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Determination of alkenylbenzenes and related flavour compounds in food samples by on-column preconcentration-capillary liquid chromatography

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ABSTRACT

A new, simple and versatile method is presented for the determination of different concentration levels of alkenylbenzenes (eugenol, isoeugenol, eugenol methyl ether, myristicin, anethole and estragole) and the related flavour compounds (coumarin and pulegone) in food samples. The method involves the use of a stationary phase (capillary column) for the enrichment with appropriate elution. After the sample had completely passed through the capillary column the eluent was changed and the separation/detection was achieved. Excellent linearity was obtained under the proposed conditions for a direct determination method and a method including on-line preconcentration. The limits of detection were in the ranges 97–148 and 9.5–14.2 ng/mL, respectively. Evidence for a matrix effect was not found and recoveries between 92 and 110% were obtained. The precision of the method, expressed as relative standard deviation values, was below 5% in all cases. The applicability of this methodology was tested by analyzing synthetic and real food samples.

1. Introduction

Present-day society is increasingly demanding food products made of natural ingredients. This trend is related to the consumer perception that "natural equals safe", which has resulted in an increased use of botanical products as bioactive ingredients in functional food, as food supplements and as herbal teas as well as food flavours. However, in spite of a long history of use, botanical or herbal-based preparations may contain individual ingredients that are known to be toxic and even genotoxic or carcinogenic. One of these categories of botanical ingredients is the alkenylbenzenes (Fig. 1) [1]. From a chemical point of view, these analytes can be split into two different classes: the allylbenzenes (with a 2,3-double bond) such as eugenol, eugenol methyl ether, estragole or myristicin, which are genotoxic carcinogens in rodents [2,3], and propenylbenzenes (with a 1,2-double bond) such as isoeugenol or anethole, which are non-genotoxic. Other flavour-related compounds, including coumarin and pulegone (Fig. 1), also exhibit potentially harmful effects. Coumarin, a benzopyrone compound, has been shown to cause hepatoxicity in animals and has been banned for use as a food additive in the US since 1956 [4,5]. In rodents, the metabolism of pulegone, a monoterpene ketone, causes irreversible destruction of cytochrome P450 [6], depletes glutathione [7] and causes the death of liver and lung cells [8]. The high probability of human exposure to these compounds has raised the need to identify and determine them by using appropriate analytical methods. The sample preparation is the bottleneck in most of these analytical protocols, and a survey on chromatographic analysis showed that sample preparation contributed to about two-thirds of the total time of analysis [9]. Many classical extraction techniques such as liquid–liquid extraction (LLE) and Soxhlet used as preconcentration and clean-up steps often use large solvent volumes and require long extraction times. Consequently there is an increasing demand today for new extraction techniques with automation possibilities and decreased time and solvent consumption [10]. Solid phase extraction (SPE) makes this step and is highly recommendable for laboratory use [11]. On-line automatic SPE is a better alternative, which allow lowering the detection limits, increasing precision and selectivity and minimizing human intervention through valve-switching devices and automatic cartridge exchange, in addition to improving analysis frequency [12–15].

Several analytical methods have been developed to separate and detect some of these compounds in different samples such as foods, scented products, cosmetics and tobacco [16–18,2,19–21]. Few publications of them use UV–vis detection combined with LC [16–18]. In one of these works [16], the study has been focused on the analysis of the 24 volatile chemicals belonging to different classes of compounds with different polarities in scented products and cosmetics. The limit of detections (LODs)

