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Journal of Chromatography A 819 (1998) 25–33

JOURNAL OF CHROMATOGRAPHY A

Review

Synergistic approaches based on nonchromatographic continuous separation techniques (solid-phase extraction and pervaporation) and chromatography couplings

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Abstract

Approaches based on continuous separation units coupled to either liquid or gas chromatography for improving the features of analytical methods are proposed. Examples of solid-phase separation–liquid chromatography for the determination of fat-soluble vitamins and their metabolites in clinical samples, and pervaporation–gas chromatography for the determination of volatile compounds in solid environmental samples are described. The clean-up and preconcentration effect achieved by the former coupling and the easy and effective solid-sample pretreatment in the latter clearly show their utility. The use of pervaporation as an advantageous alternative to headspace is demonstrated.

Keywords: Sampling handling; Pervaporation; Vitamins; Organomercuric compounds; Volatile organic compounds

Contents

1. Introduction

Despite the well-known discriminatory power of chromatographic techniques, the complexity of natural samples often calls for prior separation steps aimed at obtaining a simpler, treated sample to be introduced into the chromatograph. Nonchromatographic continuous separation techniques constitute the most suitable way for development of the pretreatment steps as they can be coupled on-line with the chromatograph. These arrangements enable full automation of the analytical process providing the appropriate interface is available [1]. Two types of nonchromatographic continuous separation techniques can be distinguished depending on the features of the target analytes.

(a) Nonvolatile species can be removed from either solid or liquid matrices by a number of

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0021-9673/98/$19.00
PII: S0021-9673(98)00431-2

continuous techniques which range from leaching techniques (usually called extraction techniques), such as supercritical fluid extraction (SFE) [2], accelerated solvent extraction (ASE) [3], microwave-assisted extraction [4], and ultrasound-assisted extraction [5], to dialysis [6], liquid–liquid extraction (LLE) [7] and liquid–solid extraction (known as solid-phase extraction, SPE) [8], including both ion exchange and sorption; stripping, continuous precipitation, etc.

SPE is one of the separation techniques which is gaining more and more popularity as a step prior to chromatography both in a discontinuous (particularly at the microscale level, solid-phase microextraction, SPME) [9] and continuous fashion. The expansion of the use of continuous SPE has promoted the commercialization of automated and semiautomated devices for a faster and unattended development of pretreatment steps, the aim of which is sample clean-up, analyte(s) preconcentration or both. They can be easily coupled to liquid chromatography (LC).

(b) Volatile analytes have been traditionally isolated from liquid matrices by gas-diffusion and distillation. Cold mercury vapour and hydride generation are techniques restricted to certain analytes, the latter enabling the conversion of nonvolatile species into volatile products. A well-spread, commercialized technique applicable to both liquid and solid samples is headspace, both in the conventional and purge and trap modes [10]. This technique has for long been used as a useful tool for removal of volatile species prior to their introduction into gas chromatography (GC).

Analytical pervaporation has emerged as a new laboratory-scale continuous separation technique which opens promising expectations in the development of separation procedures for volatile analytes (or volatile reaction products) from both liquid and solid samples [11]. Semisolid materials and liquids with solids in suspension can also be introduced into a pervaporation unit.

Both continuous SPE and pervaporation have been used by our research team. The former has been coupled to a liquid chromatograph for the clean-up and preconcentration of nonvolatile analytes (namely, vitamins and their metabolites) from clinical samples. The latter has been used for pretreatment of solid samples from which volatile species were removed and led to a gas chromatograph coupled to the pervaporator.

2. Solid-phase extraction–liquid chromatography

The quantitation of vitamins and their hydroxy metabolites in human fluids has been considered one of the most difficult goals in clinical chemistry. According to Shimada and Kobayashi [12] and Ortiz Boyer et al. [13], the low concentration of circulating hydroxy metabolites, the presence of a number of metabolites which exhibit similar chemical behaviour to one another, the large amount of other related neutral lipids and the high instability of their chemical structures in the presence of UV light and heat, are the main reasons which justify this assertion.

Sample pretreatments proposed so far have been based on the use of different procedures such as deproteinization [14–16], saponification [17–20], LLE [21–23] or SPE [20,24–31]. The efficiency of the procedures depends on the analytical requirements and nature of samples and target analytes. The use of SPE has proved to be the most efficient way for increasing clean-up and preconcentration in this field. The marriage between SPE and LC constitutes an effective tool for the quantitation of very low concentrations of analytes in the clinical field.

