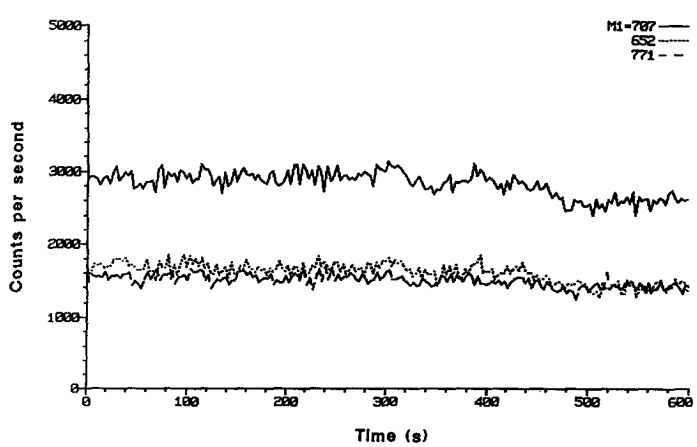


Fig. 5. ES response of myoglobin *versus* time. Conditions: 0.5 μM equine myoglobin in DDW–methanol (90:10) containing 0.05% acetic acid; liquid flow-rate, 50 μl/min. See Fig. 4 for ion assignment.

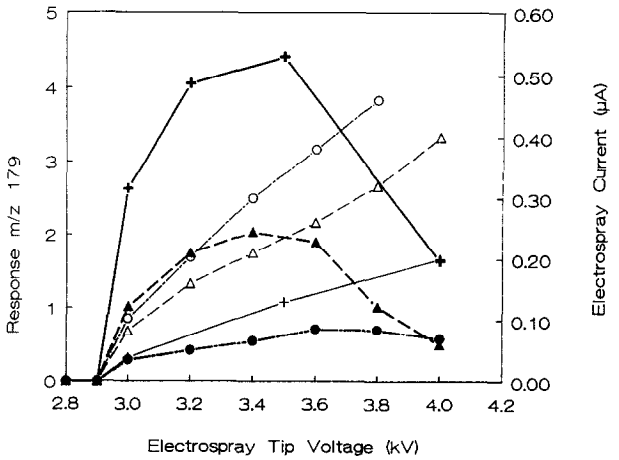


Fig. 6. ES results of AB solutions containing various concentrations of matrix ions. Open symbols, current; closed symbols, response; all solutions were 25 μM AB in DDW–methanol (80:20) plus the following: + and +, no ammonium citrate, no sodium octanesulphonate; ▲ and △ 10 mM ammonium citrate, 1 mM sodium octanesulphonate; ● and ○, 100 mM ammonium citrate, 1 mM sodium octanesulphonate.

AB, AC and TMA are organoarsenic species known to exist in marine fauna. The most powerful and often employed technique of these involatile species is LC–inductively coupled plasma (ICP) MS [16,17]. In solution, AC and TMA exist as simple cations. AB is a zwitterion in the crystallilne state, but is easily protonated in solution and exist as a cation. These ionic species are best separated by ion pairing or dynamic ion exchange. We preferred the use of poly(styrene–divinylbenzene)-type packing for AC and TMA to circumvent their strong interaction with surface silanol

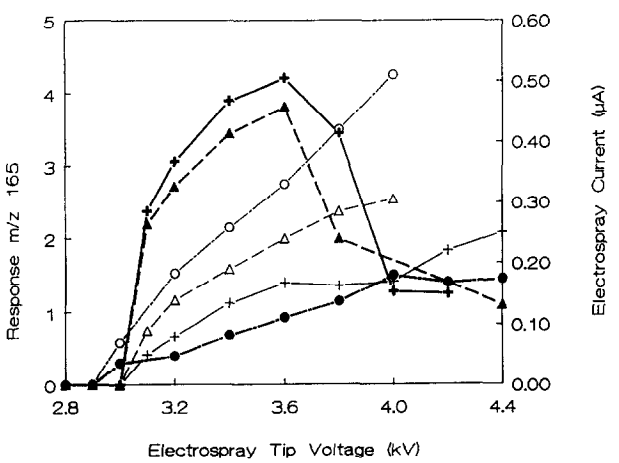


Fig. 7. ES results of AC solutions containing various concentrations of matrix ions. All solutions were 17 μM AC, all other conditions were identical to those in Fig. 6.

