Communications

Analysis of Phenols in Water at the ppb Level Using Direct Supercritical Fluid Extraction of Aqueous Samples Combined On-line With Supercritical Fluid Chromatography–Mass Spectrometry

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A high pressure multivalve switching system has provided a means of interfacing a direct aqueous SFE vessel to a packed column SFC-MS system. Using full scan negative ion atmospheric pressure chemical ionization, the combined SFE–SFC–MS instrumentation enabled the analysis of phenols in water at the 40 ppb level.

A recent review article[1] indicates, from the number of applications cited, that SFE continues to represent a growth area in analytical chemistry. Amongst the potential benefits associated with SFE is the reduction in the number of sample handling stages and the possibility of its direct coupling to a wide range of separation techniques,[2,3] of which SFC was first described.[4] Both of these features serve to promote high sample throughput.

At present most of the published reports dealing with SFE involve the study of solid matrices. Strategies for the SFE analysis of liquid based matrices can be divided into two categories:[5] (*i*) indirect procedures which require the loading of the liquid sample onto support media such as solid phase sorbents or solid phase membrane extraction discs prior to SFE, or (*ii*) direct SFE whereby the liquid matrix is directly extracted with a supercritical fluid in the absence of a support medium. Few studies involving the use of liquid SFE–SFC have been reported. Amongst these, indirect aqueous SFE–SFC has enabled the analysis of prostaglandins[6] and mitomycin C[7] whereas direct aqueous SFE–SFC procedures have been described for the analysis of phenols,[8] diisopropyl methylphosphonate[9] and two organic bases.[9]

This report represents the first account of the analysis of organic contaminants in water using a direct aqueous SFE–SFC–MS system. A novel high pressure multivalve arrangement was designed to enable these studies. The SFE vessel used in these studies has a relatively large internal volume. This feature addresses a previously identified[10] hindrance to the development of direct aqueous SFE sample preparation procedures involving trace analysis of organics in water. Large volume samples, typically 100 ml to 1 l need to be extracted to recover sufficient amounts of compounds to enable their detection by established analytical procedures.[10] The direct aqueous SFE–SFC–MS system described within this paper readily facilitated the detection of three phenols each at the low ppb level using full scan negative ion atmospheric pressure chemical ionization (APCI).

Experimental

A stainless-steel vessel (patented) with an internal volume of 300 ml, pressure tested to 43.1 MPa, housed within a Pye 104 gas chromatograph (Unicam, Cambridge, UK) was used for all aqueous supercritical fluid extractions. A Hewlett-Packard G 1205A supercritical fluid chromatograph was used to supply liquid carbon dioxide (BOC, Manchester, UK) to the extraction vessel and perform all SFC–MS studies. A Rheodyne 7010 valve (Supelco, Poole, Dorset, UK) was used to connect or isolate the vessel from a multivalve switching system in a manner previously described.[11] Schematics of the multivalve system configurations (Rheodyne valve model numbers included) used for all SFE-SFC-MS studies are shown in Figs. 1(*a*) and (*b*). The tee pieces were Valco ZTIC (Phase Separations, Deeside, Clwyd, UK), all filter assemblies were Valco ZUFR1 with Valco TSS110 stainless-steel tubing providing all connections. The multivalve system incorporated two Apex amino RP 5 μ 150 × 4.6 mm columns (Jones Chromatography, Hengoed, Mid Glamorgan, UK). The primary function of the first column was to serve as an analyte trap. Both columns were housed within the supercritical fluid chromatograph oven. Prior to SFE, both columns connected in series *via* the SFC–MS valve settings, Fig. 1(*b*), were dried *in situ* at 60 °C using carbon dioxide at a rate of 1.5 ml min^{-1} at a pressure of 30.89 MPa. At the end of the drying cycle the valves were switched to the SFE configuration, Fig. 1(*a*), such that the second amino column, now primed with carbon dioxide, became isolated.