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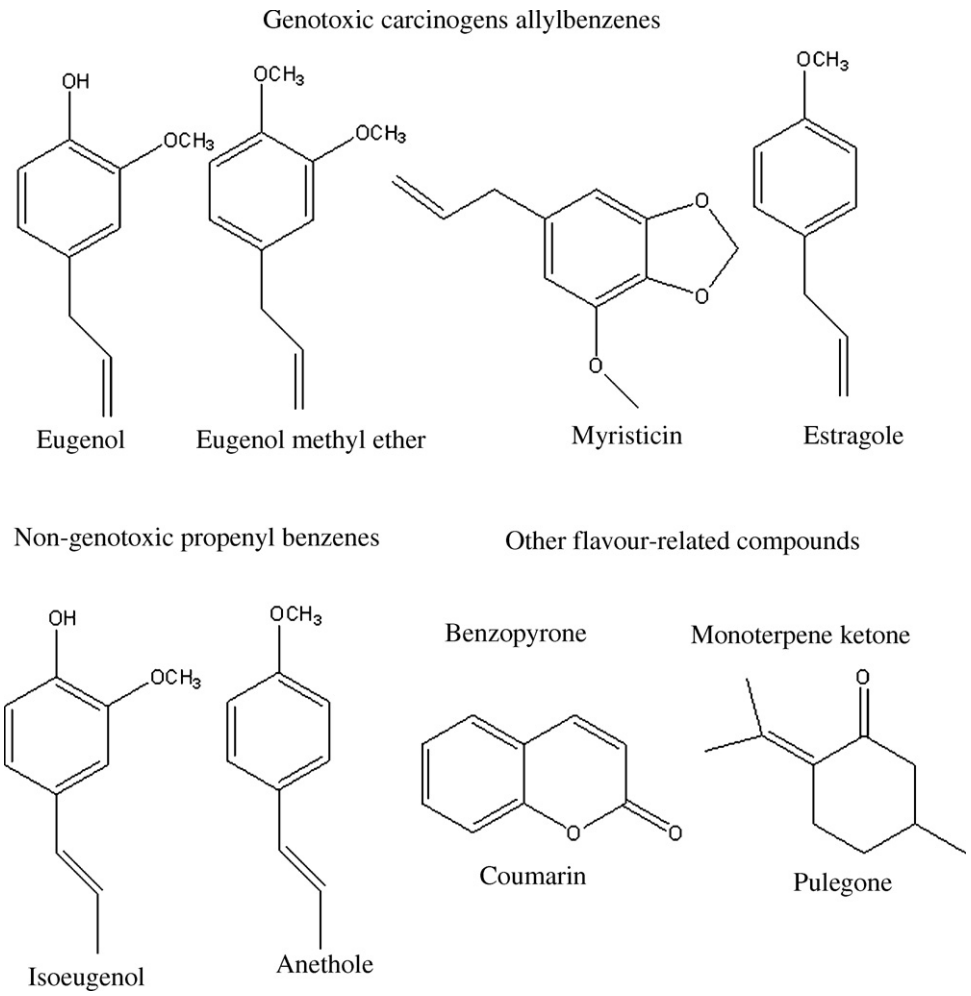

Fig. 1. Chemical structures of analyzed compounds.

obtained for, eugenol, isoeugenol and coumarin were 0.11, 0.10 and 0.07 $\mu g\,mL^{-1}$, respectively. Li et al. [17] determined eugenol and isoeugenol in bioconversion broth by LC with UV detection. The LODs obtained in this method were 6.79 $\mu g\,mL^{-1}$ for eugenol and 0.51 $\mu g\,mL^{-1}$ for isoeugenol.

The most commonly method for the determination of alkenylbenzenes and related flavour compounds is usually based on GC–MS [2] or LC–MS [19] in conjunction with pre-treatment methods, such as solid phase microextraction (SPME) [2,19,20] or simultaneous distillation extraction (SDE) [21]. SPME is the most widely used method due to its simplicity and relatively good efficiency, as well as its ability to preconcentrate the components to be analyzed and clean-up matrices from the sample prior to analysis. The increasing desire for environmentally friendly analysis has led to miniaturization of separation columns in LC since the 1970s and the development of capillary LC has been described [22]. Owing to its smaller diameter, i.e. approximately 0.1–0.8 mm I.D. compared to a conventional column of 4.0–6.0 mm I.D., the use of microcolumns in LC has advantages such as increased mass sensitivity due to a decrease in cross-sectional area of the column, low consumption of solvent and sample and lower consumption of reagents and stationary phase packing material. However, decreasing the size of the separation column also decreases its concentration sensitivity due to the limit in the sample injection volume. In order to overcome this drawback, sample enrichment has been developed and this technique has been widely employed in capillary LC in order to achieve higher sensitivity, for example with on-line SPME [23,24]. On-line in-tube SPME, a modality of this technique, was introduced by Eisert and Pawliszyn [25] and this approach enables continuous extraction, concentration, desorption and injection using an autosampler, which not only shortens the total analysis time but also provides better accuracy and precision relative to manual techniques, as reviewed by Kataoka [26] and Saito and Jinno [27]. In this technique, an open tubular fused-silica capillary column is usually used as an extraction device. The ratio of the surface area of the coated layer in contact with sample solution to the volume of the capillary column is insufficient for mass transfer. Repeated draw/eject cycles of sample solution are needed to improve extraction efficiency. Another sample enrichment technique is the use of micro-precolumns combined with column switching techniques [28,29], but these require an additional pump and injection valve for sample loading and flushing of the precolumn. One simple possibility for sample enrichment is to inject a large volume of sample in a lower strength solvent and elute the retained analytes with the higher strength mobile phase [30–32].