A clean-up/preconcentration–LC method for the determination of vitamin D_3 hydroxy metabolites has been developed using the integrated system for continuous clean-up/preconcentration and HPLC separation shown in Fig. 1 [29,31]. The clean-up/preconcentration subsystem permits simultaneous clean-up of the sample and concentration of the target analytes prior to their injection into the separation subsystem. The aminopropyl-silica preconcentration minicolumn inserted in the sample loop of a low-pressure injection valve was conditioned by sequential washing with methanol, 50 m*M* phosphate buffer, pH 6.5 and water for 2 min. Then, the pretreated sample was passed through the minicolumn (SV in the position for sample aspiration), and both the target analytes and interferents with similar features were retained. After a 20-min preconcentration time, the minicolumn was sequentially washed in 2-min cycles with 50 m*M* phosphate buffer, pH 6.5 and methanol–water (70:30, v/v) in

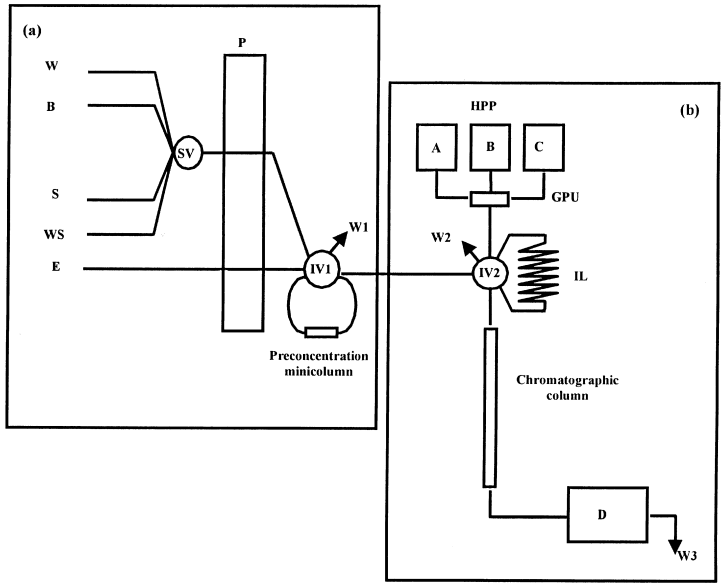

Fig. 1. Integrated continuous assembly for the determination of vitamin D_3 hydroxy metabolites. (a) Clean-up/preconcentration flow subsystem, (b) modular chromatograph for separation and UV detector. P, peristaltic pump; SV, selecting valve; W, water; B, buffer; S, sample; WS, washing solution; E, eluent; IV_1, low-pressure injection valve; HPP, high-pressure pump; A, B, C, solvent reservoirs; GPU, gradient programmable unit; IV_2, high-pressure injection valve; IL, injection loop; D, detector and W1, W2 and W3, waste.

order to remove the interferents by switching valve SV. Then, the analytes were eluted with a methanol solution by switching the low-pressure injection valve and driven to the injection valve of the chromatograph. This valve had been modified by changing the conventional loop for a 100-cm length tubing of 0.25 mm I.D. The volume of methanol containing the eluted analytes was trapped by switching the HPLC injection valve 95 s after switching the low-pressure injection valve and the analytes were thus introduced into the column. The gradient started simultaneously with injection. The analytes were removed from the column as a function of their relative polarity, monitored photometrically at 270 nm and the data treated for presentation.

The method provides enough sensitivity and precision for the direct determination of 24,25-$(OH)_2$-D_3 and 25-(OH)-D_3 in human plasma with excellent preconcentration factors. However, the sensitivity is insufficient for quantitation of 1,25-$(OH)_2$-vitamin D_3 (normal values in plasma <60 pg ml^{-1}). The method was validated by applying it to the determination of these compounds in plasma with acceptable recoveries in all instances (R.S.D. values between 86 and 104%).

The synergistic effect of the marriage between SPE and chromatographic separation with UV detection is demonstrated by the following advantages over previous methods: (a) shorter sample pretreatment with lower cost per analysis; (b) continuous clean-up and preconcentration of the target analytes; (c) miniaturization of the clean-up step which reduces sorbent consumption as the minicolumn can be reused for at least 200 times without loss of capacity and/or deterioration; (d) higher preconcentration factor, which makes the determination of 24,25-$(OH)_2$-D_3 and 25-OH-D_3 in plasma possible; (e) simpler and more versatile automation of the experimental set-up which includes easy adaptation to commercial devices such as chromatographs, low-pressure injection valves, etc.; and (f) dramatic reduction of the derivatization cost as compared with techniques such as MS and radioimmuno assay.

