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Capillary extractors for ''negligible depletion'' sampling of benzene, toluene, ethylbenzene and xylenes by in-tube solid-phase microextraction

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Abstract

The suitability of ''capillary extractors'' is demonstrated for the ''negligible depletion'' extraction of benzene, toluene, ethylbenzene and xylenes in a clean-water matrix. Extraction set-up and major extractor parameters (length, internal diameter, and film thickness) are chosen to allow rugged analysis by GC with flame ionization detection. With the selected negligible extraction conditions, the efficiency for every consecutive extraction is about 2–3% of the dissolved amount.

Keywords: Sample preparation; Negligible depletion sampling; Benzene; Toluene; Ethylbenzene; Xylenes

1. Introduction

Free concentration of target compounds dissolved in water samples is usually recognized as a more meaningful parameter than their total concentration [1] owing to the existence of several interdependent equilibria among the various components of the matrix. If the free analyte concentration is not appreciably influenced by an extraction process, we may speak about a ''negligible depletion'' (ND) extraction.

Although solid-phase microextraction (SPME) [1] is suitable to perform ND extractions [2], it presents some remarkable problems: one drawback is the presence of a headspace in the extraction vial; another may be the rather long time (sometimes several hours) which might be required to reach extraction equilibrium. Out-of-equilibrium extractions are more critical for the user because they require precise timing. As a whole, however, SPME has been well accepted by analytical chemists, because it is a sensitive solvent-less technique with good commercial support.

Other small-scale extracting–preconcentrating techniques have appeared which use similar principles {e.g. stir-bar sorptive extraction (SBSE), capillary extraction [3–7]}, founded on the partition law. If we define: K_d, partition coefficient; C_f, concentration of analyte in the extracting phase, at equilibrium; C_w, analyte concentration in the aqueous phase, at equilibrium; n_f, mol extracted at equilibrium into extracting phase; C_o, initial concentration into aqueous phase; V_f, volume of polymeric phase (extractant), V_w, volume of aqueous phase, the partition law in an extraction system, in the absence of headspace, may be written as follows [8,9]:

$$n_f = (K_d V_f C_o)/(1 + K_d V_f/V_w) \quad (1)$$

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Supposing $K_d V_f / V_w \gg 1$, then $n_f = V_w C_o$, i.e. target analyte is ''totally'' depleted from the aqueous sample. In contrast, $n_f = K_d V_f C_o$ whenever:

$$K_d V_f / V_w \ll 1 \quad (2)$$

A ND extraction is the realm of Eq. (2); in particular, it is assumed by common consent that an extraction is considered a ND one if extraction efficiency is $\leq 5\%$ [10].

''Capillary extraction'' is complementary to both SPME and SBSE concerning V_f, extraction kinetics, and mildness of GC injection–desorption [4–7]. The technique uses a glass capillary with embedded press-fit ends, internally coated with immobilized silicone stationary phase (''capillary extractors'').

The ND extraction of aromatics of major importance from an environmental point of view [11] is investigated here, at least in a clean-water matrix, to verify the suitability of the capillary extraction approach.

2. Materials and methods

2.1. Capillary extractor preparation

Glass, thanks to its transparency and cheapness, was the preferred support material to make capillary extractors; however, fused silica might replace glass supports if the polyimide cladding is clear enough. Preparation of glass capillary extractors consisted of the following steps: (1) preparing glass high-resolution (HR) GC columns [12] of suitable film thickness and internal diameter, (2) cutting them into pieces of suitable length (see below), and (3) giving rise to press-fits into their ends [13].

2.1.1. Drawing of raw glass capillaries and their coating

Coiled borosilicate glass capillaries were drawn from 8-mm O.D. Duran glass rods by means of a laboratory-made glass-drawing machine. The drawing-coiling process gave capillaries of outer and inner diameters equal to 0.1 times those of the original Duran rods, and produced turns of 0.50 m/coil. Among others, capillaries of 0.21 mm I.D. were prepared. They were leached with 20% HCl, rinsed with 0.5% HCl, dehydrated under a vacuum at 250 °C, persilylated overnight at temperatures near 400 °C with hexamethyldisilazane mixed 1:1 (v/v) with diphenyltetramethyldisilazane (2% of divinyltetramethyldisilazane was added to enhance stationary phase binding on the support), then they were statically coated with PS255, a Petrarch Systems polydimethylsiloxane gum with ~1% vinyl groups, purchased from Fluka (Milan, Italy). The coating solution (0.092%, w/v, of PS255 dissolved in pentane–dichloromethane (1:1, v/v), plus addition of 0.3% (w/w) of dicumylperoxide with respect to the PS255 phase) was evaporated under vacuum according to classic static coating [12]. The resulting film thickness was 48 nm (phase ratio β of 1087).