The aim of the work described here was to develop a simple, selective and reliable capillary LC analytical procedure suitable for the simultaneous determination of the most representative flavours in food samples. The study focused on the analysis of eight chemicals belonging to four classes of compounds with different polarities: genotoxic carcinogenic allylbenzenes (eugenol, eugenol methyl ether, myristicin and estragole), non-genotoxic propenylbenzenes (isoeugenol and anethole), benzopyrone (coumarin) and

Table 1
Optimization of chromatographic and preconcentration conditions of the proposed methods.

Chromatographic conditions			Preconcentration conditions		
Variable	Tested range	Optimum value	Variable	Tested range	Optimum value
Flow-rate (μl/min)	5–20	10	Flow-rate (μl/min)	5–20	10
Injection volume (μl)	0.2–8	8	Injection volume (μl)	0.2–8	8
Mobile phase A	Water 0.1% acetic acid in water 0.5% acetic acid in water	Water	Mobile phase A	Water 0.1% acetic acid in water 0.5% acetic acid in water	Water
Mobile phase B	Methanol Acetonitrile	Acetonitrile	Number of injection	1–15	10

monoterpene ketone (pulegone). To the best of our knowledge, a capillary LC method has never been applied for this purpose. The method developed here was applied to the detection of these analytes in seven representative commercial food products: basil, nutmeg, clove, anis, cassia, pesto sauce and tomato sauce. Samples with high levels of compounds (spices) did not need any preconcentration step, whereas those with low concentrations (sauces) were concentrated in the capillary column prior to detection. This on-line preconcentration was performed by accumulating the compound at the head of capillary column and then using an appropriate elution solvent. After injection of the sample onto the column was complete, the eluent was changed and the chromatographic separation was achieved.

2. Experimental

2.1. Chemicals, materials and samples

Eugenol, coumarin, anethole, estragole, myristicin and 3′,4′-(methylenedioxy)acetophenone (MDA) (used as internal standard) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Isoeugenol, eugenol methyl ether and pulegone were purchased from Fluka (Steinheim, Germany). Standard stock solutions were prepared in methanol and stored in the dark at 5 °C until use. The concentration of the standard stock solutions was in the range from 1 to 4 mg mL^{-1}, depending on the particular analyte. Working solutions were prepared fresh each day by diluting each stock solution with the solvent [water/acetonitrile (1/1)]. Water was purified with a Milli-Q system (Millipore). All solutions prepared for capillary LC were passed through a 0.45 μm Nylon filter before use. Dry samples (basil, nutmeg, clove, anis and cassia) and wet samples (tomato and pesto sauce) were obtained from local supermarkets.

2.2. Sample preparation

For natural and spiked dry samples (basil, nutmeg, clove, anis and cassia), 0.1 g of material was finely chopped, sonicated in an ultrasonic bath for 10 min and macerated for 12 h at 50 °C with 15 mL of methanol in a closed vessel. The suspension was then filtered and washed with 25 mL of water, the extract was collected in 100 mL calibrated flask and the process was repeated on the solid residue with 10 mL of methanol. Finally, the extracts were combined, filled up to the calibration mark with water, poured through a sintered filter and stored in the dark at 5 °C until the analysis. For the samples with very high content of some analytes, the sample extracts were diluted with [water/acetonitrile (1/1)] to reach linear range of direct capillary LC method.

The spiking procedure of dry samples was as follows: 1 g of finely chopped sample was weighed on aluminum foil and a microvolume of stock solutions of the analytes was slowly added dropwise, the solution being spread over the sample and allowed to stand for 60–120 min to allow methanol to evaporate.

For wet samples (pesto and tomato sauce), 1 g samples of each sauce were extracted with 25 mL of methanol and diluted to 100 mL with water under the conditions used for dry samples.