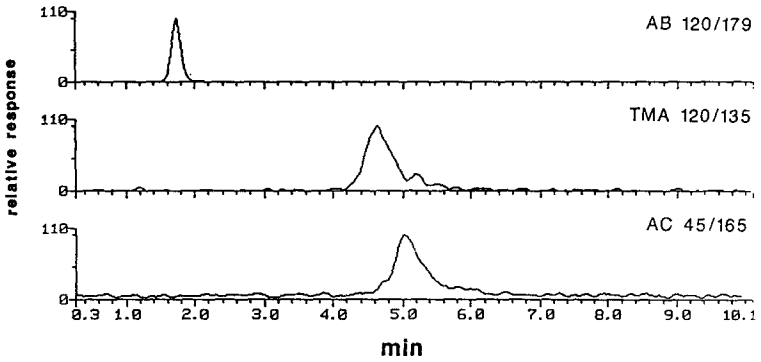


Fig. 8. Selected reaction monitoring of AB, AC and TMA. Analyte amounts, 1 ng each; column, PRP-1,5 μm; eluent, DDW–methanol (80:20) containing 1 mM sodium octanesulphonate and 10 mM ammonium citrate. The daughter/parent pair monitored are indicated in the figure.

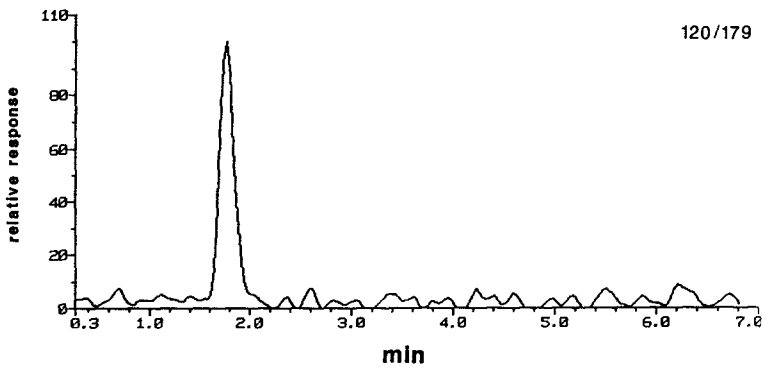


Fig. 9. Selected reaction monitoring of 100 pg AB. See Fig. 8 for conditions.

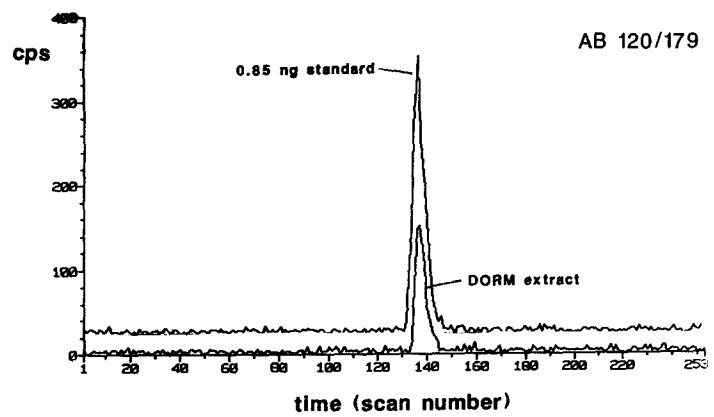


Fig. 10. Determination of AB in DORM. Column, Novapak C_{18}, 4 μm; eluent, DDW–methanol (70:30) containing 10 mM sodium octanesulphonate and 0.5% acetic acid.

groups on silica-based materials. The collision-induced dissociation pathways of the three organoarsenic species are well characterized [7,18]; the principal daughter ion for both protonated AB (*m/z* 179) and TMA (*m/z* 135) is the trimethylarsine ion (*m/z*