Freshly-coated capillaries were filled with helium, ends were closed by fusion, then the coated polymer was immobilized by heating from 160 to 200 °C at a rate of 0.3 °C/min. Coated and crosslinked capillaries were conditioned under a helium flow by heating from 60 to 320 °C (rate 10 °C/min) for not less than 40 min. Conditioning profiles (bleed curves) were recorded as proof of sound static coatings. Detailed recipes for the above treatments are described in Ref. [12].

2.1.2. Press-fit realization

Although *glass* press-fits [13] are usually embedded in glass capillary extractors, in this study the extractors were provided with PTFE press-fit ends, prepared in the laboratory from tracts of PTFE tube (0.86 mm O.D.×0.4 mm I.D.) made shrinkable by the method described by Grob [14]. In practice shrinkable PTFE unions ~2 cm long were shrunk for a length of about 6 mm on the fire-polished ends of short pieces of apolar GC column. The PTFE press-fit ends allowed the easy leak-proof connection with 0.32 mm I.D. fused silica (FS) capillaries, which are the ''transfer-lines'' of the cryofocusing injector device [15].

2.1.3. Measure of extractant amount

The amount of extracting phase inside any extractor was measured by filling it with pure water and measuring the mass increase (± 0.1 mg), which is related to the volume V_g of coating solution evaporated during static coating.

V_g is linked to the known phase ratio β and to the

coated phase volume V_{PDMS} by the following equation:

$$V_{PDMS} = V_g/\beta$$

2.2. Benzene, toluene, ethylbenzene and xylenes (BTEX) sample preparation

2.2.1. Pure BTEX mix

Benzene (Rudi Pont, purity >99.5%), toluene (redistilled, high purity, single peak by GC analysis), ethylbenzene (Fluka, >99%), *ortho*-, *meta*-, and *para*-xylenes (Aldrich, purities >99%) were used as primary substances. A BTEX stock standard mixture (six compounds) was prepared by mixing 2.00 ml of each solvent, and the mixture was stored at room temperature in a screw-closed glass bottle.

2.2.2. Aqueous BTEX standards

A BTEX working standard solution was prepared by adding 1 μl of the BTEX stock solution (Section 2.2.1) to 1.14 l of Milli-Q water with moderate magnetic stirring. The final concentration of each aromatic compound was 146 ppb.

2.3. In-tube SPME sampling

Capillary extractors were charged with aqueous BTEX sample (Section 2.2.2) by a fast-squeezed extraction [6,7] using a couple [16] of 5-ml Valco glass syringes equipped with PTFE-tipped plungers. During extraction, syringe needles were connected to any extractor through its PTFE press-fits. The BTEX sample (5.00 ml) was delivered by one of the two syringes, while the other acted as a ''slave'' collecting the sample at the extractor exit, and storing it for the next ND extraction. This way, all extractions were carried out on the same aliquot of sample, which could be re-extracted tens of times. Extractions required about half a minute for the 5.7- and 13.5-cm extractors. More time was needed for the extractor of 170 cm length, because of the increased hydrodynamic resistance during sampling. Operations were carried out at ambient temperature (20–23 °C).

2.4. GC injection and BTEX elution

After sampling, the BTEX-charged extractor (Section 2.3) was emptied from sample excess by detaching one of the sampling syringes from the extractor and slowly sucking out the liquid (meniscus moving at 2–4 cm/s) within the other syringe. The suction was *timely* stopped; this avoided breakthrough of volatiles. The extractor was then mounted at once (press-fit ends) as the GC precolumn on the cryofocusing device [6,15], and the extracted compounds analysed by HRGC after a desorption time of 30 s. Liquid nitrogen was used to focus analytes inside a deactivated 0.32 FS movable capillary, while a temperature of 200 °C was maintained to reinject them. Overall suitability of GC injection by capillary extraction was tested; stable baseline, good peak shape and symmetry, and absence of carryover were proved.

2.5. GC instrumentation

GC analyses were carried out with a Perkin-Elmer 8500 gas chromatograph equipped with a flame ionization detection (FID) system set at 200 °C and a laboratory-made cryofocusing-injector device [15]. The Duran glass separation column (3 m×0.16 mm I.D., 0.5 μm PS255) was laboratory-made [12] following the general outline of Section 2.1.1. The carrier was helium at 10 p.s.i.g., with pressure regulation (1 p.s.i.=6894.76 Pa). The oven isothermal temperature was 40 °C. Borwin software from Jasco (Italy) at a sampling frequency of 25 Hz was employed to acquire raw GC data and to store chromatograms.

3. Results and discussion

Often techniques of preconcentration and analysis are complementary, so the user should know their pros and cons to make the best choice. For example, coating thickness selection is rather limited for SPME fibers, whereas capillary extractors can be prepared with a wide choice of length, internal diameter and phase ratio [4–7,15,16]. Hundreds of capillary extractors of average length might be prepared by trimming a single HRGC capillary

Table 1
Minimal sample volume required, by calculation, for various SPME fibers (PDMS) to extract a hypothetical analyte with a K_d value of 1000 according to negligible depletion criteria

Fiber coating thickness (μm)	Total volume[a] (μl)	Extractant volume[a] (V_{PDMS}; nl)	Minimal sample amount for ND extraction (ml)
100	0.707	612	12.2
30	0.227	132	2.6
7	0.121	27	0.53

[a] Data from Ref. [17].

column, so capillary extraction is also a remarkably cheap technique, which performs efficiently with volatile organic compounds [7,16] as well as semivolatiles using temperature programmed GC [4,6]. Because the film thickness of the extracting phase coated inside capillary extractors is much thinner than that of any commercial SPME fiber coatings, equilibrium capillary extraction is the rule, being reached in a few seconds: the capillary extractors used here had a film thickness of only 48 nm, i.e. 2084 times less than the 100 μm SPME fibers. For analytes which are not extremely hydrophobic, capillary extractors of general use require just 1 ml of aqueous sample, delivered in-tube, to ''equilibrate'' the extraction device. This is due to the very small amount of stationary phase, usually 10^{-1}–10^{-3} of that used by standard SPME fibers [6,7]. Moreover, with classic SPME, or SBSE, the stirring of sample liquid is a major limiting factor (magnetic stir bars cannot be rotated too quickly) whereas the ''squeeze'' mode of capillary extraction (i.e. the fast delivering of sample with a syringe, perhaps with a pressure of several bars) reaches tremendous relative speed between the immobilized coating and the liquid sample [6,7]: about 400 cm/s in the present study. This way, during the extraction step, the attainment of partition equilibrium of many target compounds, including the BTEX, is a matter of seconds.

Other advantages come from the extractor desorption modality. GC injection, in fact, concerns 100% of the extracted amount because the capillary extractor is mounted as a precolumn. In this study, owing to a very short HRGC column (3 m) the cryofocusing device helped to reduce the band broadening resulting from the injection process [15], an extremely mild solventless kind of on-column injection which gives inherently symmetrical peaks [6,7,16]. Headspaces, which are in practice unavoidable with SPME, are absent in the capillary extraction technique; unwanted losses are thus minimized, which is important during ND measurements.

By following Eqs. (1) and (2), with the inclusion of the 5% ND threshold value, the condition to be required to perform a ND extraction is:

$$K_d V_f / V_w \leq 0.05 \quad (3)$$

Suppose a target analyte has a K_d value of 1000, which is roughly that of ethylbenzene and xylenes [16]. The minimal sample amounts required to perform ND extraction by commercial apolar SPME fibers [17] can be calculated by Eq. (3) (Table 1). It is interesting to compare these values with those *calculated* for the capillary extractors of concern (Table 2). This comparison is sound because capillary extraction and SPME (at least with polysiloxane coatings) both rest on the same partition law. On

Table 2
Minimal sample volumes required, by calculation, for ND extractions with some capillary extractors

Extractor length[a] (cm)	Hold-up volume (V_g; μl)	Extractant volume (V_{PDMS}; nl)	Minimal sample amount for ND extraction (ml)
5.7	2.0	1.84	0.037
13.5	4.7	4.32	0.086
170.0	59.6	54.8	1.096

[a] All extractors had an internal diameter of 0.21 mm and a phase-ratio of 1087.

average, the capillary extractors would need a volume of sample 18 times lower than that required with standard SPME. This factor might be a major argument both in life and forensic sciences, for which sample volume can be quite limited.

In this experimental study, the shortest extractor (5.7 cm long) allowed detection of BTEX components only with a detection limit (3σ) of about 180 ppb, whereas the extractor 13.5 cm long gave enhanced BTEX sensitivity and allowed accurate peak integration; so it was the extractor of reference for the present study. Fig. 1 reports the overlapping chromatograms that resulted from three consecutive capillary extractions carried out on the same sample aliquot with this extractor.

3.1. Extended negligible depletion test

To prove further the effectiveness of the reference extractor (13.5 cm long) as ND in-tube SPME sampler, several consecutive extractions were carried out on a sample aliquot (5 ml). Fig. 2 shows a comparison between two runs that are separated by about 16 consecutive extractions. To obtain this effect, the 170-cm extractor was used one time [with an overall effect equal to 13 repeated extractions hypothetically carried out with the 13.5-cm extractor (170/13.5 = 12.6)], plus three extra ND extractions which were directly carried out with the 13.5-cm extractor. Quantitative results, reported in Table 3, demonstrate that the extractions satisfied the *negligible depletion* criterion.

4. Conclusion

Capillary extraction is able to perform ''negligible depletion'' solid-phase microextraction of diluted aqueous solution of BTEX with ease, neatly and quickly, at least with clean water as the matrix. Analytical conditions (in-tube extractions plus GC analysis) are rather flexible and easy to perform, and even the cryofocusing step might be unnecessary [6,7] whenever the GC column used is not too short with respect to the extractors used. In particular, for

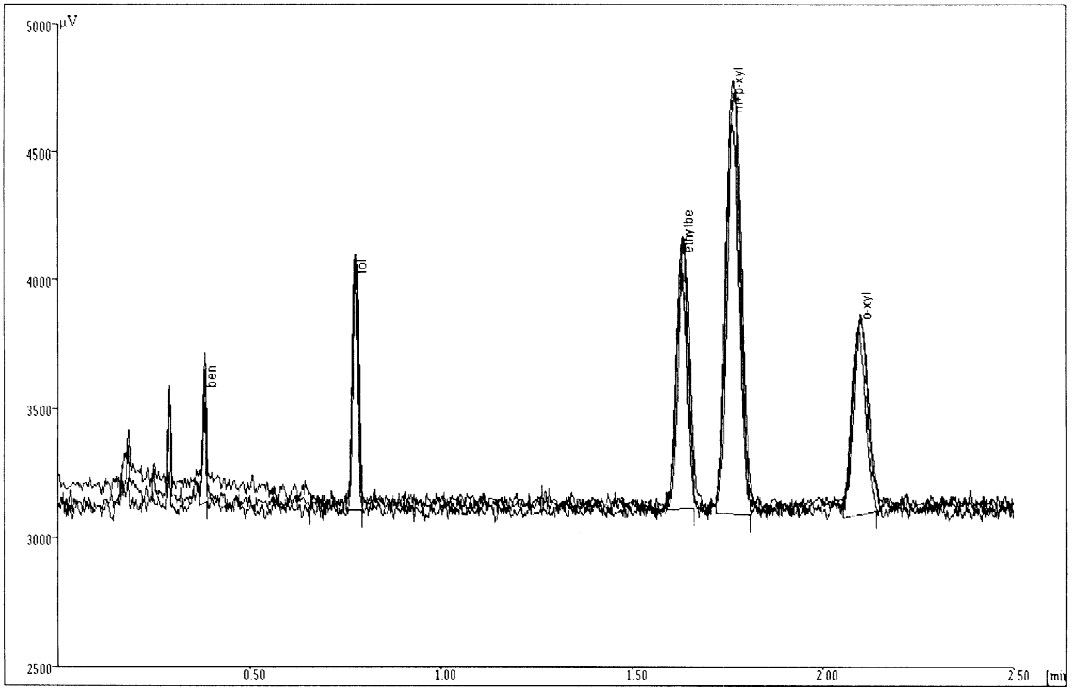

Fig. 1. Overlapping of three successive chromatograms derived from consecutive ''negligible depletion'' capillary extractions. Extractor: 13.5 cm × 0.21 mm I.D., PS255 $\beta = 1087$. Sample: 5 ml of aqueous BTEX at 146 ppb (v/v) each compound.