2.3. Instruments and apparatus

The modular capillary chromatographic system (Agilent Series 1200) consisted of a vacuum degasser, capillary LC pump, microwell-plate autosampler (8 μl injection loop), thermostatted column compartment and a diode-array detector. The detector was coupled to a data system (Agilent, HPLC ChemStation) for data acquisition and calculation. The analytical column was a reversed-phase C18 column (Luna) (250 mm × 0.5 mm I.D., 5 μm) from Phenomenex. A thermostatic bath and an ultrasonic bath were used for sample preparation.

2.4. Chromatographic, preconcentration and detection conditions

The chromatographic conditions involved the use of a gradient method: (i) for 1 min to obtain 40% acetonitrile and maintaining this for 9 min; (ii) from 40 to 60% in 45 min. The total chromatographic analysis time was 55 min. A direct determination method was used to analyze samples with high levels of alkenylbenzenes whereas a method involving in-line preconcentration would be used to analyze samples with lower levels of these compounds.

Water was used as the solvent for the sample injection step during preconcentration. The optimum sample injection volume was 8 μL and the optimum number of injections in this case was 10 (nine under optimum preconcentration conditions, followed by a final injection under optimum chromatographic conditions). The latter value was chosen taking into account the preconcentration factor required and the time needed to achieve this. The time for the preconcentration step was 5 min for each injection. A total of 45 min was required for the multiple injections in the preconcentration step.

The wavelength with the highest intensity (215 nm) was used for quantitative analysis except for pulegone (260 nm). Furthermore, UV/vis spectra between 210 and 400 nm were recorded in order to verify the peak identity.

3. Results and discussion

The principal characteristic of the proposed method is the possibility of adapting the preconcentration level in an automatic way. Furthermore, it is well-known that spices are one of the most important sources of alkenylbenzenes in the human diet and they can be present in very different levels according to the analyzed sample. For this reason, we considered this family of compounds to be appropriate to demonstrate the versatility and applicability of the method. Consequently, we propose a direct determination

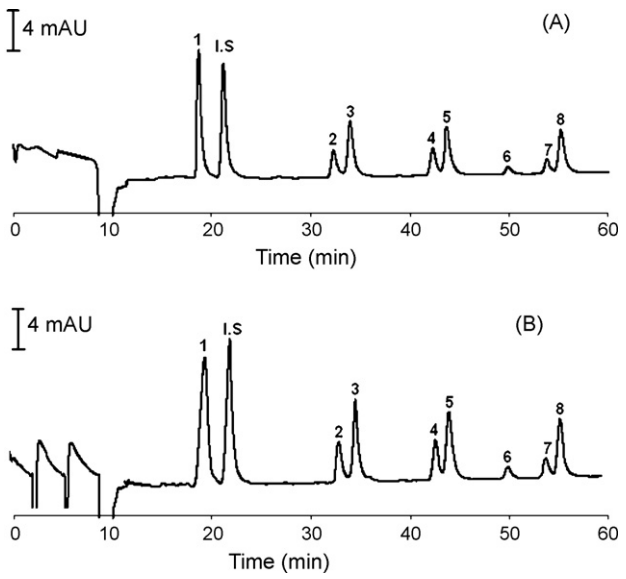

Fig. 2. Chromatograms for standards. (A) Direct determination method, analyte concentration was 5 μg/mL, detection wavelength was 260 nm; (B) method including in-line preconcentration, analyte concentration was 0.5 μg/mL, detection wavelength was 260 nm. Peaks: (1) coumarin; (IS) internal standard; (2) eugenol; (3) isoeugenol; (4) eugenol methyl ether; (5) pulegone; (6) myristicin; (7) anethole; (8) estragole.

method to analyze samples with high levels of alkenylbenzenes whereas a method involving in-line preconcentration would be used to analyze samples with lower levels of these compounds.

3.1. Direct determination method

The optimum chromatographic conditions (separation–detection) were determined in order to obtain the shortest analysis time, the best separation between all analytes and the highest sensitivity. The separation variables optimized were flow-rate, injection volume, solvent-sample composition and solvents employed as the mobile phase. The ranges studied for these variables and their optimum values are shown in Table 1 (chromatographic conditions). The monitoring wavelength was 215 nm (260 nm for pulegone).

The proposed method is a gradient mode for which the optimum chromatographic conditions were described in Section 2.4. The chromatogram obtained for one injection under these optimum chromatographic conditions is shown in Fig. 2A.

3.2. Method including in-line preconcentration

According to the results obtained by Jiménez and Luque [32], on-column sample preconcentration can be developed only if a capillary LC column is used and the retention factor of the solutes in the initial mobile phase is higher than 20. Therefore, the retention factors of the analytes must be determined prior to the development of the method involving preconcentration.

The retention factors of alkenylbenzenes and related flavour compounds in the initial mobile phase were determined using a standard solution containing 50% acetonitrile as a solvent front marker and all analytes, at 10 μg mL^{-1}, were prepared in water and injected onto the chromatograph. The injection volume was 8 μL and the isocratic mobile phase used for the retention of studied analytes was acetonitrile (10%). The solvent front was observed at 10 min and the analytes remained in the column after 205 min. The retention factor, calculated from expression (1), was higher than 20 for all analytes. Therefore, it is possible to inject several vol-

Table 2
Figures of merit for the proposed methods.

Analyte	Direct determination method						Method including in-line preconcentration					
	$a \pm S_a$	$b \pm S_b$	RSD	R^2	LOD (mg L^{-1})	LOQ (mg L^{-1})	$a \pm S_a$	$b \pm S_b$	RSD	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
Coumarin	0.061 ± 0.009	0.204 ± 0.002	3.2	0.9997	0.132	0.441	0.003 ± 0.006	0.0019 ± 0.0001	4.8	0.9996	9.474	31.579
Eugenol	0.082 ± 0.008	0.227 ± 0.002	4.1	0.9994	0.106	0.352	−0.009 ± 0.009	0.0019 ± 0.0002	4.7	0.9996	14.211	47.368
Isoeugenol	0.074 ± 0.011	0.223 ± 0.006	3.5	0.9994	0.148	0.493	−0.032 ± 0.008	0.0019 ± 0.0001	4.6	0.9992	12.632	42.105
Eugenol Methyl ether	0.079 ± 0.012	0.250 ± 0.003	4.3	0.9994	0.144	0.480	−0.031 ± 0.010	0.0022 ± 0.0003	5.0	0.9990	13.636	45.455
Pulegone	0.089 ± 0.028	0.668 ± 0.003	2.9	0.9999	0.126	0.419	−0.013 ± 0.021	0.0061 ± 0.0004	4.5	0.9999	10.328	34.426
Myristicin	0.049 ± 0.007	0.217 ± 0.003	1.9	0.9994	0.097	0.323	0.009 ± 0.009	0.0019 ± 0.0001	3.9	0.9994	14.211	47.368
Anethole	0.039 ± 0.005	0.109 ± 0.004	4.7	0.9999	0.138	0.459	0.079 ± 0.004	0.0012 ± 0.0002	4.2	0.9992	10.000	33.333
Estragole	0.043 ± 0.009	0.193 ± 0.002	4.2	0.9995	0.140	0.466	−0.013 ± 0.008	0.0017 ± 0.0001	4.1	0.9994	14.118	47.059

a: intercept; S_a: standard deviation of the intercept; *b*: slope; S_b: standard deviation of the slope; *R*: regression coefficient; LOD: limit of detection; LOQ: limit of quantification, RSD: relative standard deviation ($n = 11$).

ume samples onto the column, concentrate the analytes in the head of the column and then elute them with an appropriate gradient composition:

$$RF = \frac{t_R}{t_{fs}} \quad (1)$$

t_{fs}, solvent front time; t_R, retention time of each analyte.

The variables optimized in this step were flow-rate, solvent-sample composition, mobile phase composition and number of injections. The tested range and the optimum values of these variables are shown in Table 1 (preconcentration conditions). Moreover, the micro-metering device on the autosampler provides injection volumes in the range 0.01–8 μL with the standard loop capillary and from 0.01 to 40 μL with the extended loop capillary. The extended loop capillary (40 μL) would be the most appropriate system for the injection of the largest amount of sample in the lowest number of injections. Unfortunately, it was not possible to carry out the analysis due to the over pressure detected in the system after each injection. Therefore, a standard loop (8 μL) was selected because changes in the system pressure were not observed on switching the valve.

Furthermore, the chromatogram obtained from ten consecutive injections, nine under optimum preconcentration conditions, followed by a final injection under optimum chromatographic conditions is shown in Fig. 2B.