The previous method has been expanded and applied to the determination of vitamins A, D_2, D_3, E, K_1, K_3 and several hydroxy metabolites of

vitamin D_3 [namely, 25-(OH)-D_3, 24,25-$(OH)_2$-D_3 and 1,25-$(OH)_2$-D_3)] [32]. The modified procedure required the introduction of several changes in the above described clean-up/preconcentration procedure in order to both improve the retention capacity of other fat soluble vitamins in the minicolumn and facilitate the elution towards the separation subsystem.

The manifold and the procedure used were similar to that depicted in Fig. 1 and quite similar to that described above, respectively. The following compromises were adopted and differences were introduced.

(1) The target analytes exhibited absorption maxima that made a compromise wavelength for monitoring necessary. Vitamins (A, K_1, K_3, D_2, D_3 and their metabolites 24,25-$(OH)_2$-D_3, 1,25-$(OH)_2$-D_3 and 25-(OH)-D_3 exhibit absorption maxima within 250–290 nm. The maximum of vitamin A appears at longer wavelength (325 nm), but it also absorbs appreciably at 270 nm; so this value of the instrumental variable was selected for monitoring.

(2) In order to obtain a good separation of the target analytes the gradient and the composition of the mobile phase were optimized. The influence of a polarity modifier was studied using methanol, acetonitrile and isopropanol mixtures. An isopropanol–methanol mixture provided an excellent separation of the target analytes. An acetonitrile–50 m*M* phosphate buffer, pH 6.5 (20:80, v/v) mixture was used as initial mobile phase, and a linear gradient was programmed to obtain a methanol–isopropanol (90:10, v/v) mobile phase in 1.7 min. The system was finally stabilized with methanol–isopropanol (90:10, v/v) for 9 min. Under these conditions the analytes were separated in 28 min. Fig. 2 shows the chromatograms obtained from a solution containing 100 ng ml^{-1} of each analyte with and without preconcentration step. The preconcentration factor, established as the ratio between the peak area obtained in both cases, was excellent in all instances (in the range of 55.2% for 1,25-$(OH)_2$-D_3 to 8.1% for vitamin K_3).

The clean-up/preconcentration step endows the method with enough sensitivity for the determination of vitamins E, D_2, D_3, 24,25-$(OH)_2$-D_3, 25-(OH)-D_3, K_1 and K_3 in human plasma, as their normal levels (even at concentrations much lower than the limits at which a deficiency of these vitamins can be determined. In short, the method exhibits linear ranges between 0.005 and 100 ng ml^{-1} for vitamins D_2, D_3 and their hydroxy metabolites; between 0.1

