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Pervaporation–gas chromatography coupling for slurry samples Determination of acetaldehyde and acetone in food

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Abstract

The use of pervaporation for the removal of volatile species from slurry samples, with a full automated introduction of the sample into the lower chamber of the pervaporation unit prior to their individual separation and determination by gas chromatography–flame ionisation detection, is presented for the first time. For this purpose, the upper chamber of the pervaporator is situated in the loop of an HPLC injection valve and the only requirement of the experimental setup for being used with slurries is to have adequately sized diameters for the units of the dynamic manifold assisting the donor chamber in order to avoid clogging by the suspended particles. The method developed was applied to the determination of acetaldehyde and acetone in food samples with different solids contents, such as yoghurt, Actimel and different kind of juices.

Keywords: Food analysis; Pervaporation; Milk; Fruit juices; Acetaldehyde; Acetone

1. Introduction

Analytical pervaporation emerged in the late 1980s as a non-chromatographic continuous separation technique competing with other membrane-based separation techniques thanks to the absence of sample–membrane contact, which makes it useful for dirty and/or aggressive samples [1–3]. The later integration of steps in the analytical process other than separation (viz. derivatising chemical [4,5] and biochemical reactions, either preceding [6,7] or following [8,9] the pervaporation step) increased its potential and miniaturisation capability, which was enlarged with its use as an alternative to headspace systems (both static and dynamic or purge and trap modes) [10–13]. All the described steps can be implemented in an automated continuous fashion with the help of a proper flow injection (FI) manifold assisting both the donor, sample chamber and the upper, acceptor chamber when liquid samples are involved. Nevertheless, with the direct use of solid samples, the overall process becomes a hybrid discontinuous–continuous process as the sample is weighed in the donor chamber; then both chambers, and the membrane between and spacers, if required, are fastened with screws. Once the analytes cross the membrane, the subsequent steps are developed in a continuous, automated manner.

From the beginning, pervaporation provided a number of advantages as compared with headspace that can be summarised as follows: (a) the thin air gap layer above the sample requires very small amounts of the analytes to establish equilibrium with

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the solid or liquid sample phase and mass transfer across the membrane; (b) continuous removal of the volatilised analytes across the membrane from the air gap displaces the mass transfer equilibrium and increases the separation efficiency; (c) continuous removal of the pervaporated analytes to the preconcentration column, if used, allows fresh portions of acceptor gas to continuously come into contact with the diffused species, thus displacing the mass transfer equilibrium; (d) the separation step can be totally or partially automated (when liquid or solid samples, respectively, are involved) for laboratory use with minimal purchase and maintenance costs; (e) unlike purge and trap, no water vapour condenser is required, nor is a hydrophobic sorbent, as no water-crossing through the (hydrophobic) membrane occurs at the usual working temperatures.

The formation of a slurry from the solid sample allows full automation of the performance of the pervaporator–gas chromatograph coupling, thus circumventing the drawback of total unattendance of the overall analytical process. The only requirement of the experimental setup is for adequately sized diameters of the units of the dynamic manifold for assisting the donor chamber in order to avoid clogging by the suspended particles. The performance of such an approach has been studied in this research using samples with different solids contents in order to demonstrate its general applicability to food analysis.

2. Experimental

2.1. Instruments and apparatus

The flow manifold–pervaporator–gas chromatograph assembly used is shown in Fig. 1. The flow manifold consists of a four-channel Gilson Minipuls-3 peristaltic pump (Gilson, Middleton, WI, USA) fitted with a rate selector, two Rheodyne (Elkay, Galway, Ireland) on–off valves, PTFE tubing of 1.5 mm I.D. (Análisis Vínicos, Tomelloso, Spain).