3.3. Analytical features of the analytical method

A combination of capillary liquid chromatography with simpler on-column preconcentration proved to be an excellent way to determine alkenylbenzenes and related flavour compounds in food samples. Analytical figures of merit used to characterize this proposed method included its linear dynamic range and sensitivity (expressed as the detection limit). Individual calibration graphs for coumarin, eugenol, isoeugenol, eugenol methyl ether, pulegone, myristicin, anethole and estragole were obtained for all analytes by plotting concentration against peak area, following linear regressions within a concentration range of 0.5–20.0 μg/mL for the direct method and 0.05–2.0 μg/mL for the method including in-line preconcentration. Each solution was injected in triplicate. The precision of the method, expressed as RSD, for the determination of 1 μg/mL of each analyte was <5% (n = 11) in all cases. This represents an acceptable level for this type of application, including the on-column preconcentration.

The intercept and slope of the regression line are shown in Table 2 along with the regression coefficient for the proposed methods and for each individual analyte. On the basis of these results, excellent correlation coefficients were obtained for both methods, with the outstanding result being the improvement in the theoretical LOD (defined as the concentration of analyte that gives a signal equivalent to the blank signal plus three times its SD), which is in the range 97–148 ng/mL for the direct method and 9.5–14.2 ng/mL for the method including in-line preconcentration. Moreover, slopes for all analytes in both methods captured the enhancement in sensitivity. Accordingly, a preconcentration factor of approximately 10 is obtained with a prior preconcentration step of 10 injections and this process increased the total analysis time by 45 min. Consequently, different preconcentration factors could be obtained by changing the number of injections carried out under preconcentration conditions, according to the required sensitivity and the time available for the determination.

The applicability of in-line preconcentration method was checked by analyzing synthetic samples of alkenylbenzenes and related flavour compounds. The results obtained are shown in Table 3. The trueness of the proposed instrumental method was

Table 3
Determination of alkenylbenzenes and related flavour compounds in synthetic samples by in-line preconcentration method.

Sample	Added (ng/mL)	Coumarin		Eugenol		Isoeugenol		Eugenol methylether		Pulegone		Myristicin		Anethole		Estragole	
		Found (ng/mL)	R(%)	Found (ng/mL)	R(%)	Found (ng/mL)	R(%)	Found (ng/mL)	R(%)	Found (ng/mL)	R(%)	Found (ng/mL)	R(%)	Found (ng/mL)	R(%)	Found (ng/mL)	R(%)
S1	50	49	98.2	49	98.8	57	114.8	57	113.8	55	109.4	48	96.6	55	109.6	58	116.6
S2	100	96	95.7	92	91.9	102	101.7	94	94.0	102	101.8	97	97.2	99	98.7	97	96.8
S3	250	249	99.6	255	101.9	248	99.1	248	99.2	250	99.9	246	98.3	262	104.9	239	95.5
S4	500	507	101.4	514	102.7	508	101.6	489	97.9	467	93.4	483	96.6	522	104.4	470	93.9
S5	1000	984	98.4	1000	100.0	1011	101.2	1020	102.0	1007	100.7	1004	100.4	1022	102.2	1016	101.6
t(exp)		0.91		0.63		2.41		0.35		0.61		1.43		2.39		0.58	

t_{crit} = 2.78 (4 degrees of freedom at the 95% confidence level).

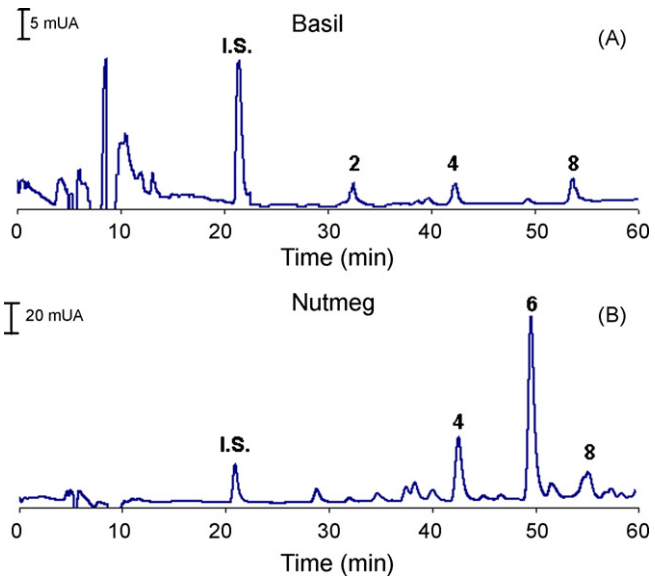

Fig. 3. Analysis of real samples by direct determination method. (A) Chromatogram of basil extract and (B) chromatogram of nutmeg extract. Peak assignment is as reported in Fig. 2. Detection wavelength was 215 nm.