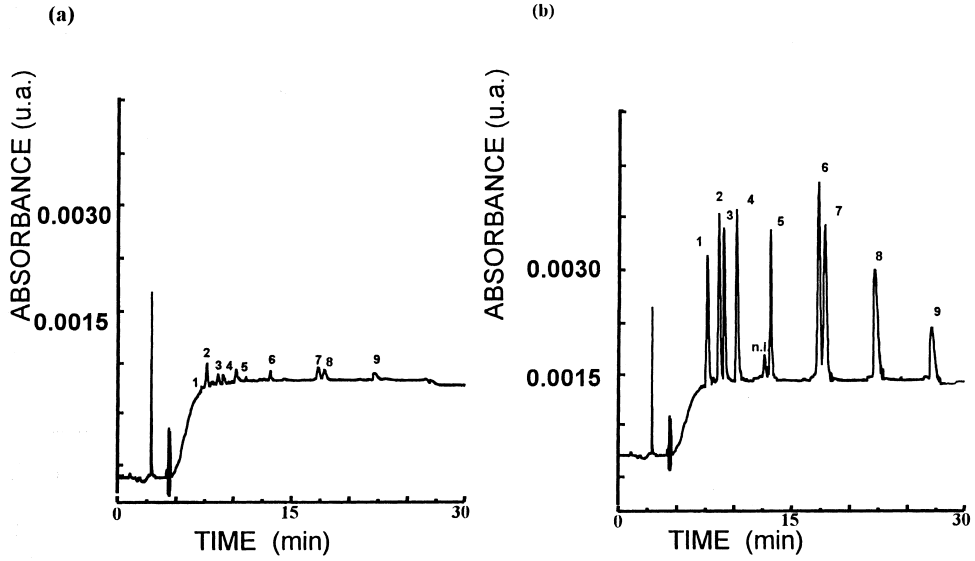

Fig. 2. Chromatograms obtained for 100 ng ml^{-1} of each analyte (a) without continuous preconcentration and (b) after preconcentration step. 1=Vitamin K_3; 2=24,25-$(OH)_2$-vitamin D_3; 3=1,25-$(OH)_2$-vitamin D_3; 4=25-(OH)-vitamin D_3; 5=vitamin A; 6=vitamin D_2; 7=vitamin D_3; 8=vitamin E and 9=vitamin K_1; n.i.=not identified.

and 100 ng ml^{-1} for vitamins A, K_1 and K_3 and between 1 and 100 ng ml^{-1} for vitamin E; with excellent regression coefficients ($\geq$0.99).

The proposed method was validated by applying it to the determination of the analytes in plasma in two ways, namely: (a) determination of the analytes in ten plasma samples from a hospital and (b) study of the recovery afforded after addition of two standard solutions containing 1 and 10 ng ml^{-1} of each analyte in three plasma pools. Acceptable recoveries, between 78 and 109% were obtained in all instances. The method provides enough information for quantitation of the main fat-soluble vitamins in human samples.

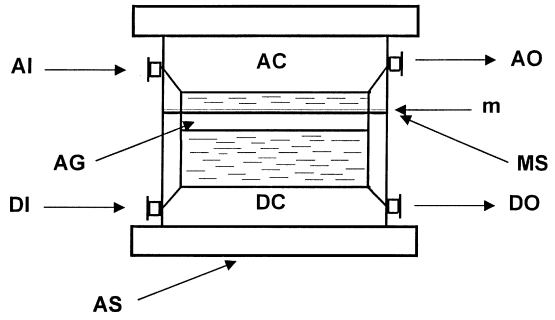

Fig. 3. Conventional pervaporation module. AC, acceptor chamber; DC, donor chamber; MS, membrane support; m, membrane; AG, air gap; AS, aluminium support; AI/AO, acceptor inlet/outlet; DI/DO, donor inlet/outlet.

3. Pervaporation–gas chromatography

Pervaporation, a membrane-based separation technique, has for long been used in the industry and recently proposed for analytical purposes [33,34]. Analytical pervaporation can be defined as the integration of an evaporation and gas diffusion steps in the same module. The volatile analyte (or its reaction product) present in a heated donor phase evaporates through a porous membrane and the vapours condenses in contact with a cool acceptor phase on the other side of the membrane. The temperature difference, which results in a vapour pressure difference across the membrane, is the driving force for the separation. A key characteristic of analytical pervaporation is the presence of an air gap between the sample in the donor chamber and the membrane, thus avoiding any contact between them and circumventing the problems associated with membrane clogging or deterioration. A cross-sectional view of a laboratory-made pervaporator is shown in Fig. 3 and its different parts have been described elsewhere [33,34]. The versatility of the design of an analytical pervaporator, attributed to its changeable donor volume and the air-gap present above the sample, has enabled its use with both liquid [35–37] and solid samples [4,38–41]. The configurations used are shown in Fig. 4.

The introduction of liquid samples to the lower, donor chamber of the pervaporation cell can be done either by means of a peristaltic pump (by continuous aspiration or by injection, using a low-pressure injection valve) or by a hypodermic syringe, through a septum located at the entrance of the chamber. The former sample introduction mode is employed when the sample is available in relatively large quantities, whereas the latter is used when it is scarce; in this case, the configuration used is a hybrid between a continuous and a discontinuous manifold.

