Short Communication

Supercritical fluid extraction and chromatography of aromatic amines

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Abstract

Supercritical fluid extraction (SFE) was used for the isolation of antioxidant aromatic amines from tyre rubber. The efficiency of SFE was comparable with that of Soxhlet microextraction. Aromatic amines were also extracted from different matrices (inert silanized material, sand and humic soil) with supercritical carbon dioxide. It was found that the extraction efficiency depends on the nature of the matrix. Whereas the recovery of amines from the inert material approached 100%, that from acidic matrices was poor and some amines were not recovered.

1. Introduction

Supercritical fluid extraction (SFE) has shown much promise for the isolation of organic compounds from various samples [1]. The use of SFE in the context of environmental analysis and some illustrative applications have recently been reviewed [2]. It has also been shown that not only is SFE suitable for the isolation of organic compounds from a given matrix but that it can also be utilized for clean-up [3,4] or fractionation [5] of complex organic mixtures.

In environmental analysis, attention is mostly focused on priority pollutants and related (usually chlorinated) compounds. Hence, little is known about the SFE of amines from various matrices as they are not common environmental pollutants, generally being found only near production sites or in areas of intense use.

Good solubility of the analytes in the supercritical fluid is the basic prerequisite of successful SFE; the partitioning step during the SFE is mainly controlled by the solubility of the analyte in the extraction fluid, kinetic limitations and the ability of the extraction fluid to interrupt matrix-analyte interactions [6]. However, the sources of solubility data for amines in supercritical fluids are limited; diphenylamine is less soluble [7] in pure supercritical carbon dioxide than the aromatic hydrocarbon of comparable molecular mass, naphthalene. It has also been shown [8] that some amines are not extractable by supercritical carbon dioxide. The highly basic primary and secondary aliphatic amines probably react with carbon dioxide under SFE conditions to form either insoluble products (carbamates) not extractable by supercritical carbon dioxide, or reaction products not measurable by gas chroma-

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tography used for the final analysis [8]. Better SFE results were obtained by using nitrous oxide as the supercritical fluid [8]. In this instance all the compounds tested (heptyl-, tributyl-, dodecyl-, octadecyl-, diphenyl- and N-methyl-heptylamine and 2-ethylaniline) were found in the SFE extract. However, aromatic and tertiary amines (2-ethylaniline, tributylamine and diphenylamine) were also extractable by carbon dioxide.

It has also been reported that the use of nitrous oxide modified with an amine significantly improved the SFE of aromatic amines from soil [9], but nitrous oxide and the addition of a small amount of 5% 1,6-hexanediamine in methanol solution directly to the extraction chamber provided the best recoveries of aromatic amines [10]. However, the recovery of some aromatic amines was still poor.

Supercritical fluid chromatography (SFC) has been employed in analyses for aromatic amines: the amines can be separated using supercritical CO_2 on propylcyano- or propylamino-bonded phases with flame ionization (FID), ultraviolet or Fourier transform infrared detection [11,12], and/or using supercritical N_2O using both packed and capillary columns [8]. It has been shown that the SFC separation has a much higher degree of resolution and that SFC method development is easier than for HPLC procedures [12]. SFC with diode-array detection [13] and the use of ammonia as a mobile phase for SFC of amines [14] have also been investigated.

The aim of this work was to investigate the extractability of some relevant amines: diphenylamine (DFA), p-isopropyldiphenylamine (4-iso-DFA), N-phenyl-N'-isopropyl-p-phenylenediamine (AOCD) and N-phenyl-N'-2-methyl-4-pentyl-p-phenylenediamine (AO13). These amines are used either as rubber antioxidants or the intermediate products in their industrial synthesis. Carbon dioxide was used as the supercritical fluid because some matrices tested contained a high content of oxidizable organic matter. The use of nitrous oxide can result in explosions [15].

2. Experimental

SFE was performed in the off-line mode in an apparatus consisting of a piston pump (HPP 5001, Laboratorní Přístroje, Prague, Czech Republic) for liquid carbon dioxide connected to a stainless-steel extraction chamber (volume 0.6 ml; 2.5 cm × 4 mm I.D.). The extraction chamber was placed in a heated jacket. The pressure of the supercritical carbon dioxide in the extraction chamber was maintained by means of a 30 × 25 μm I.D. fused-silica restrictor. The time of SFE was 30 min, pressure of carbon dioxide 20 MPa and temperature of the extraction chamber 50°C, if not stated otherwise. The outlet of the restrictor was immersed in a small amount of a suitable organic solvent (usually 1 ml of isopropyl alcohol) to trap the extracted analytes. After SFE, the solvent was analysed by GC and/or SFC–FID. The decrease in the solvent volume during the SFE was corrected for by addition of an internal standard (tetrahydronaphthalene). The same amount of internal standard was added to the reference "100% recovery" solution used for calculation of the SFE recovery.