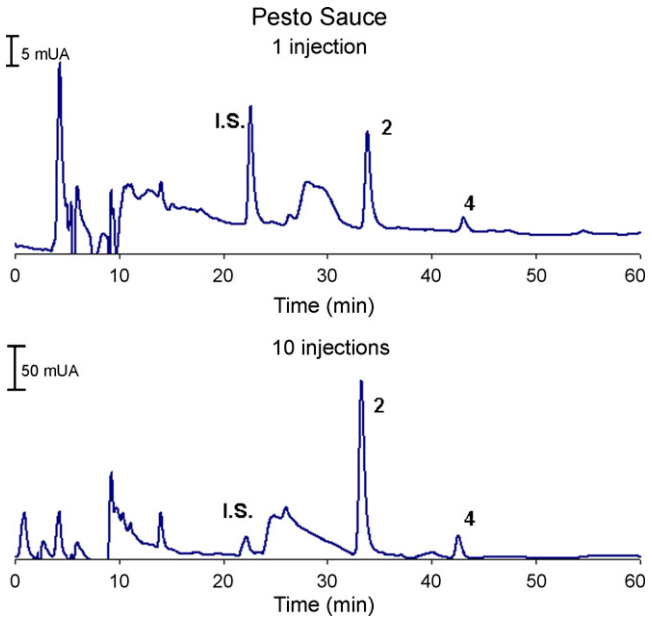

Fig. 4. Analysis of pesto sauce extract by direct determination method (1 injection) and method including in-line preconcentration (10 injections). Peak assignment is as reported in Fig. 2. Detection wavelength was 215 nm.

evaluated by means of paired samples tests. The statistics (t_{exp}) values were 0.91, 0.63, 2.41, 0.35, 0.61, 1.43, 2.39 and 0.58 for coumarin, eugenol, isoeugenol, eugenol methyl ether, pulegone, myristicin, anethole and estragole, respectively. These values are lower than the corresponding tabulated t_{crit} values (2.78 for 4 degrees of freedom at the 95% confidence level). As a result, the differences between the concentrations added and those found were statistically insignificant.

3.4. Analysis of alkenylbenzenes and related flavour compounds in synthetic and real samples

Seven different samples, most of which are used in traditional gastronomy and as additives in commercial food, were also chosen to demonstrate the real applicability of the proposed method. Thus, basil, nutmeg, clove, anis, cassia, pesto sauce and tomato sauce were analyzed. Samples with high levels of alkenylbenzenes, like spices, did not require a preconcentration step, whereas those with lower concentrations, like sauces, had to be preconcentrated in the capillary column prior to detection. The quantitative levels of the studied analytes found in dry samples (spices) are shown in Table 4 and typical chromatograms for a basil extract and nutmeg extract, respectively, are shown in Fig. 3A and B. The majority of the concentrations obtained were in agreement with those found in the Refs. [21,33,34] and the differences can be explained in most cases by the different sample origin or the different extraction method employed. Finally, the trueness of the proposed instrumental method and potential matrix effects were studied by speaking these sample extracts with a known concentration and analyze them. A summary of analyte recoveries for each sample is also presented in Table 4.

Wet samples (sauces) were analyzed by both direct determination and the method involving in-line preconcentration. The chromatograms obtained from pesto and tomato sauces, respectively, both with and without a preconcentration step are shown in Figs. 4 and 5. In these figures it can observed that pesto sauce can be quantified by direct determination but for the tomato sauce a preconcentration step is essential. The presence of eugenol, eugenol methyl ether and estragole in these samples is due to the basil used in their preparation. The amounts of these compounds found in basil extract, pesto sauce and tomato sauce are shown in Table 5 along with the most suitable mode for their determination according to the concentration levels present in the samples.

Table 4
Analysis of some selected food samples by the direct determination method.