The pervaporation unit consists of a lower donor chamber and an upper acceptor chamber, both fitted with inlet and outlet orifices, a membrane support, a membrane and spacers, if required, located between the two chambers. Close contact between parts was achieved by screwing them between aluminium supports with four screws. The upper chamber of the pervaporator was located in the loop of an HPLC valve in order to keep the acceptor gas static or circulating to the chromatographic column when in its filling or injection position, respectively. The diameter of the inlet and outlet orifices of the lower chamber were modified in order to adapt them to the PTFE tubing of 1.5 mm I.D. PTFE membranes (47 mm diameter and 1.5 mm thickness from Trace (Braunschweig, Germany) and a Selecta (Barcelona, Spain) Ultraterm 6000383 water bath were also used.

A modification of the gas chromatograph [Varian Star 3400 CX equipped with a flame ionisation

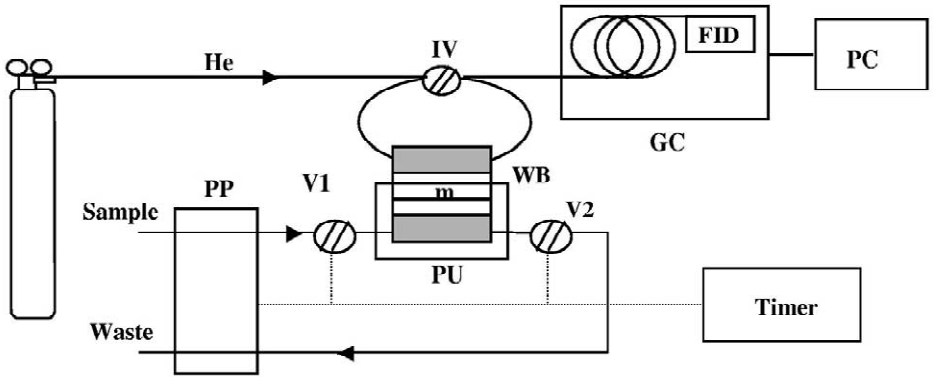

Fig. 1. Experimental setup used for the proposed method. PP, peristaltic pump; V1, V2, on–off valves; PU, pervaporation unit; WB, water bath; m, membrane; IV, HPLC injection valve; GC, gas chromatograph; FID, flame ionisation detector; PC, personal computer.

detection (FID) system, P.S. Analytical, UK] was necessary in order to drive the content of the loop of the HPLC valve (IV in Fig. 1), to the column top (Carbowax, MFE-20, 25 m×0.25 mm, 0.25 μm, Análisis Vínicos) by direct connection of the He tubing to the column without passage through the GC injector. A computer with a Varian Star Chromatographic Workstation software was used for data collection and treatment.

2.2. Reagents and solutions

Aqueous standard solutions were prepared by appropriate dilution from: 7800 μg ml^{-1} acetaldehyde (Merck, Darmstadt, Germany) solution, 7900 μg ml^{-1} acetone (Panreac, Barcelona, Spain) and 7800 μg ml^{-1} 2-propanol (Scharlau, Barcelona, Spain).

All solutions were prepared using doubly distilled water of high purity obtained from a Millipore (Bedford, MA, USA) Milli-Q plus system.

Solutions of the analytes in octanol were used for preliminary chromatographic studies involving no pervaporation.

The helium gas used was supplied by Carburos Metálicos (Barcelona, Spain).

2.3. Samples

(a) Yoghurt was used both in the optimisation of the pervaporation process and in the application of the method to natural samples.

(b) Milk was used for studying the features of the method after adding 7 μl ml^{-1} lactic acid to simulated a fermented milk product. Appropriate volumes of standard solutions of the analytes and the internal standard (I.S.) 2-propanol were added too.

(c) Actimel is a commercial product, a hybrid between yoghurt and milk, thicker than milk because it contains orange pulp.

(d) Juices: three different kind of juices were used for the application of the method: apple juice, peach+grape juice and tomato juice. Filtration or dilution of the samples to eliminate the pulp of the fruits were not necessary.