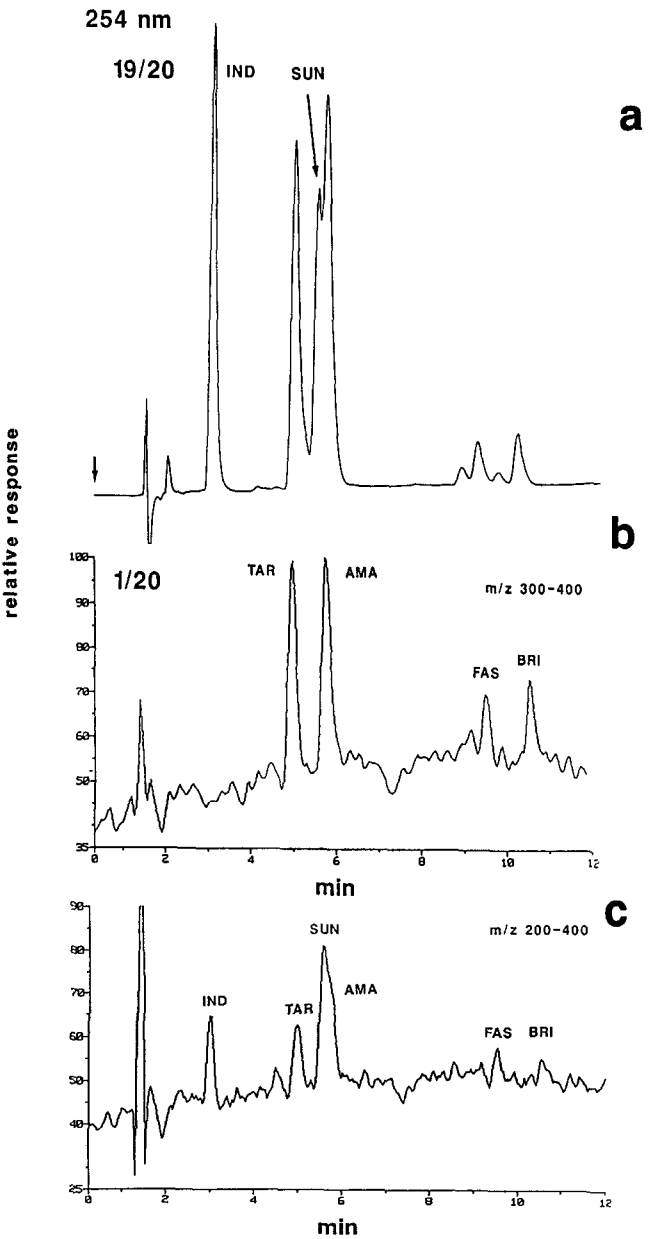


Fig. 11. Ion-pairing separation of food dyes. (a) UV–visible detection at 254 nm; (b) total ion chromatogram in the negative-ion detection mode from *m/z* 300 to 400; (c) total ion chromatogram from *m/z* 200 to 400. The split ratio between the UV–visible detector and the mass spectrometer was 19:1. Column, Novapak C_{18}, 4 μm; eluent, linear gradient from 50:50 to 40:60 DDW–methanol containing 5 m*M* TBAP in 12 min. See Fig. 2 for structures of the dyes.

120), and that for AC is at m/z 45, which is believed to be protonated ethylene oxide.

Fig. 8 shows selected reaction monitoring of 1 ng each of AB, AC and TMA via their parent/principal daughter pairs. Relatively strong interaction with the stationary phase was still apparent for TMA and AC, which were partially resolved. The best sensitivity was observed for AB whose minimum detectable amount (MDA) (defined as two times over noise) was estimated to be about 20 pg (Fig. 9).

This technique has been applied to the determination of AB in a dogfish muscle reference material (DORM-1, National Research Council of Canada) (Fig. 10). A weaker eluent was used to better retain AB. AB was extracted from the tissue by sonicating 0.3 g DORM-1 in 10 ml eluent for 30 min. The measured concentration of 15.7 ± 0.4 μg arsenic per g (four replicate analyses employing both standard calibration and additions) is practically identical to that obtained by LC–ICP-MS [16].