Sample	Analyte	Conc. in the sample (mg/g)	Conc. added (mg/g)	Recovery(%) for $n = 3$
Basil	Eugenol	0.8 ± 0.2	0.5	91.1
	Eug-methyl-ether	0.9 ± 0.2	0.5	94.1
	Estragole	1.2 ± 0.3	1.0	90.3
Nutmeg	Eugenol	0.6 ± 0.1	0.5	94.4
	Isoeugenol	0.68 ± 0.01	0.5	95.7
	Eug-methyl-ether	1.4 ± 0.2	1.0	100.2
	Myristicin	16.9 ± 0.6	10.0	94.4
Anis	Anethole	33.9 ± 0.3	10.0	103.4
	Eugenol	–	4.0	105
	Eug-methyl-ether	–	4.0	110
Clove	Eugenol	28.5 ± 0.4	10.0	101.5
	Myristicin	–	1	107
	Anethole	–	6	114
Cassia	Coumarin	2.6 ± 0.3	1.0	97

Table 5
Analysis of some selected food samples by the direct determination method and method including in-line preconcentration.

Sample	Eugenol (mg/kg)	Eugenol methyl ether (mg/kg)	Estragole (mg/kg)	Method
Basil	540	560	710	DDM
Pesto sauce	268	48	–	DDM/ILPM
Tomato sauce	27	4	–	ILPM

DDM: direct determination method; ILPM: in-line preconcentration method.

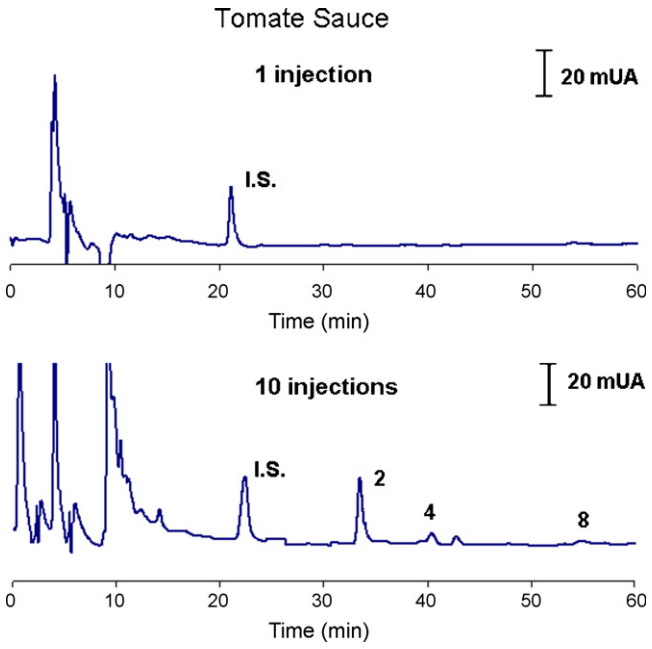

Fig. 5. Analysis of tomato sauce extract by direct determination method (1 injection) and method including in-line preconcentration (10 injections). Peak assignment is as reported in Fig. 2. Detection wavelength was 215 nm.

4. Conclusions

A method for the determination of alkenylbenzenes and related flavour compounds (coumarin, eugenol, isoeugenol, eugenol methyl ether, pulegone, myristicin, anethole and estragole) in food samples has been developed. The use of on-column preconcentration in capillary liquid chromatography provides appropriate sensitivity. The detection limits obtained were ranged from 0.009 to 0.014 $\mu g\,mL^{-1}$, which compares favourably with other values obtained using HPLC coupled with diode-array detector (DAD). For example, detection limit obtained by Villa et al. [16] using HPLC-DAD method for the determination of coumarin, eugenol, isoeugenol and other compounds in scented products was ranged between 0.01 and 0.74 $\mu g\,mL^{-1}$.

The proposed method is very simple and an additional pump and injection valve for sample loading and flushing of the capillary column are not required. In addition, 80 μL of sample is required. Moreover, solvent consumption and waste generation are much lower than those associated with methods involving conventional forms of analyte enrichment and separation. Finally, the versatility of the method has been demonstrated, in this case for a preconcentration factor of 10, by means of 10 consecutive injections that require only forty-five additional minutes. In cases where lower or higher preconcentration factors were required, the injection stage could be adjusted as necessary, taking into account the total analysis time and the final resolution obtained. Consequently, the in-line preconcentration-determination method could be applied in an automated approach, for different concentration levels, by simply changing the number of injections in the preconcentration step.

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