The most interesting application of pervaporation is the direct introduction and treatment of solid samples in the separation unit [4,38–41]. The relatively big volume of the donor chamber, which can be further increased by location of spacers of adequate thickness, permits direct weighing of the solid, as well as addition of the reagents necessary for analyte leaching and its possible derivatization by injection through a septum located at the entrance of the lower chamber, after the separation cell is tightly closed. The analytical pervaporator hence works simultaneously as a leaching, derivatization and separation vessel; sample manipulation is avoided or minimized and therefore no losses of analyte occur. The analytical manifold is similar to that used for scarce liquid samples, that is a combined continuous/discontinuous approach. This has been the configuration coupled to a gas chromatograph for removal of volatile species. In fact, when the pervaporator is coupled to a gas chromatograph, it works in a similar way to a headspace device, but with substantial advantages with respect to both static and dynamic(or purge and trap) headspace sampling, since the small air gap present above the sample surface in the donor chamber requires very small amounts of the

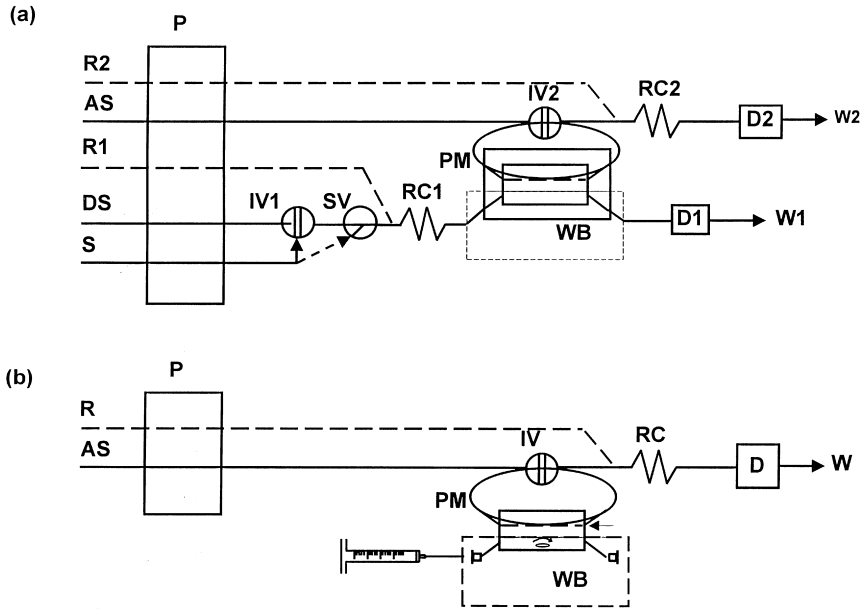

Fig. 4. Dynamic configuration-pervaporation assemblies (a) for liquid samples and (b) for solid samples. P, peristaltic pump; IV, injection valve; SV, selection valve; RC, reaction coil; PM, pervaporation module; WB, water bath; D, detector; w, waste; S, sample; DS, donor stream; AC, acceptor stream; R, derivatization reagent.

analytes for establishing equilibrium conditions with the solid or liquid phase and so short equilibration times are necessary. Moreover, it can be easily coupled to a flow system, permitting the automation of the whole process and avoiding excessive sample manipulation and, therefore, losses of the analyte; in addition, the use of a hydrophobic membrane avoids the passage of water vapours to the chromatographic column and the cost of the analysis is low, as the separation module is made of methacrylate, which is a relatively cheap material. An important advantage of the method over the static headspace mode is the fact that the time of analysis is shortened, as equilibrium conditions have not to be reached, whereas compared with the purge and trap mode, there is no need for a water condenser, as the presence of the hydrophobic separation membrane does not allow the passage of water through it.

An example of the use of pervaporation as an alternative to headspace sampling is the method proposed for the determination of organomercuric compounds (namely Me_2Hg, Et_2Hg and MeHgCl) in solid samples by GC–atomic fluorescence detection [41]. The experimental assembly used is shown in Fig. 5A. The solid sample is directly weighed in the donor chamber of the pervaporation module, which is shut after locating the hydrophobic membrane (PTFE) and the acceptor chamber. The module is placed in a waterbath at an elevated temperature for a certain time, while a flow of argon is passing through the acceptor chamber. The mercury species in the solid sample pervaporate through the membrane, collected in the argon stream and driven to a Tenax minicolumn situated in the loop of a low-pressure rotary injection valve placed in an ice bath, where they are preconcentrated during a preset time. The column is then placed in a muffle oven at a high temperature for a few seconds, followed by switching the valve to the ‘inject’ position, thus allowing a flow of helium to pass through the column and bring the desorbed species first to the injector of the gas chromatograph and, subsequently, to the semi-capillary HP-1 chromatographic column. The separated species leaving the chromatographic column are then passed through a pyrolysis unit, where the compounds are broken down to elemental mercury. This stream is then mixed with a make-up gas and a second flow stream of argon is used as a sheath gas,