The next separation attempted was the ion-pairing separation of food dyes. The

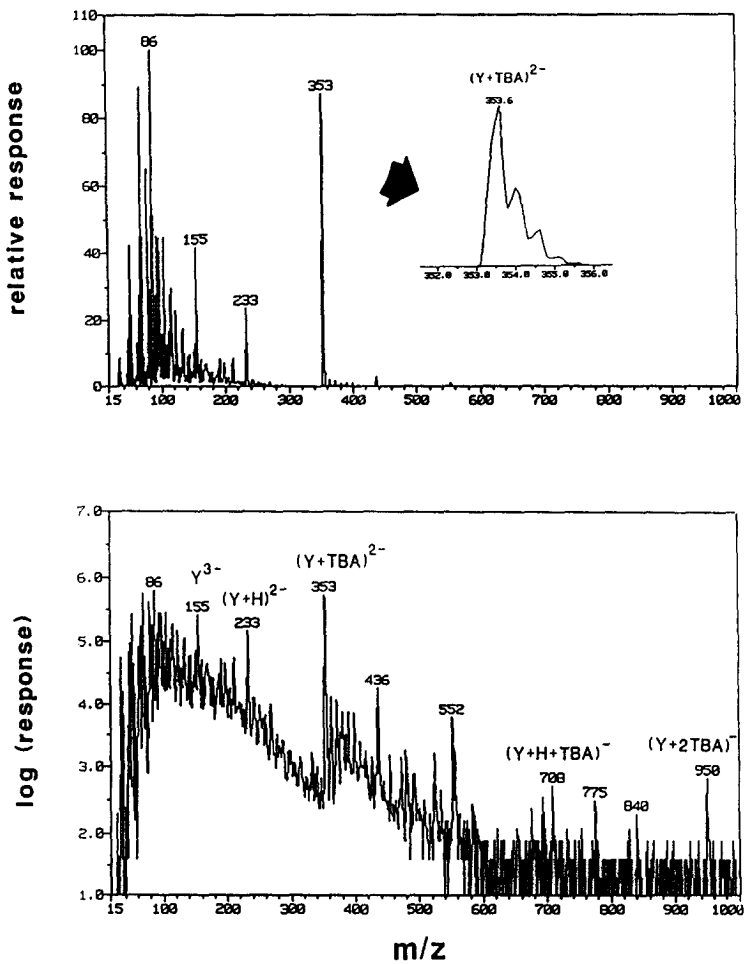


Fig. 12. Full-scan mass spectrum of a 935 μ*M* solution of Tartrazine in DDW–methanol (50:50) containing 5 m*M* TBAP. ES flow-rate, 50 μl/min; continuous infusion. Y stands for the fully ionized Tartrazine.

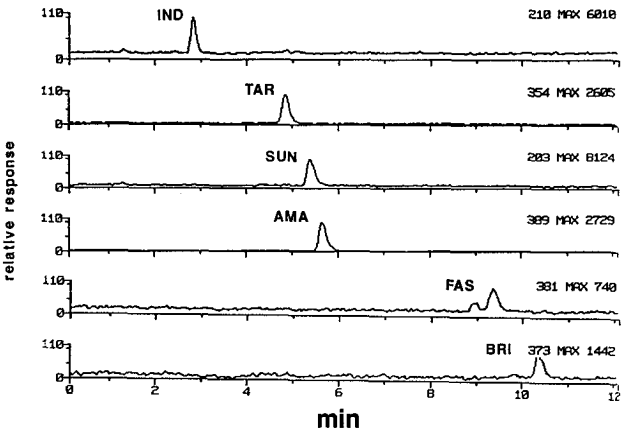


Fig. 13. Selected ion monitoring of the TBA adduct ion of food dyes. Chromatographic conditions are detailed in Fig. 11; analyte amounts, 5 ng each. The *m/z* monitored are shown in the figure.

food dyes contain good chromophores and are traditionally assayed by means of UV–visible detection after LC separation [12]. Fig. 11 shows the simultaneous output of the UV–visible detector and the mass spectrometer on injection of a mixture of the six dyes. The upper MS trace (Fig. 11b) is a total ion chromatogram in the negative-ion detection mode between *m/z* 300 and 400, which shows the presence of four of the six dyes. All six dyes were detected when the scan range was extended to measure from *m/z* 200 to 400 (Fig. 11c). The most prominent food dye ions under the given experimental conditions were the adduct ions of tetrabutylammonium (TBA) with the food dyes, which contain two to three sulphonate and/or carboxylate groups. For Tartrazine, which has three ionizable groups, the principal ion was at *m/z* 353 (Fig. 12) and identified as a doubly charged adduct ion between 1 unit of Tartrazine and 1 unit of TBA. Other Tartrazine-based ions were also present; the highest (triply) charged Tartrazine ion at *m/z* 155 is the principal ion when TBA or other ion-pair reagent is absent. The same trend was observed for the other food dyes. That the *m/z* ion of Tartrazine at *m/z* 353 is doubly charged is self-evident after the *m/z* scale is expanded to reveal resolution of the ^{13}C-containing ions by 0.5 *m/z* units. Fig. 13 shows selected ion monitoring of the TBA adduct ions of 5 ng each of the food dyes. MDAs of the food dyes were in the order of a few hundred of picograms. For Amaranth the MDA was estimated to be about 200 pg, *ca.* a factor of 10 better than that of UV–visible detection.

CONCLUSIONS

ES is a viable and valuable interface for coupling ion-exchange and ion-pairing chromatography to MS. The presence of ion-exchange buffers and ion-pairing reagents do raise the MDAs of analyte ions by suppressing analyte ion signals and/or raising background ion counts. This increase in MDAs is typically five to ten times above those obtained in the absence of matrix ions, which is the price one pays for employing ion-exchange and ion-pairing chromatography. Conboy *et al.* [19] have done impressive work in reducing the electrolyte concentration in their chromato-

graphic effluent by passing it through an ion-exchange fibre (much like Dionex ion chromatography) prior to ES. This may be the approach of choice when maximum sensitivity is desired.

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