The supercritical fluid chromatograph was interfaced to a Finnigan MAT (San Jose, CA, USA) TSQ 700 series triple quadrupole mass spectrometer. The APCI interface vaporiser heater was 500 °C with the heated capillary at 250 °C using a corona current of 5 μA. The APCI interface was modified[12] to facilitate SFC–MS using a 1/16” od stainless-steel tube of 0.005” id acting as a final restriction stage. The column eluant was split such that 25% was supplied to the APCI interface.

An aqueous solution of 2,4-dibromophenol, 2,4,5-trichlorophenol and 4-nitrophenol was prepared, pH adjusted to 4.2 *via* the addition of hydrochloric acid, such that each phenol was present at the 40 ppb level. A 250 ml aliquot of this solution, equivalent to 10 μg of each phenol, was transferred into the SFE vessel. Using the multivalve configuration shown in Fig. 1(*a*) direct aqueous SFE was performed at 65 °C at a pressure of 32.33 MPa with a flow rate of liquid carbon dioxide at 4 ml min^{-1} for a period of 45 min. During SFE the temperature of the oven housing the amino columns was maintained at 30 °C. At the end of the extraction period the mutivalve system was switched to enable SFC–MS operation.

All SFC and SFC-MS studies were conducted at 30.89 MPa with high purity grade methanol (Fisons, Loughborough, Leicestershire, UK) serving as organic modifier. The mobile phase flow rate was 1.5 ml min^{-1} and the steps of the organic modifier programme are given in Table 1.

The temperature programme used for SFC and SFC–MS studies was 1 min at 60 °C then to 70 °C at 10 °C min min^{-1} held for 1 min, then to 90 °C at a rate of 20 °C min^{-1} with this final temperature held until analyses were completed. The supercritical fluid chromatograph UV detector was set at 220 nm for off-line SFC and SFE–SFC studies.

Off-line direct valve loop APCI analyses, interface parameters as previously described, of each phenol were conducted at 30.89 MPa using carbon dioxide with 25% v/v methanol at 1.5 ml min^{-1}.

All GC–MS studies were performed in selected ion monitoring mode using a Hewlett-Packard 5971A mass-selective detector, operated using electron ionization, interfaced to a HP 5890 gas chromatograph equipped with a HP 6890 series autosampler. Three calibration standards containing each phenol, whose respective concentrations ranged from 0.2 to 1 ng μl^{-1}, were prepared with ethyl acetate as solvent. Each solution also contained anthracene, acting as internal standard, at the 0.4 ng μl^{-1} level. All GC–MS analyses were performed using a HP-5MS 30 m × 0.2 mm column of 0.25 μm film thickness with helium (MG Gas Products, Reigate, Surrey, UK) serving as carrier gas. The gas chromatograph temperature programme was 2 min at 60 °C then to 290 °C at 15 °C min^{-1}, with the final temperature held for 15 min. Splitless 5 μl injections were made at 250 °C. The quotient of responses obtained for the molecular ions of: 2,4-dibromophenol (M_r = 252), 2,4,5-trichlorophenol (M_r = 196) and 4-nitrophenol (M_r = 139) with that for anthracene (M_r = 178) were used for calibration and determination of individual phenol SFE recoveries.

Table 1 Organic modifier programme

Level	Ramp rate (% methanol)	% Methanol	Hold time/ min
0		0	2
1	1.0	1.0	1.0
2	2.0	10	2.0
3	10.0	30	40.0

Results and Discussion

In order to develop a combined dynamic aqueous SFE–SFC–MS system a high pressure multivalve switching arrangement incorporating an amino column was designed. During dynamic aqueous SFE the restrictor module was situated pre-column by selection of the appropriate valve settings as shown in Fig. 1(*a*). This arrangement resulted in the trapping of extracted phenols onto the column following decompression of supercritical fluid carbon dioxide. At the end of the extraction period, with the aqueous SFE vessel isolated,[11] the multivalve configuration was switched, Fig. 1(*b*), so that the restrictor module became situated post-column to enable SFC–MS analysis of trapped phenols.

Initial studies were conducted to investigate whether a single amino column, acting as an analyte trap, could also provide an efficient means of separating the phenols in the following SFC stage. These investigations indicated significant degradation of chromatographic performance when the results of direct SFC were compared to those obtained for off-line SFE–SFC. This is attributed to the creation of an imprecise trapping zone at some point along the column whose own back pressure serves to provide an *in situ* restriction. Due to this factor the effective column length available for SFC following the trapping stage of SFE was reduced, adversely affecting column separation efficiency. In order to circumvent this problem a second

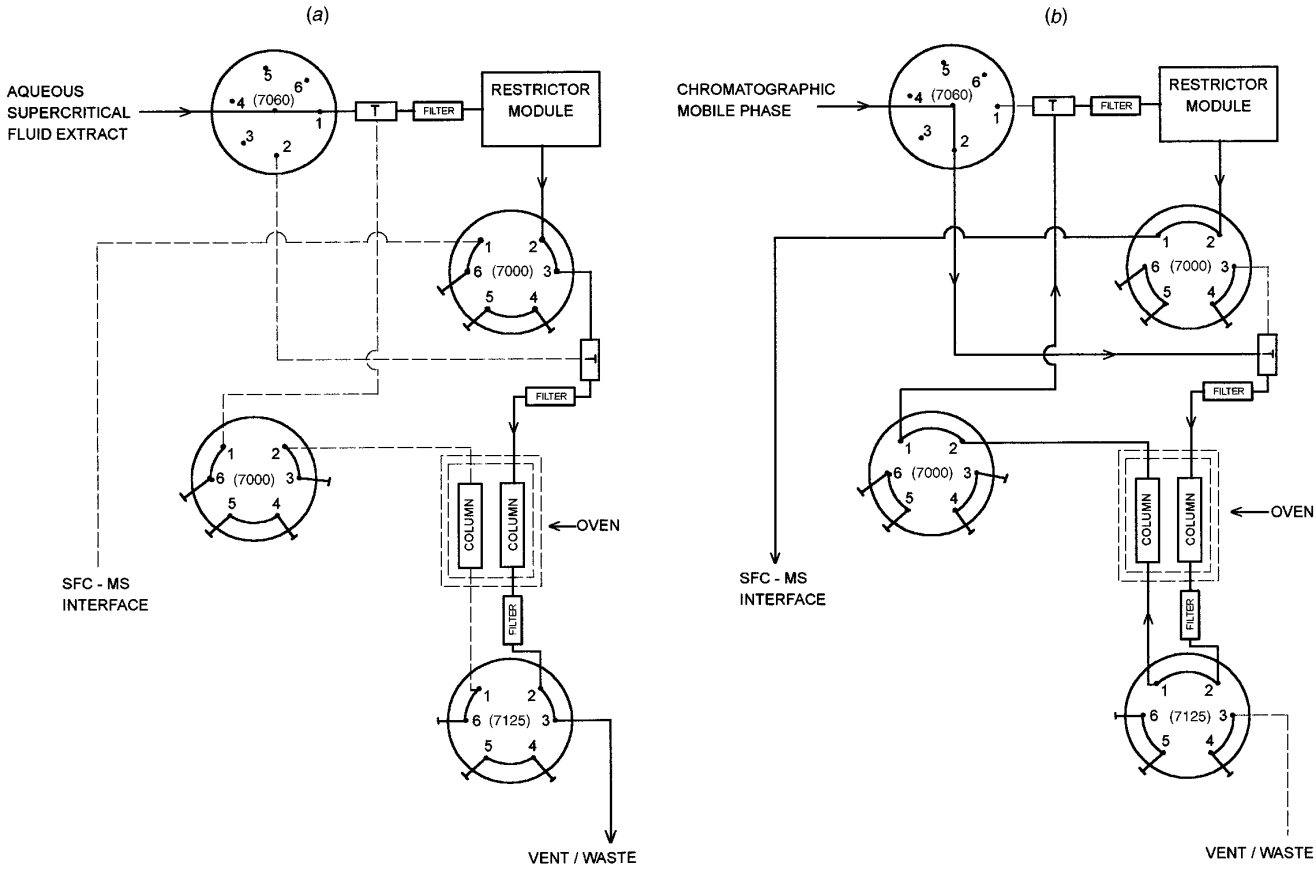

Fig. 1 Configurations of high pressure multivalve system used for (*a*) direct aqueous SFE and (*b*) SFC–MS.