For the GC analyses, an HP 5890 II gas chromatograph (Hewlett-Packard) was used. The instrument was equipped with a flame ionization detector, split–splitless injector and wide-bore fused-silica capillary column (25 m × 0.52 mm I.D., coated with RSL-150, $d_f = 1.2$ μm; Alltech). Nitrogen was used as the carrier gas. The column temperature was programmed from 70 to 300°C at 10°C/min.

Although the aromatic amines studied can be successfully analysed by capillary gas chromatography, their separation requires relatively high temperatures. Therefore, the possibility of their SFC analysis was also tested. A Dionex, Lee Scientific 600D supercritical fluid chromatograph equipped with a flame ionization detector, timed split injection valve and a 10 m × 50 μm I.D. fused-silica capillary column (SB-Biphenyl-30, $d_f = 0.25$ μm; Dionex UK) with multi-channel restrictor [16,17] was used. Carbon dioxide was used as the mobile phase. The chromatographic

conditions for the SFC separation were as follows: oven temperature, isothermal at 150°C; detector temperature, 350°C; CO_2 density programme, 0.25 g/ml for 5 min, then a linear ramp at 0.04 g/ml per min to 9 min and a linear ramp at 0.01 g/ml per min to 18 min. An example of the SFC separation is shown in Fig. 1.

Recoveries of SFE were calculated by comparison of peak areas obtained from GC (SFC)–FID analysis of the solution obtained after the SFE and the "100% recovery" reference solution.

Recovery experiments were performed using three different spiked matrices: a silanized inert support for gas chromatographic packed columns (Gas Chrom Q, 100–120 mesh; Alltech), fine silica sand (grain size 0.4 mm), and humic topsoil (0.315–0.400 mm). All matrices were first dried at 105°C before being fortified with the amines immediately before the extraction, so that these samples can be considered as fresh spikes. The spiking was accomplished by placing the matrix in a 50-ml beaker, introducing the spike solution from a syringe and allowing the solvent (isopropyl alcohol) to evaporate prior to transferring the matrix to the extraction chamber. Evaporation of the solvent was performed in a closed vessel under a slow stream of nitrogen to prevent oxidation of the amines by atmospheric oxygen. In all instances, the extraction chamber was full of the material to be extracted. Although we were aware that spike recovery studies may not be valid for the conditions necessary for the native samples [18], spiked samples were used in this SFE study because native samples with certified concentrations of analytes were not available. The recoveries from the freshly spiked samples can serve as a guide to the potential usefulness of SFE.

As AO13 and AOCD are used as rubber antioxidants (e.g., in tyres), an experiment with SFE of this matrix was also carried out. Before extraction, the rubber from a 3-year-old tyre was disintegrated into small particles by means of grinding wheel. The rubber initially contained only AOCD. A 100-mg amount of the rubber was extracted with supercritical carbon dioxide. The SFE effixciency for the isolation of AOCD from rubber was compared with that of micro-Soxhlet extraction in which 0.2110 g of rubber was extracted with 20 ml of benzene for 8 h.

3. Results and discussion

In order to find whether supercritical CO_2 extracts and does not react with the aromatic amines, the inert matrix (Gas Chrom Q silanized support) was spiked with 0.02 mg of each amine and subjected to SFE under the conditions given in Table 1. The recovery of amines in this instance approached 100% (Table 1). These experiments showed that the aromatic amines were sufficiently soluble in supercritical carbon
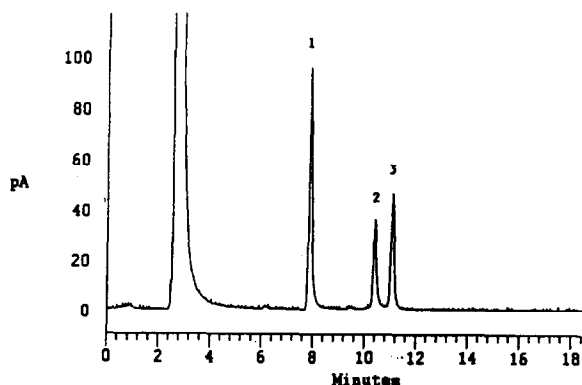

Fig. 1. SFC separation of amines. For conditions, see text. Peaks: 1 = DFA; 2 = AOCD; 3 = AO13.