2.4. Procedure

First, the on–off valves (V1 and V2) were opened, injection valve (IV) was in the filling position and a constant volume of sample was aspirated by the peristaltic pump and carried to the donor chamber of the pervaporation unit to ensure that the sample level in this chamber was constant too. Then, V1 and V2 were switched to the off position at the same time that the peristaltic pump (PP) was stopped. In this way, the stream of He gas was going to the chromatograph but the portion which was trapped in the loop of the HPLC valve remained static in the acceptor chamber while the sample was also static in the lower chamber located in a thermostat bath at 80 °C (optimised pervaporation temperature).

After 10 min (optimised preconcentration time), IV was switched to the injection position for 10 s (optimised injection time). During this interval, the evaporated analytes were led to the top of the chromatographic column by the carrier. Under the optimal working conditions the two analytes and the I.S. were chromatographed within 2 min.

Before a new sample injection, a cleaning step of the pervaporation unit was required. For this step, V1 and V2 were switched for 2 min for circulation of fresh water through the sample manifold and IV was switched to the injection position for 15 s.

3. Results and discussion

3.1. Optimisation study

Variables which influence the method here proposed can be divided into two groups: chromatography and pervaporation variables. A univariate optimisation was performed for both groups of variables. The ranges over which these variables were studied and the optimum values found are listed in Table 1.

3.1.1. Optimisation of the chromatographic separation

For chromatographic identification of the target analytes and in order to carry out the subsequent optimisations, octanol solutions 10 μg ml^{-1} of each

Table 1
Optimisation of the method

Step	Variable	Range studied	Optimum value
Chromatography	$T_{initial}$ (°C)	35–50	50
	Programme rate (°C min^{-1})	0–5	0
	Split-ratio	–	1:10
	He, flow-rate (ml min^{-1})	1–3	1
	Injection time (s)	5–20	10
Pervaporation	$T_{pervaporation}$ (°C)	50–80	80
	Preconcentration time (min)	3–15	10

analyte and 20 μg ml^{-1} 2-propanol as internal standard were prepared daily. These solutions were directly injected into the chromatographic column under the recommended conditions for this type of column [$T_{initial}$=50 °C, programme rate=4 °C min^{-1}, flow-rate (He)=1 ml min^{-1}, split ratio=1:10, injector temperature=200 °C and detector temperature=220 °C].

A solution containing both analytes and I.S. in octanol was injected into the chromatograph under the conditions mentioned above. The peaks corresponding to acetaldehyde and acetone appeared slightly overlapped. An isothermal programme was tested, obtaining a complete separation of the peaks, as shown in Fig. 2A; so this programme was selected for subsequent experiments. Under these working conditions the retention times (in min) were: 0.89, 1.05 and 1.40 for acetaldehyde, acetone and 2-propanol, respectively.

3.1.2. Optimisation of the pervaporation process

The sample used for this optimisation was yoghurt, which contains both analytes. For the preliminary experiments with pervaporation the manifold in Fig. 1 but without V1 and V2 valves was used. The problem with this manifold was that, when the injection valve IV was switched to the injection position, the He gas diffused through the membrane and was ejected through the inlet and outlet orifices of the lower chamber. For this reason, on–off valves, V1 and V2, were located at the inlet and outlet of the lower chamber in order to isolate the pervaporator from the dynamic system when required. In this way, a constant sample volume was carried to the lower chamber of the pervaporation unit, the content of which remained static when V1 and V2 were switched to the off position and, at the same time, PP was stopped.

Pervaporation variables such as temperature and the preconcentration time and a chromatographic variable such as the injection time, were optimised with the assembled manifold. Table 1 shows the ranges studied and the optimum values of the variables.

3.1.2.1. Optimisation of the preconcentration time

The time necessary for preconcentration of the analytes in the static gas (He) at the upper chamber of the pervaporation unit was optimised using values between 3 and 15 min. As shown in Fig. 3, the peak area increased with the preconcentration time, but times longer than 10 min resulted in not significant increases (less than 10% when the time changed from 10 to 15 min). Moreover, more poorly defined chromatographic peaks were obtained at 15 min. Therefore, 10 min was chosen for subsequent experiments.

3.1.2.2. Optimisation of the pervaporation temperature

The increase of temperature applied to the pervaporation unit had a positive effect on the analytical signal, since it favoured both evaporation of the analytes and their permeation through the membrane. Fig. 4 shows that for 90 °C the signal obtained was the biggest but the precision (8.5% for acetaldehyde and 10.1% for acetone, expressed as RSD, n=3) was poorer than at 80 °C (4.5% for acetaldehyde and 5.2% for acetone) because of the presence of some water vapour in the upper chamber. Furthermore, very wide and poorly defined peaks were obtained at

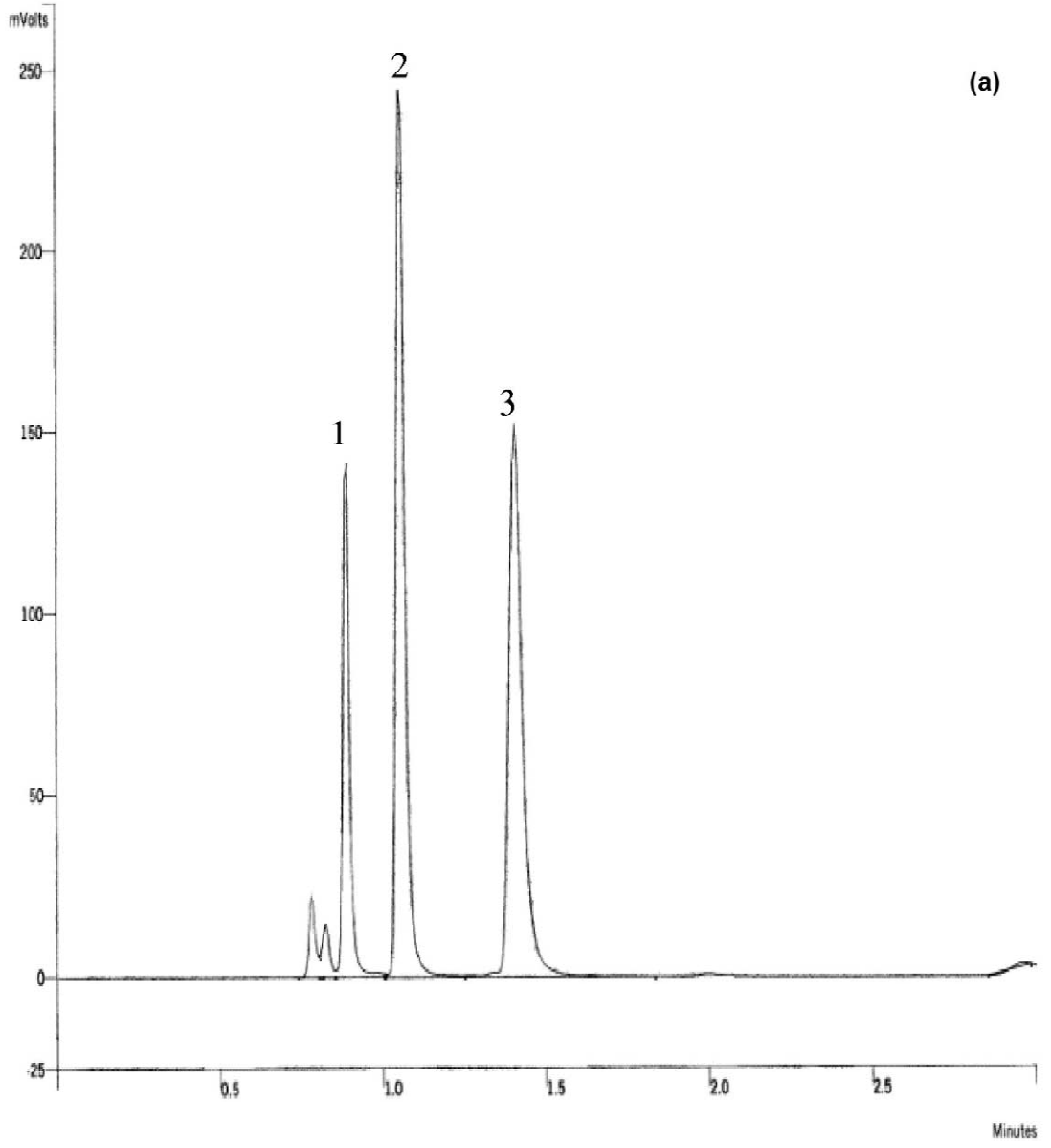

Fig. 2. Chromatograms of the target analytes obtained under optimum conditions for a direct injection of standards at 10 μg ml^{-1} (A) and a