ELSEVIER

PII: S0308-8146(98)00001-6

Food Chemistry, Vol. 63, No. 2, pp. 281-286, 1998

Printed in Great Britain
0308-8146/98 $19.00+0.00
Analytical, Nutritional and Clinical Methods Section

Analysis of volatile fruit components by headspace solid-phase microextraction

Elena Ibáñez, Sara López-Sebastián, Elena Ramos, Javier Tabera & Guillermo Reglero*

Instituto de Fermentaciones Industriales, CSIC. c/Juan de la Cierva, 3, 28006 Madrid, Spain

(Received 3 February 1997; revised version received and accepted 20 October 1997)

A method based on headspace solid-phase microextraction (HS-SPME) has been developed for the analysis of volatile compounds in fruits. Conditions of sampling have been tuned. Repeatability and recoveries obtained with this method have been determined by using a mixture of typical components of fruit aroma. Several fruits, such as raspberries, strawberries, blackberries, banana and mango, have been analysed by the proposed method. The patterns obtained include compounds typically found in fresh fruits and compounds formed during processing or storage. The results showed the use of the technique for fruit characterization and its potential as a routine method for analyzing changes in key flavour compounds during different fruit processing regimes. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Flavour study in different foods has been of increasing interest due to its relationship with the quality of a food product. In the case of fruits, aroma is one of the most appreciated characteristics. Fruit flavour is particularly sensitive to compositional alterations. The volatile compounds that form the fruit flavour are produced through metabolic pathways during ripening, harvest, post-harvest and storage and depend on many factors related to the species, variety and type of technological treatment (Rizzolo *et al.*, 1992). Sometimes it is important to know the typical chromatographic pattern of a fresh product in order to identify changes in the volatile composition. That way, the modifications produced during, for example, processing (Douillard and Guichard, 1990) or storage (Shamaila *et al.*, 1992) can be detected.

Outstanding works of identification of volatile components of different fruits and studies of the effect of processing on fruit flavour have been carried out (Robertson *et al.*, 1995; Koulibaly *et al.*, 1992; Ueda *et al.*, 1992; Boresjsza-Wysocky *et al.*, 1992; Young *et al.*, 1992; Shamaila *et al.*, 1992; Macku and Shibamoto, 1991; Douillard and Guichard, 1990; Georgilopoulos and Gallois, 1987; Macku and Jennings, 1987).

Different methods have been used for flavour analysis. The most typically utilised for extraction and preconcentration are headspace techniques (Macku and Jennings, 1987), purge-and-trap (Miszczak *et al.*, 1995), liquid–liquid extraction (Kok *et al.*, 1987) and simultaneous distillation and extraction (Blanch *et al.*, 1991). Methods based on the use of solvents have severe drawbacks, such as the possibility of sample contamination, the lost of analytes during the concentration process and environmental problems related to the use of large amounts of organic solvents. On the other hand, headspace sampling and purge-and-trap techniques are simpler, less laborious, faster and, even more especially, solvent-free techniques. Nevertheless these methods have some disadvantages, such as the risk of cross-contamination and the use of high flow rates that can sometimes be incompatible with the on-line operation, mainly when GC-MS is used with narrow bore columns (Shirey, 1994).

In the fruit processing industry, good analytical methods are crucial to the success of any process control and/or product development studies. Quality control can be difficult if inappropriate methods are used. In these particular cases, a rapid, simple and inexpensive technique for extracting and preconcentrating fruit flavour can be very useful. One such technique is a new sample preparation method called Solid-Phase Microextraction (SPME) (Arthur and Pawliszyn, 1990). SPME is a solvent-free, inexpensive, rapid and versatile method for the extraction of organic compounds. It consists of a fused-silica fibre, coated with a polymeric stationary phase introduced into a liquid or gas sample. The method involves two processes: the partitioning of the analytes between the coating and the sample and the

---

*To whom correspondence should be addressed.

thermal desorption of the analytes into the gas chromatograph. This method has been used by several authors for the analysis of volatile compounds in food samples (Hawthorne *et al.*, 1992; Pelusio *et al.*, 1995), demonstrating its utility for flavour analysis. Also, the use of an SPME device for headspace and liquid extraction of some real samples, such as ground coffee, fruit juice beverage and butter flavour, has been studied (Yang and Peppard, 1994).

The objective of the present work was to demonstrate the utility of the HS-SPME method for fruit flavour analysis. Several fruits, such as raspberries, strawberries, blackberries, banana and mango, have been analysed by the proposed method. The patterns obtained include compounds typically found in fresh fruits and compounds formed during processing or storage.

MATERIALS AND METHODS

Headspace solid-phase microextraction

An SPME holder (Supelco, Bellefonte, PA, USA) was used to perform the experiments. A fused silica fibre, coated with a 100 μm layer of dimethylpolysiloxane, was chosen to extract the volatile components of the fruits.

SPME conditions selection

A sample of commercial dry milled leaves of rosemary (*Rosmarinus officinalis* L.) was used in order to select the variables that influence the HS-SPME process and to tune the operating conditions. This sample was used since it contains volatile compounds of similar chemical nature to those generally found in the aroma of fruits, is an homogeneous material, easy to conserve and provides reproducible results.

The fibre was exposed to the headspace of 0.1 g of sample placed into a 20 ml vial and capped with a plastic film. In order to evaluate the possibilities of the method for the recovery of the compounds under study, the experiments presented in Table 1 were carried out. The desorption of the compounds in the fibre took place in the GC injector at 200°C for 15 min. