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Journal of Chromatography A, 1020 (2003) 189–197
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Evaluation of multiple solid-phase microextraction as a technique to remove the matrix effect in packaging analysis for determination of volatile organic compounds

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Received 9 May 2003; received in revised form 11 August 2003; accepted 12 August 2003

Abstract

Multiple solid-phase microextraction (SPME) is an useful technique for the direct quantification of solid samples removing any matrix effect. The volatile organic compounds formed in the extrusion–coating process of multilayer packaging materials have already been quantified by multiple HS-SPME coupled to gas chromatography (GC)–mass spectrometry (MS) using volatile organic compound (VOC) solutions in hexadecane for calibration. In this article, water is proposed as solvent to prepare the calibration solutions because it provides a shorter calibration time, better linearity, better reproducibility, and lower detection limits than hexadecane. Besides, the extraction of VOCs from aqueous solutions is exhaustive and avoids the extrapolations needed to calculate the total peak areas, as they can be calculated as the sum of the individual areas of each extraction. Finally, it is checked whether the two solvents provide the same mean values for the total peak areas.
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Keywords: Solid-phase microextraction; Packaging materials; Multiple solid-phase microextraction; Volatile organic compounds

1. Introduction

The new needs of the industry have increased the demand and use of packaging materials mainly for food and beverages, but also for drugs, cosmetics, farming products, etc. Packaging materials must be inert and safe in order to preserve the properties and quality of the packaged product. However, the volatile organic compounds produced in the extrusion–coating process can migrate from the packaging to its content and modify its organoleptic properties [1]. These compounds have been mainly identified as carbonyl compounds in a number of reports [2–8].

Nowadays, the most recommended technique for the analysis of volatile organic compounds in liquid or solid samples is solid-phase microextraction (SPME) [9,10], as it is a simple technique for the direct analysis of samples without using solvents.

Hexadecane has been reported [11] as solvent for quantitative analyses of multilayer packaging materials by HS-SPME–GC–MS, but a matrix effect appears when the method used is external standard calibration or standard addition calibration.

The composition of the sample matrix usually affects the sensitivity of the SPME method by the so-called “matrix effect”. On the one hand, when the

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doi:10.1016/j.chroma.2003.08.066

extraction is carried out at constant temperature, under equilibrium conditions and using constant phase volumes, the slope of the calibration graph for the analyte changes because the distribution constants fibre/calibration solvent and fibre/sample matrix are different. Therefore, there are many aspects involved in the matrix effect, namely ionic strength, percentage of organic solvent/matter in the sample, presence of compounds that can react with the analyte, etc. On the other hand, if the SPME is carried out under non-equilibrium conditions, other parameters related to kinetic aspects (e.g. viscosity differences between calibrants and samples) may be also involved in the matrix effect.

If the sensitivity of the method is different between calibrants and samples, the results will be affected by systematic errors. The matrix effect can be overcome in liquid samples by standard addition, but this calibration method does not take into account the recovery differences between spiked and native analytes when solid samples are extracted.

Multiple SPME can be used to avoid any matrix effect in the quantification of solid samples [12]. This technique involves sampling repeatedly the same vial by HS-SPME, with several subsequent consecutive extractions of volatile organic compounds (VOCs) at equilibrium. The total peak area of an exhaustive extraction of the analytes from the matrix can be estimated using the peak areas obtained in each individual extraction. Theoretically, it has been shown [13] that the total peak area (A_T) can be calculated with the following expression:

$$A_T = \frac{A_1}{1-\beta}$$

where A_1 is the peak area in the first extraction and β is calculated using the logarithm of the individual peak areas:

$$\ln A_i = (i-1)\ln\beta + \ln A_1$$

The main drawback of using hexadecane as solvent is the long analysis time needed to obtain the total peak area. In this work, water has been used to prepare standard solutions of VOCs. Note that to avoid degradation problems or losses by evaporation [11], it is necessary to store the stock solutions at 4 °C in sealed vials without any headspace and introduce the aqueous solutions to be analysed in the vials just before the analysis. The influence of the extraction time on the amount of analyte extracted and the features of the method have been studied. Besides, the method has been checked to verify whether the results obtained for hexadecane are statistically equal to those obtained using aqueous solutions.

2. Experimental

2.1. Samples

The sample analysed was a multilayer flexible packaging provided by Amcor Flexibles Tobepal S.A. (Logroño, Spain) consisting in a external layer of cellulose, a layer of extruded polyethylene, a layer of aluminium and another layer of extruded polyethylene.

2.2. Chemicals

The following chemicals were used to prepare the standard solutions in water: 3-methylbutanal (≥98%), pentanal (≥98%), 2,4-pentanedione (≥99.5%), hexanal (≥98%), cyclohexanone (≥99.5%), 3-heptanone (≥99.5%), heptanal (≥95%), 2-ethylhexanal (≥97%), octanal (≥98%), nonanal (∼97%), decanal (∼97%), and undecanal (∼97%), from Fluka (Buchs, Switzerland), and toluene (99.8%) from Carlo Erba (Rodano, Italy). Firstly, stock solutions were made in methanol, and dilutions in water were prepared using the same content of methanol (0.53%) in all the solutions. In addition, the solutions were stored at 4 °C in sealed vials without any headspace because some analytes like octanal, nonanal, and decanal have low polarity and tend to pass from the aqueous solution to the headspace.

Standard solutions in hexadecane (≥98%) from Fluka (Buchs, Switzerland) containing pentanal, hexanal, heptanal, 2,4-pentanedione, 3-heptanone, octanal, nonanal, decanal, and toluene were also prepared and used in the validation study.

2.3. Instruments and materials

A SPME holder from Supelco (Bellefonte, PA), together with a hot plate from Corning (Supelco, Bellefonte, PA), was used to perform HS-SPME manually.

The GC–MS equipment consisted of a Varian 3900 gas chromatograph and a Varian Saturn 2100T mass spectrometer detector (Walnut Creek, California, USA). Chromatographic peaks were assigned using a GC–MS mass spectral library (US National Institute of Standards and Technology, NIST).

2.4. Sampling procedure

VOCs were extracted by multiple HS-SPME using a 75 μm CAR-PDMS fibre (Supelco, Bellefonte, PA) and 15 ml sealed vials. The rest of the conditions for each kind of sample are described in the following sections.

2.4.1. VOC solutions in hexadecane

A 10 μl of solution was sampled four times, each vial was pre-heated for 5 min, and then extracted for 60 min at 100 °C.

2.4.2. VOC solutions in water

The amount of solution was 10 μl, the number of extractions was 2–4 (until all the analytes had been completely removed), the pre-incubation time was 5 min, the extraction time was 30 min, and the incubation temperature was 63 °C.

2.4.3. Packaging material

A 4 cm^2 of packaging were sampled four times, pre-heated for 5 min, and then extracted at 100 °C for 60 min.

2.5. Chromatographic conditions

The injector port was equipped with an insert for SPME of 0.8 mm i.d., the temperature of the injector was maintained at 280 °C, with a 1:20 split ratio at the initial time, followed by a 1:50 split ratio after 0.5 min. Helium (99.996%) at a flow of 1.0 ml/min was the carrier gas used. The column used was a CP5860 wall-coated open tubular (WCOT) fused-silica column (30 m × 0.25 mm i.d. with a 0.25 μm 95% dimethyl–5% diphenylpolysiloxane (CP-SIL8 CB) low-bleed/MS phase) (Varian, Walnut Creek, California, USA). The oven temperature was held at 35 °C for 5 min, followed by an increase at a rate of 10 °C/min up to 230 °C.

The masses scanned in the mass spectrometer ranged from 40 to 150 *m/z* at one cycle per second, ionisation was performed by electronic impact, the ion trap temperature was 200 °C, and the electron multiplier voltage was set at 1700 V.

The following ions were selected to quantify the compounds: 58 for 3-methylbutanal, 44 for pentanal, 91 for toluene, 100 for 2,4-pentanedione, 72 + 85 for 3-heptanone, 55 + 98 for cyclohexanone, 72 for 2-ethylhexanal, 41 for hexanal, heptanal, octanal, nonanal, decanal and undecanal.

3. Results and discussion

3.1. Extraction time of VOCs in aqueous solutions

To perform multiple HS-SPME, it is necessary to reach equilibrium, and thus the first step was to study the influence of the extraction time on the VOCs in aqueous solutions and obtain the equilibrium time for each analyte. The concentration of VOCs in the solutions ranged between 1.37 ppm (pentanal) and 8.63 ppm (2,4-pentanedione), while the extraction time ranged from 1 to 45 min. Fig. 1 shows the variation of the peak area versus the extraction time (the results are the mean of four replicates). For each compound, a value of 100 was assigned to the maximum peak area (the rest of areas are related to this value).

The peak area (proportional to the amount of analyte extracted) increased by increasing the extraction time until it reached a maximum, remaining constant onwards. This means that no competition among the analytes for the SPME fibre-sites occurred at the analyte concentration levels and for the exposition times studied ($\leq$45 min).

In spite of the lower temperature used, equilibrium was reached faster in aqueous solutions than in hexadecane. This might be due to the lower viscosity of water and the higher diffusion coefficient of the analytes. An extraction time of 30 min was selected for the aqueous solutions.

3.2. Features of the method

After studying the extraction time, a linearity study of the total peak area versus the VOCs mass in aqueous solutions was performed. Ten μl of VOC standard

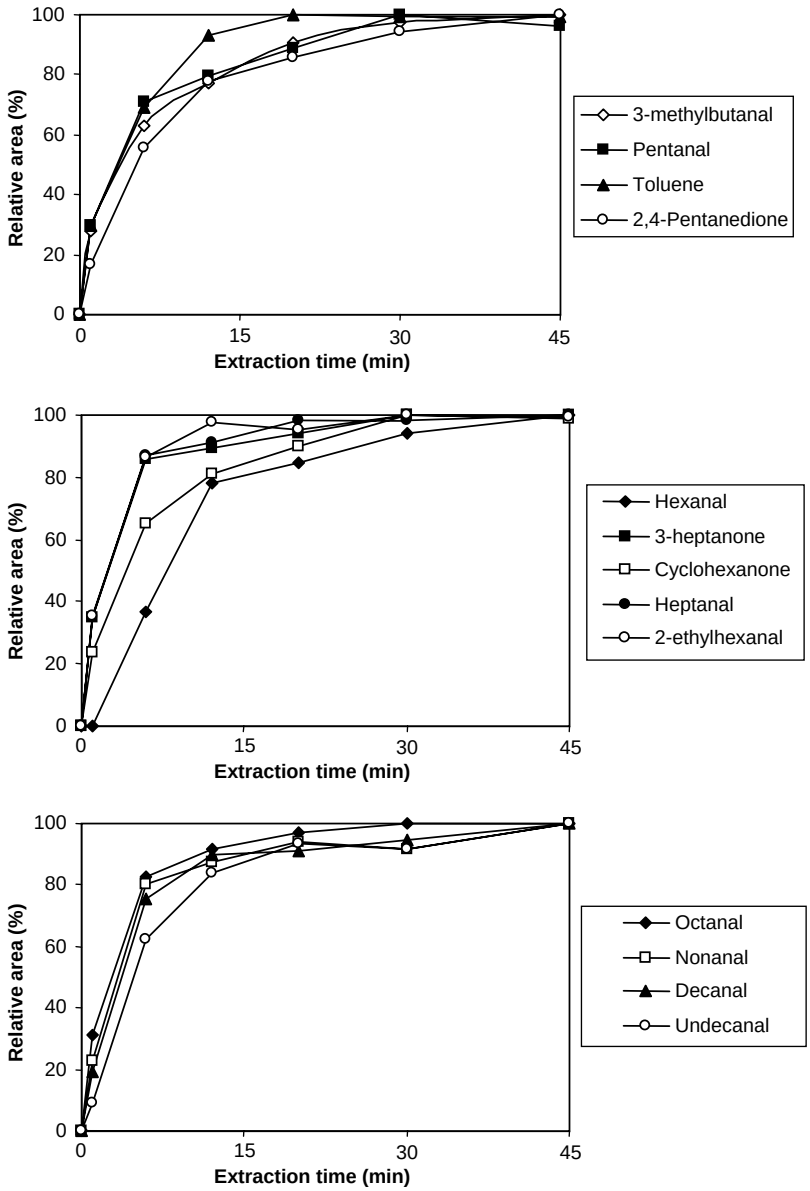

Fig. 1. Influence of the extraction time on the HS-SPME of VOCs from aqueous solutions. For HS-SPME and GC–MS conditions, see text.

solution in water were placed in a 15 ml sealed vial and processed using a CAR-PDMS 75 μm fibre as described in Section 2. The number of HS-SPME extractions performed depended on the VOC concentration of each aqueous solution; four extractions were necessary for the most concentrated solution and two for the most diluted one. In this way, all the analytes are completely removed from the vial and it is not necessary to calculate β from the logarithm of the individual peak areas to obtain the total peak area, as it can be calculated as the sum of the individual peak areas. As expected, the distribution constant between the coating and the matrix was larger in aqueous solutions than in hexadecane solutions because the polarity of

the fibre coating is much more different from water than hexadecane. The analytes (organic compounds) are more prone to be dissolved by an organic phase (fibre coating or hexadecane) than by water. The distribution constant can be considered as the ratio of analyte solubility in the fibre coating and in the solvent used. Since the analyte solubility was lower in water than that in hexadecane, the distribution constant for water was higher than that for hexadecane.

Fig. 2 shows the chromatograms obtained for four successive HS-SPME–GC extractions from the same aqueous standard solution with the following VOC concentration: 10.4 μg/ml of 3-methylbutanal, 10.8 μg/ml of pentanal, 6.2 μg/ml of toluene, 13.8 μg/ml of 2,4-pentanedione, 18.1 μg/ml of hexanal, 12.3 μg/ml of cyclohexanone, 10.9 μg/ml heptanal, 10.7 μg/ml of 2-ethylhexanal, 5.9 μg/ml of 3-heptanone, 18.1 μg/ml of octanal, 17.5 μg/ml of nonanal, 20.1 μg/ml of decanal, and 21.7 μg/ml of undecanal.

Table 1 shows the ranges of the VOC masses studied, the linear ranges, the limits of detection (LOD), the slope and intercept with their standard deviations, the correlation coefficients (R), and the relative standard deviation found. All the compounds studied responded linearly to the ratio total peak area to mass. The relative standard deviations of the total peak area ranged from 1 to 14%, being the heaviest aldehyde (undecanal) the compound that showed less reproducibility.

The standard deviations of the intercept values were large considering the good linear fit implied by the R values. This was the result of a centroid close to the high concentration edge of the linear range.

If these results are compared to those obtained for hexadecane solutions [13], it is observed that water provides better results in terms of linearity, reproducibility, and detection limits. The use of water allows to quantify the following compounds: 3-methylbutanal (there is no exponential decay of the peak area when hexadecane is used), cyclohexanone, and 2-ethylhexanal (there is no linear response of the total peak area to the analyte mass when hexadecane is used). However, pentanoic acid and hexanoic acid cannot be quantified in this way as these acids are dissociated in water but not in hexadecane.

Table 2 shows the peak areas of the centroid ($\bar{y}$) and the standard error (s_{xy}) in the calibration graphs for water and hexadecane. The standard error related to the centroid ($100s_{xy}/\bar{y}$) was calculated in the calibration graphs for VOCs in aqueous solutions and in hexadecane solutions. Water proved to be more precise as solvent except for pentanal and 2,4-pentanedione.

3.3. Comparison of total area obtained for aqueous and hexadecane solutions

In order to show that aqueous standard solutions provided the same results than hexadecane ones, the total peak area of VOCs in aqueous and hexadecane

Table 1
Features of the multiple HS-SPME method using aqueous solutions of VOCs

Compound	Studied range (ng)	Linear range (ng)	Slope ± s_m (counts × s/ng)	Intercept ± s_b (counts × s)	LOD (ng)	R	R.S.D.[a] (%) (mass level, ng)
3-Methylbutanal	0–104	1.1–104	297 ± 5	(3 ± 3) × 10^2	0.4	0.9992	5 (29)
Pentanal	0–108	0.3–108	420 ± 7	(−4 ± 4) × 10^2	0.12	0.9993	12 (30)
Toluene	0–62	0.5–62	(251 ± 5) × 10	(−2 ± 15) × 10^2	0.3	0.9991	3 (31)
2,4-Pentanedione	0–138	2.2–138	222 ± 11	(−36 ± 10) × 10^2	1.1	0.996	8 (38)
Hexanal	0–182	0.5–182	439 ± 13	(−21 ± 12) × 10^2	0.18	0.998	11 (52)
3-Heptanone	0–59	0.2–59	989 ± 16	(−13 ± 5) × 10^2	0.07	0.9993	5 (30)
Cyclohexanone	0–124	0.1–124	1386 ± 20	(−1 ± 13) × 10^2	0.05	0.9995	10 (34)
Heptanal	0–109	0.5–109	325 ± 7	(−11 ± 4) × 10^2	0.22	0.9990	1 (30)
2-Ethylhexanal	0–108	0.1–108	898 ± 21	(8 ± 12) × 10^2	0.06	0.9990	8 (30)
Octanal	0–182	0.6–182	276 ± 11	(−18 ± 10) × 10^2	0.3	0.996	7 (26)
Nonanal	0–175	2.4–175	317 ± 11	(−7 ± 10) × 10^2	0.9	0.997	12 (25)
Decanal	0–202	6–202	252 ± 8	(6 ± 9) × 10^2	2.2	0.997	11 (29)
Undecanal	0–217	8–217	159 ± 9	(15 ± 10) × 10^2	3	0.992	14 (62)

s_m: standard deviation of the slope; s_b: standard deviation of the intercept.
[a] Calculated from three replicates.

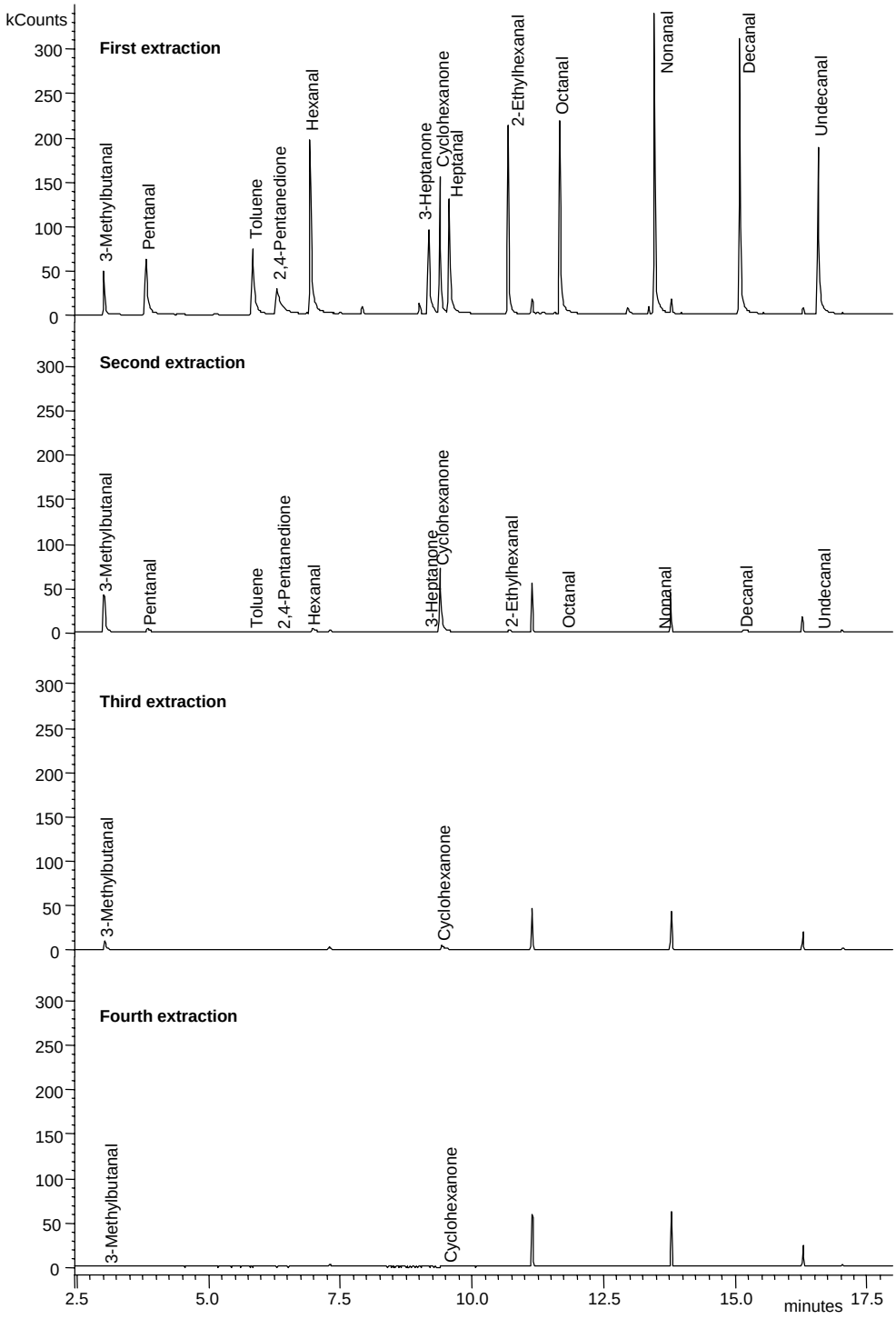

Fig. 2. Chromatograms of four successive HS-SPME extractions from 10 μl of an aqueous VOC standard solution. For the VOC concentrations, see text.

Table 2
Total peak area of the centroid (y), standard error (s_{xy}), and standard error related to the total peak area of the centroid ($100s_{xy}/\bar{y}$) in aqueous solutions and in hexadecane solutions

Compound	Aqueous solutions			Hexadecane solutions		
	$\bar{y}$ (counts × s)	s_{xy} (counts × s)	$100s_{xy}/\bar{y}$	$\bar{y}$ (counts × s)	s_{xy} (counts × s)	$100s_{xy}/\bar{y}$
Pentanal	27.3×10^3	1.1×10^3	4	36.1×10^3	0.8×10^3	2.2
Toluene	112×10^3	4×10^3	4	29×10^4	3×10^4	9
2,4-Pentanedione	9.2×10^3	1.9×10^3	21	11.6×10^3	0.9×10^3	8
Hexanal	24.8×10^3	2.0×10^3	8	35×10^3	3×10^3	10
3-Heptanone	52×10^3	3×10^3	6	61×10^3	5×10^3	9
Heptanal	19.3×10^3	1.2×10^3	6	30×10^3	5×10^3	15
Octanal	15.6×10^3	0.5×10^3	3	45.7×10^3	1.4×10^3	3
Nonanal	17.0×10^3	0.7×10^3	4	49×10^3	4×10^3	9
Decanal	16.2×10^3	1.0×10^3	6	41×10^3	3×10^3	8
Undecanal	12.8×10^3	0.9×10^3	7	33×10^3	3×10^3	10

Table 3
Total peak areas[a] (k counts × s) obtained for different VOC masses in hexadecane and water by multiple HS-SPME

Compound	20 ng		40 ng		60 ng	
	Hexadecane	Water	Hexadecane	Water	Hexadecane	Water
Pentanal	21 ± 4	19.9 ± 1.4	40.0 ± 2.2	43.7 ± 1.1	69 ± 4	66.6 ± 1.7
Toluene	110 ± 18	111 ± 8	240 ± 22	243 ± 14	354 ± 24	332 ± 20
2,4-Pentanedione	5.5 ± 2.0	4.7 ± 0.6	13 ± 4	16.1 ± 1.9	20 ± 5	25 ± 3
Hexanal	17 ± 4	16.6 ± 1.3	38 ± 6	35.7 ± 1.0	61 ± 15	58 ± 3
3-Heptanone	35 ± 4	41.1 ± 1.5	89 ± 8	91 ± 4	134 ± 16	130 ± 3
Heptanal	13 ± 3	11.7 ± 0.8	31 ± 3	26.3 ± 1.3	45 ± 7	41.5 ± 2.5
Octanal	11.0 ± 0.9	13.8 ± 1.8	28.9 ± 1.9	27 ± 3	44 ± 9	37 ± 5
Nonanal	16 ± 4	15.5 ± 1.1	36 ± 6	32 ± 3	46 ± 8	46 ± 5
Decanal	15 ± 3	17.5 ± 2.2	27 ± 4	34 ± 4	45 ± 8	47 ± 6

[a] Mean value ± standard deviation (three replicates).

solutions were calculated for different VOC masses (20, 40 and 60 ng). Table 3 shows the mean total peak areas obtained and their standard deviations. First, a test of homogeneity of variances (F-test) was applied to determine whether the variances were homogeneous ($s_1^2 = s_2^2$). The variances are homogeneous when $F_0 < F_C$ and considering that for $n_1 = 3$, $n_2 = 3$ and $\alpha_C = 0.05$ the value of F_C is 39.00, it can be concluded that the variances are homogeneous for these values.

Then, the t-test for homogeneous samples was applied to determine whether the mean values were the same ($\bar{x}_1 = \bar{x}_2$). For 4 d.f. ($n_1 + n_2 - 2$) and $\alpha_C = 0.05$, the value of t_C is 2.776, and applying the test it was verified that the two methods provide the same values ($t_0 < t_C$). Table 4 shows the values of F_0 and

Table 4
F_0 and t_0 values

Compound	20 ng		40 ng		60 ng	
	F_0	t_0	F_0	t_0	F_0	t_0
Pentanal	6.70	0.657	3.71	2.639	4.96	1.194
Toluene	5.31	0.060	2.54	0.200	1.34	1.235
2,4-Pentanedione	9.81	0.649	3.53	1.240	2.74	1.680
Hexanal	9.08	0.202	36.52	0.489	29.88	0.418
3-Heptanone	8.40	2.182	3.41	0.326	24.64	0.318
Heptanal	14.86	0.919	7.09	2.200	7.59	0.870
Octanal	3.98	2.334	2.12	0.910	3.08	1.165
Nonanal	11.61	0.334	3.82	1.113	2.94	0.040
Decanal	1.59	1.199	1.27	2.000	1.67	0.372

Critical values ($n_1 = 3$, $n_2 = 3$, $\alpha_C = 0.05$): $t_C = 2.776$, $F_C = 39.00$.

t_0 calculated. They were calculated as

$$F_0 = \frac{s_1^2}{s_2^2}$$

and

$$t_0 = \frac{\bar{x}_1 - \bar{x}_2}{[s^2(1/n_1 + 1/n_2)]^{1/2}}$$

where variance s^2 was:

$$s^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}$$

The results in Table 4 show that the use of the total peak area obtained by multiple HS-SPME removes the matrix effect.

3.4. Analysis of multilayer packaging materials

A sample of multilayer flexible packaging material was quantified by multiple HS-SPME under the conditions described under Experimental. The analyses were performed in triplicate, and the total peak areas were interpolated in the calibration graphs obtained using aqueous standard solutions.

Table 5 shows the VOC concentrations found (expressed as μg of VOC per m^2 of packaging) using aqueous standard solutions of VOCs as calibrants. These values are in good agreement with those previously obtained using hexadecane as solvent [13,14] and are within the range of those reported in the literature [5].

Table 5
VOC concentrations[a] in a multilayer packaging sample by multiple HS-SPME using water solutions

Compound	Concentration (μg/m^2)
3-Methylbutanal	28.3 ± 0.4
Pentanal	45 ± 2
Toluene	5 ± 1
2,4-Pentanedione	63 ± 11
Hexanal	(10 ± 3) × 10
3-Heptanone	14 ± 3
Cyclohexanone	10 ± 2
Heptanal	20 ± 10
2-Ethylhexanal	8.6 ± 0.4
Octanal	75 ± 13
Nonanal	(24 ± 4) × 10
Decanal	276 ± 16
Undecanal	125 ± 19

[a] Mean value ± standard deviation (three replicates).

4. Conclusions

Water reduces the extraction time compared to hexadecane and the number of extractions that must be performed is also lower. Therefore, the calibration time is also significantly shorter when water is used.

If water is used as solvent, no more VOCs were extracted after two or four extractions, and the total peak areas can be calculated as the sum of the individual peak areas. Since the calculation of β from logarithms is no longer necessary, this calibration method improves the reproducibility and linearity coefficients. Also, the increased distribution constant provides lower detection limits than hexadecane.

The total peak areas of VOCs obtained by multiple HS-SPME in hexadecane solutions are statistically equal to those obtained in aqueous solutions. This fact proves that the matrix effect has been removed.

Acknowledgements

The authors thank Amcor Flexibles Tobepal S.A. (Logroño, Spain) for financing this study through Contract OTEM001218. Óscar Ezquerro also thanks the Comunidad Autónoma de La Rioja for his grant.

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