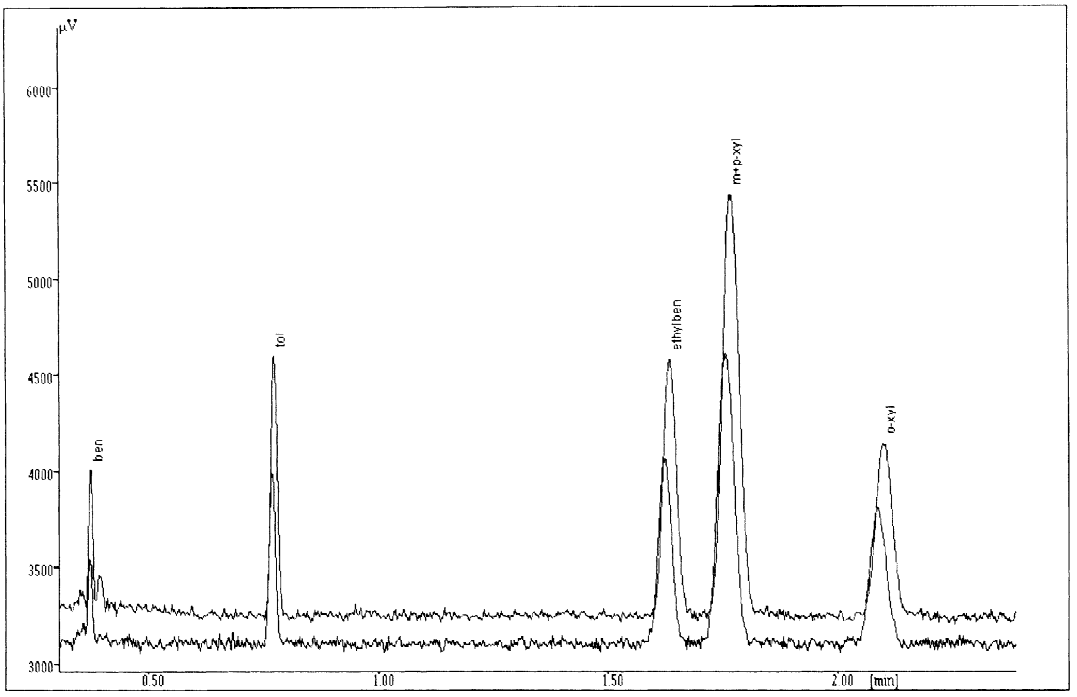

Fig. 2. Comparison between neat sample and the same specimen after several capillary extractions. The aqueous BTEX (5 ml, 146 ppb, v/v) was extracted one time with the 170-cm extractor, and three additional times with the 13.5-cm extractor (see Results and discussion for details).

Table 3
Peak response by height of peaks for the runs shown in Fig. 2

Extraction number	Benzene (µV)	Toluene (µV)	Ethylbenzene (µV)	Xylene group (µV)	BTEX average±SD
1	734	1363	1328	3511	
16	407	881	953	2229	
% depletion (overall)	45	35	28	37	36±7.0
% depletion (per single extraction)	2.8	2.2	1.8	2.3	2.3±0.44

For single extraction, % of depletion is also indicated; SD, standard deviation ($n=4$).

the extraction of volatile organic compounds, the application of ND capillary extraction to real matrices of environmental or biological origin might be worthwhile, owing to the complementary character of capillary extraction with respect to alternative techniques like SPME or SBSE. Capillary extraction does not require more skill than that required for the sister techniques, but it is remarkably cheap, fast, furnishes good peak shapes and stable chromatogram baselines due to the optimal use of a very low amount of extracting phase.

References

[1] J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction (RSC Chromatography Monographs), Royal Society of Chemistry, Cambridge, UK, 1999, pp. 129–130.

[2] J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction (RSC Chromatography Monographs), Royal Society of Chemistry, Cambridge, UK, 1999, Ch. 9.
[3] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcol. Sep. 11 (1999) 737.
[4] L. Nardi, in: P. Sandra (Ed.) Proceedings of the 23rd International Symposium on Capillary Chromatography, on CD-ROM, Riva del Garda, Italy, 5–10 June 2000.
[5] L. Nardi, Italian Pat. RM2000A000301 (2000).
[6] L. Nardi, Am. Lab. 34 (2002) 30.
[7] L. Nardi, J. Chromatogr. Sci., submitted for publication.
[8] L. Urruty, M. Mountury, J. Chromatogr. Sci. 37 (1999) 277.
[9] H. Lord, J. Pawliszyn, J. Chromatogr. A 902 (2000) 17.
[10] J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction (RSC Chromatography Monographs), Royal Society of Chemistry, Cambridge, UK, 1999, p. 132.
[11] S.A. Scheppers Wercinski (Ed.), Solid Phase Microextraction: A Practical Guide, Marcel Dekker, New York, 1999, pp. 8–10, 14, 23–24, 69, 71–72, 136–137, 148–152, 173, 235.
[12] K. Grob, Making and Manipulating Capillary Columns for Gas Chromatography, Hüthig, Heidelberg, 1986.
[13] L. Nardi, J. High Resolut. Chromatogr. 21 (1998) 625.
[14] K. Grob, Making and Manipulating Capillary Columns for Gas Chromatography, Hüthig, Heidelberg, 1986, pp. 85, 57.
[15] L. Nardi, J. Chromatogr. A 985 (2003) 67.
[16] L. Nardi, J. Chromatogr. A 985 (2003) 39.
[17] S.A. Scheppers Wercinski (Ed.), Solid Phase Microextraction: A Practical Guide, Marcel Dekker, New York, 1999, p. 62.