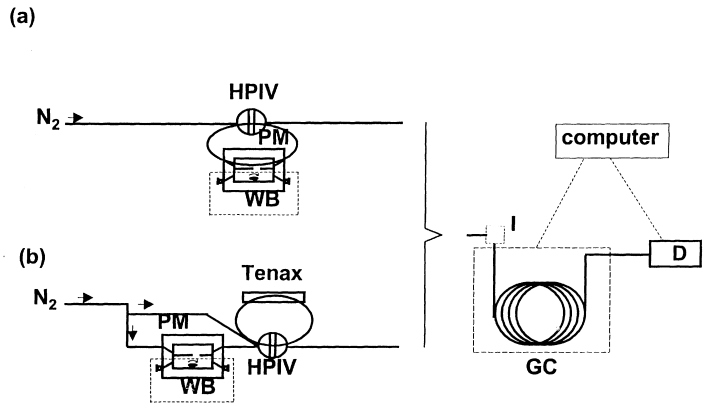

Fig. 5. Gas chromatography–pervaporation coupling for determinations in solid samples; (a) without and (b) with preconcentration. LPIV, low pressure injection valve; HPIV, high pressure injection valve; I, injector (gas chromatograph); GC, gas chromatograph; D, detector. Other abbreviations as in Fig. 4. A pyrolyser is located between the chromatographic column and the detector for mercury speciation analysis.

improving significantly the reproducibility of the results. The usefulness of the proposed assembly for this task is demonstrated by the good linear ranges for the three analytes, the low detection limits and the validation study, which includes both a sandy soil and a sewage sludge certified reference material, and with good precision, expressed as R.S.D. The separation process of Me_2Hg, Et_2Hg and MeHgCl is highly efficient, as the continuous removal of the analytes through the membrane from the air gap above the sample surface displaces the mass-transfer equilibrium. In addition, the speciation of organomercuric compounds in solid samples is achieved without any sample pretreatment or derivatization, thus avoiding losses of the analyte and simplifying the whole analytical procedure.

The versatility of the pervaporator–GC coupling allows its easy adaptation to the target system. Such is the case of the determination of volatile organic compounds (VOCs) in solid samples [40]. The preparation of the pervaporation cell is done in the same way as for the organomercuric compounds described before. The location of an HPLC injection valve between the inlet of the carrier gas to the split ratio flow controller and the inlet of the carrier gas to the injector of the gas chromatograph permits working in two modes, as shown in Fig. 5A and B. In the first mode, the pervaporation cell is connected to the loop positions of the injection valve and the latter is turned to the ‘inject’ position as soon as the cell is placed in the thermostatic magnetic stirrer, allowing the passage of the nitrogen (carrier) stream through the acceptor chamber and driving the analytes directly to the capillary TRB-1 chromatographic column and subsequently to the electron-capture detector. The calibration curves for the three analytes studied, namely chloroform, carbon tetrachloride and trichloroethene, are characterized by wide linear ranges and low limits of detection. In the second mode, the flow of nitrogen from the gas cylinder is divided into two streams, one of them entering the injection valve as the carrier stream and passing through the GC column and the other passing through the acceptor chamber of the pervaporation module, the outlet of which is connected to the sample inlet of the injection valve. A Tenax minicolumn is situated at the loop of the injection valve, in which, when the HPLC injection valve is in the ‘filling’ position, the pervaporated analytes are preconcentrated for a previously determined time. The desorption of the analytes from the minicolumn is then achieved in the same way as for the organomercuric compounds. The injection valve is switched to the ‘inject’ position and the desorbed analytes are driven to the chromatographic column, without passing through the injector of the gas chromatograph. In this case, the linear ranges are shifted to lower values, and the detection limits significantly improved. The method

can be easily automated with minimal costs, permitting the introduction of the sample either continuously or by injection, and, when compared to both static and dynamic headspace modes already applied to the determination of the same analytes in solid matrices, it improves significantly both the recovery and repeatability.

The main shortcoming involved in the use of the pervaporation separation process is that it can only be applied to the determination of volatile analytes and to the determination of analytes, which can be transformed to volatile products by means of a (bio)chemical reaction.

4. Conclusions

Nonchromatographic continuous separation techniques are versatile enough to provide appropriate solutions to the drawbacks which appear in the analysis of natural samples, both liquids and solids, prior to their introduction into a chromatograph.

Acknowledgements

The Comisión Interministerial de Ciencia y Tecnología (CICyT) is thanked for financial support (project No. PB97/0505).

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