dedicated chromatographic amino column was incorporated into the multivalve system as shown in Figs. 1(*a*) and 1(*b*). During SFE this column, which had been previously conditioned, was isolated by the appropriate valve settings from the trapping column. This arrangement using a second amino column resulted in retention of chromatographic integrity.

Prior to any SFE–SFC–MS studies, each of the three individual phenols were subjected to direct APCI analysis in positive and negative ion modes *via* direct valve loop injections, equivalent to 500 ng of each phenol, without any intervening chromatography. All three phenols provided an intense response in negative ion APCI mode with each mass spectrum being dominated by the presence of an $[M - H]^-$ ion. Since none of the three phenols provided a good response using positive ion APCI, with the previously specified conditions, all further SFE–SFC–MS studies were conducted in negative ion mode.

In order to establish the feasibility of dynamic aqueous SFE–SFC– MS for the analysis of phenols in water, the vessel was loaded with 250 ml of water spiked with each phenol at the 40 ppb level. The water had been previously acidified, pH adjusted to 4.2 with hydrochloric acid. The results of a previous direct aqueous SFE study[12] involving a range of phenols (including three chlorinated species) indicated that lowering of pH suppressed phenol dissociation which served to promote SFE recovery. After 45 min direct aqueous SFE the vessel was isolated and the valves were switched to allow SFC–MS analysis.

The SFE–SFC–MS negative ion APCI total ion chromatogram obtained for the analysis of the three phenols at the 40 ppb level is shown in Fig. 2(*a*). Despite all the phenols' full scan response providing poor S/N, each could be readily detected by inspection of the respective $[M - H]^-$ ion profiles, Figs 2(*b*)–(*d*). Acidification of the mobile phase may help reduce peak tailing which was also observed in off-line SFC studies of the three phenols. The full scan SFE–SFC–MS negative ion APCI mass spectrum obtained for 2,4,5-trichlorophenol is shown in Fig. 3. No attempt was made to determine the full scan negative ion APCI detection limit of each phenol in these preliminary SFE–SFC–MS studies. The intensity of the mass spectrum obtained for each phenol suggests that determining the presence of these compounds at levels lower than 40 ppb should be readily achievable in full scan mode.

In order to determine individual phenol extraction efficiencies, the vessel was emptied and flushed with 100 ml of deionised water. The pH of the combined aqueous fractions was adjusted to 2 *via* the addition of concentrated hydrochloric acid and then extracted with two 100 ml aliquots of ethyl acetate. The combined ethyl acetate fractions were then blown almost completely to dryness under a stream of nitrogen before being transferred to a 5 ml calibrated flask. The contents of the flask were then made up to 5 ml using ethyl acetate and an ethyl acetate solution of anthracene. The final concentration of anthracene acting as internal standard was equivalent to 0.4 ng μl^{-1}. Results of quantitative GC–MS indicated the following recoveries: 2,4-dibromophenol 88%, 2,4,5-trichlorophenol 81% and 4-nitrophenol 67%. These extraction efficiencies correlate with the phenol SFC retention times, both parameters relating to polarity. It should be possible to improve the SFE recoveries for each phenol by using a longer extraction period or by using a pump capable of delivering a flow of liquid carbon dioxide greater than 4 ml min^{-1} to the vessel.