yogourt sample without standard addition (B) and with standard addition at 10 μg ml^{-1} (C). (1) Acetaldehyde; (2) acetone; (3) 2-propanol.

90 °C. For these reasons the temperature of the thermostat was adjusted to 80 °C.

3.1.2.3. Optimisation of the injection time

By the injection time of IV the volume of gas entering the chromatographic column can be controlled. For long injection times high but very wide and overlapped peaks were obtained, whereas very short injection times gave better defined chromatographic peaks but smaller. The range studied for this variable was 5–20 s and the best signal, which means well defined and high peaks, was obtained for 10 s. Fig. 5 shows the influence of this variable on the peak area.

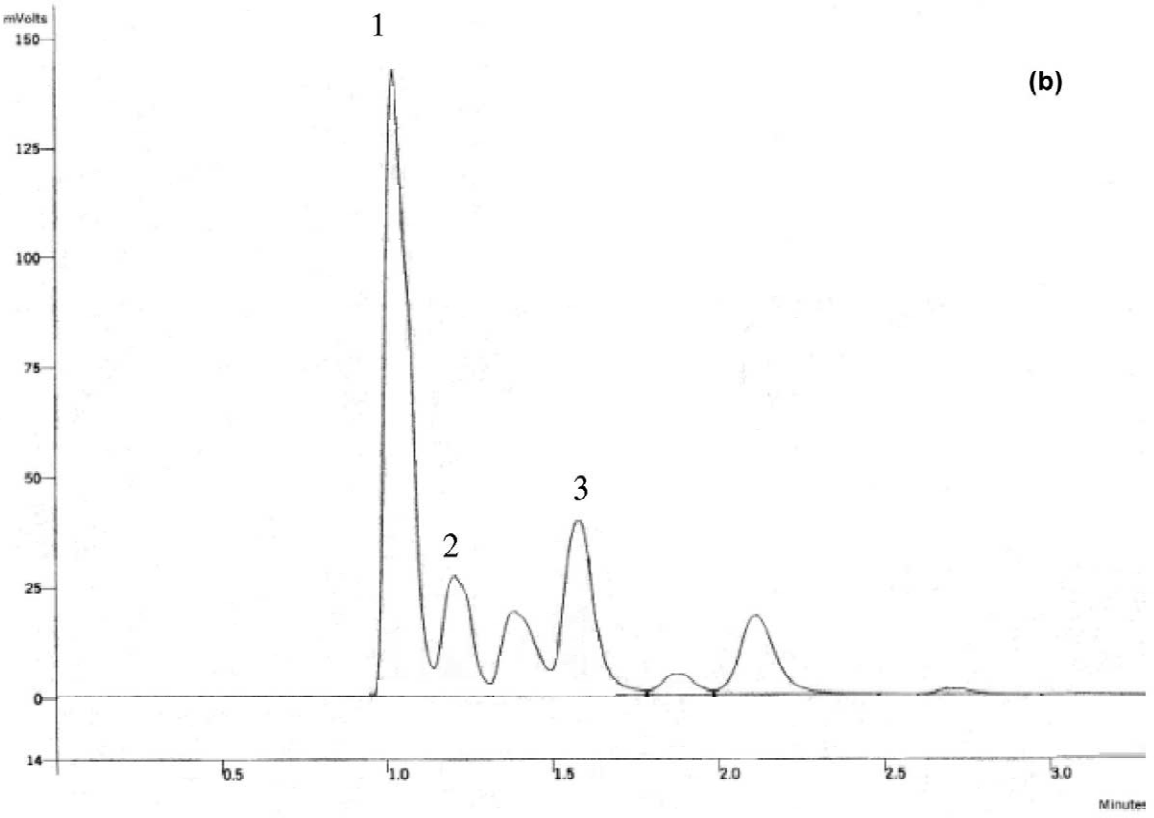

Fig. 2. (*continued*)

3.1.3. Features of the method

The features of the proposed method were estimated using milk samples spiked with lactic acid to simulate yoghurt and 20 μg ml^{-1} 2-propanol as internal standard. The results obtained in terms of calibration equations, correlation coefficients, linear ranges, detection limits and repeatability, calculated with seven samples and expressed as relative standard deviation, are summarised in Table 2.

3.1.4. Evaluation of the precision of the method

To evaluate the precision of the proposed method, within-laboratory reproducibility and repeatability were estimated in a single experimental setup with duplicates [14]. The experiments were carried out using milk samples modified with lactic acid and spiked at 10 μg ml^{-1} of each analyte and 20 μg ml^{-1} 2-propanol. In all experiments, the optimal values obtained for the variables were used. Two measurement of each analyte per day were carried out on 7 days.

To determine the variance due to the between-day effect, Eqs. (1) and (2) were used:

$$s^2_{\text{between}} = (\text{MS}_{\text{between}} - \text{MS}_{\text{within}})/n_j \quad (1)$$

where n_j is the number of replicates per day. The within-laboratory reproducibility, s^2_{WR}, is equal to:

$$s^2_{\text{WR}} = s^2_{\text{r}} + s^2_{\text{between}} \quad (2)$$

As shown in Tables 3 and 4, the repeatability, expressed as relative standard deviation, was 3.8 and 4.6% for acetaldehyde and acetone, respectively; meanwhile the within-laboratory reproducibility was 4.3 and 5.0% for acetaldehyde and acetone, respectively.

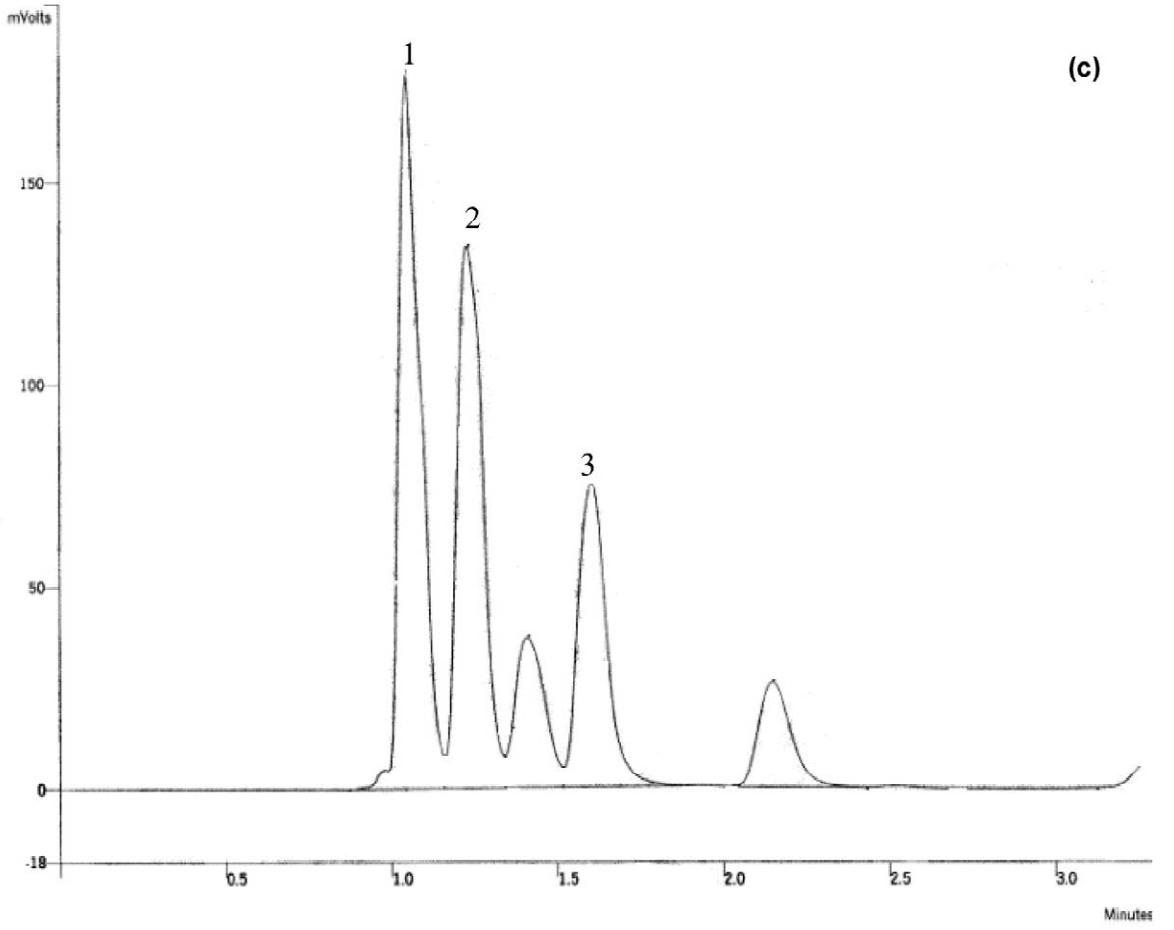

Fig. 2. (*continued*)

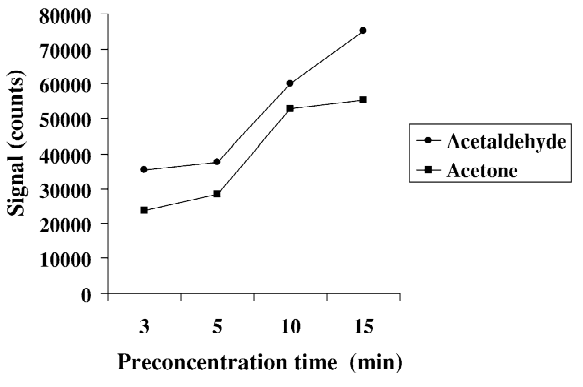

Fig. 3. Variation of the signal with the preconcentration time for the target analytes.

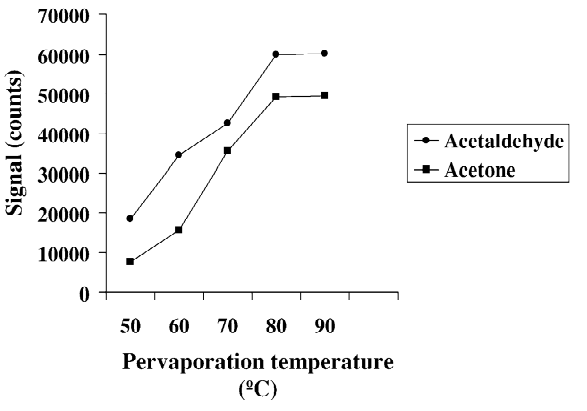

Fig. 4. Variation of the signal with the pervaporation temperature for the target analytes.

Table 2
Features of the method

Analyte	Calibration equations[a]	r ($n=6$)	Linear range ($\mu g\ ml^{-1}$)	Detection limit ($\mu g\ ml^{-1}$)
Acetaldehyde	$y=-0.01+0.16x$	0.9999	0.1–100	0.03
Acetone	$y=0.09+0.14x$	0.9998	0.8–100	0.30

[a] Where y indicates the peak area and x is the concentration of the analytes in $\mu g\ ml^{-1}$.

Table 3
Experimental setup and results obtained from the evaluation of the precision of the proposed method: analysis of variance table

Compound	Source	Sum of squares	Degrees of freedom	Mean of squares
Acetaldehyde	Between days	70.32	6	11.72
	Within days	26.54	7	3.79
	Total	96.86	13	
Acetone	Between days	95.86	6	13.69
	Within days	32.12	7	4.59
	Total	127.98	13	

Table 4
Repeatability relative standard deviation and within-laboratory reproducibility relative standard deviation obtained for each analyte

Parameter	Acetaldehyde	Acetone
$\%s_r$	3.79	4.59
$\%s_{WR}$	4.28	5.06

$\%s_r$, repeatability relative standard deviation; $\%s_{WR}$, within-laboratory relative standard deviation.

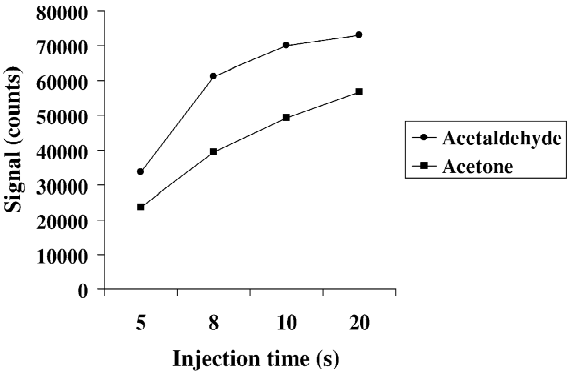

Fig. 5. Variation of the signal with the injection time for the target analytes.

Table 5
Application of the method to natural samples

Sample	Acetaldehyde		Acetone	
	SAM[a]	ISM[b]	SAM[a]	ISM[b]
Yoghurt	17.87	18.00±0.06	4.86	4.77±0.04
Actimel	8.19	8.10±0.04	1.07	1.05±0.03
Apple juice	1.00	0.94±0.01	0.00	0.00±0.00
Peach juice	3.44	3.31±0.10	1.50	1.36±0.05
Tomato juice	2.44	2.37±0.06	1.50	1.57±0.02

SAM, standard addition method; ISM, internal standard method.

3.1.5. Application of the method to natural samples

In order both to validate and show the applicability of the proposed method for slurry samples, it was used for the determination of the analytes in different kind of fruit juice, Actimel and yoghurt. Pretreatment of the sample was unnecessary in all cases. Three replicates were obtained for each sample (spiked with 20 $\mu g\ ml^{-1}$ 2-propanol) and the results were compared with those provided by the standard addition method, in which two different amounts of the analytes were added to aliquots of the same sample. As can be seen in Table 5, the results obtained by both methods are in agreement. Fig. 2B,C shows the chromatograms of a yoghurt sample before and after standard addition, respectively, as compared with the chromatogram of a direct injection of standards of both analytes (Fig. 2A). As can be seen, the retention times are slightly different for samples and standard due to the different time for transporting to the top of the chromatographic column (direct injection of the standards on the top of the column and transport by the carrier gas from the

acceptor chamber of the pervaporated compounds from the samples.

4. Conclusions

This is the first time that pervaporation has been used instead of dynamic headspace (purge and trap) for slurries from solid or semi-solid samples with fully automated introduction of the sample into the pervaporator. In previous works with solid and semi-solid samples pervaporation was used as alternative to headspace but in a hybrid discontinuous–continuous process as the sample was weighed in the donor chamber [15]. The proposed method requires a simple and inexpensive equipment and the preconcentrating effect in the loop of the auxiliary injection valve (IV) makes possible the determination of small quantities of the analytes. Moreover, higher preconcentration factors can be achieved by locating a sorption minicolumn at the outlet of the pervaporator acceptor chamber, if required. In order to enlarge the field of application to non-volatile compounds, on-line pre-pervaporation reactions can be developed, in a way similar to those carried out in the lower chamber with solid samples [16].

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