Table 1
Recoveries of aromatic amines from Gas Chrom Q

Compound	Recovery (%)	R.S.D. (%)
DFA	96	3.5
4-isoDFA	95	4.5
AOCD	97	4.5
AO13	96	3.7

Pressure of carbon dioxide, 20 MPa; temperature of the extraction chamber, 50°C (density of carbon dioxide, 0.8 g/ml); time of SFE, 30 min; spiking level, 0.02 mg of each amine in extraction chamber packing; averages of triplicate determinations.
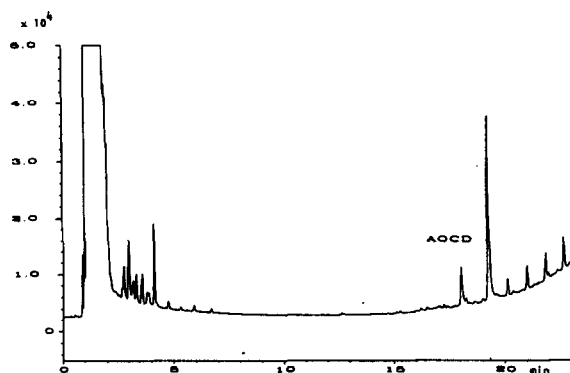
Fig. 2. Chromatogram from SFE and GC of rubber. For conditions, see text.

dioxide, that solvent trapping of the amines after the SFE at the outlet of the restrictor was efficient and that no losses of the analytes occurred in this step.

In further experiments, the content of antioxidant in a 3-year-old tyre rubber was determined by off-line SFE and GC and by Soxhlet extraction and GC. A chromatogram of the isopropyl alcohol solution after the SFE is shown in Fig. 2. Both methods provided essentially the same result: the concentration of AOCD in rubber was 0.23 mg/g according to the results from the Soxhlet extraction and 0.21 mg/g from the SFE (both values are averages from triplicate determinations). Co-extracted compounds shown

in Fig. 2 were not identified or determined in this work.

The recoveries of the amines from sand are given in Table 2. The recovery of AO13 and particularly AOCD decreased with decreasing concentration in the sand matrix. Even if the recoveries of different amines were not complete, the reproducibility was good. It is apparent that active acidic sites on the sand surface are responsible for the lower recovery of amines. To interrupt or decrease matrix interaction, various SFE conditions were tested. It was found that pressures of CO_2 higher than 20 MPa, temperatures of the extraction chamber higher than 50°C (up to 35 MPa and 95°C) and extraction times longer than 30 min had no effect on the recovery of aromatic amines from the sand. Addition of 5% of methanol as a polarity modifier to the supercritical fluid did not improve the recovery of aromatic amines in those instances where the SFE with pure CO_2 gave poor recoveries.

The matrix interactions were found to be even stronger when humic soil was extracted (Table 3). Very poor or negligible recoveries of aromatic amines from soil were also obtained by Oostdyk et al. [10] when only a pure or methanol-modified supercritical fluid was used for SFE. Only modification by addition of highly basic 1,6-hexanediamine improved the recoveries satisfactorily. Unfortunately, highly basic primary amines cannot be used as carbon dioxide polarity modifiers because of carbamate formation [8].

Table 2
Recoveries (%) of aromatic amines from sand

Compound	Amount of amine in sand (mg)			
	1[a]	0.1[b]	0.08[a]	0.06[a]
DFA	98 (2.6)	99 (2.5)	95 (3.1)	95 (3.5)
4-isoDFA	94 (5.1)	99 (3.6)	90 (4.2)	101 (6.1)
AOCD	84 (3.8)	69 (4.8)	50 (6.2)	48 (7.1)
AO13	86 (6.6)	86 (4.6)	75 (5.3)	65 (6.8)

Amount of the sand in extraction chamber, 0.94 g; pressure of carbon dioxide, 20 MPa; temperature of extraction chamber, 50°C (density of carbon dioxide, 0.8 g/ml); time of SFE, 30 min.
[a] Averages of triplicate determinations with relative standard deviations (%) in parentheses.
[b] Averages from five determinations and relative standard deviations (%) in parentheses.

Table 3
Recoveries (%) of aromatic amines from soil

Compound	Amount of amine in soil (mg)	
	1.0	0.06
DFA	86 (5.8)	80 (4.5)
4-isoDFA	83 (6.1)	81 (7.8)
AOCD	3 (25.1)	0
AO13	8 (20.6)	0

Amount of the soil in extraction chamber, 0.53 g; pressure of carbon dioxide 20 MPa; temperature of the extraction chamber, 50°C (density of carbon dioxide, 0.8 g/ml); time of SFE, 30 min; averages of triplicate determinations with relative standard deviations (%) in parentheses.

4. Conclusions

It has been shown that aromatic amines are extractable with pure supercritical carbon dioxide. The recovery depends on the matrix type; the best recoveries were obtained for an inert matrix and the more acidic the matrix, the lower were the recoveries. The extraction efficiencies of SFE and Soxhlet extraction for the antioxidant AOCD from tyre rubber were comparable.

5. Acknowledgement

6. References

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