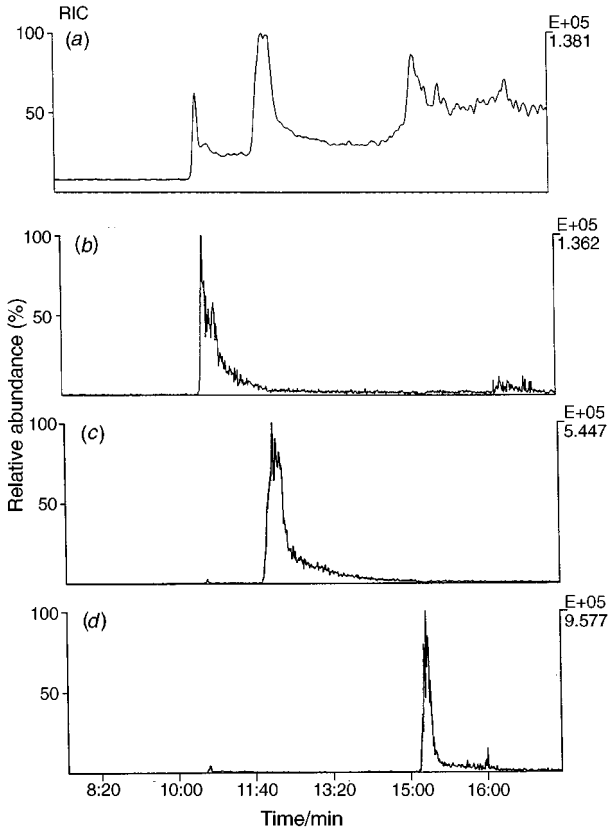

Fig. 2 (*a*) Total ion SFE–SFC–MS chromatogram obtained for a 250 ml water sample, spiked with three phenols each at the 40 ppb level. Ion profile responses obtained for the $[M - H]^-$ species of individual phenols; (*b*) *m/z* 251 for 2,4-dibromophenol (*c*) *m/z* 195 for 2,4,5-trichlorophenol; and (*d*) *m/z* 138 for 4-nitrophenol.

Conclusion

This study has established the feasibility of direct aqueous SFE–SFC–MS. The relatively large sample capacity of the vessel helped facilitate the detection of three phenols each at the 40 ppb level. The SFE–SFC–MS full scan negative ion APCI mass spectrum obtained for each phenol was characterized by the

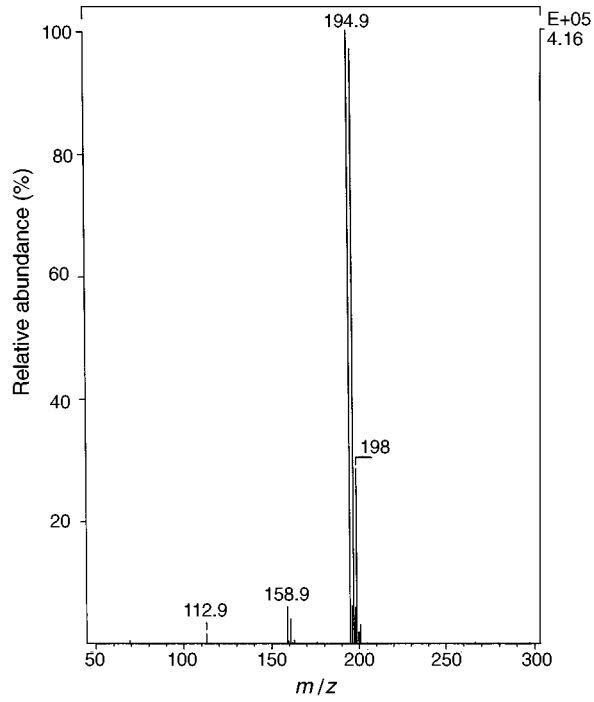

Fig. 3 SFE–SFC–MS negative ion APCI mass spectrum obtained for 2,4,5-trichlorophenol (M_r = 196).

production of an intense $[M - H]^-$ species. By resorting to selected ion monitoring negative ion APCI, it is anticipated that ppt or lower detection limits should be possible for each phenol using the current SFE–SFC–MS system.

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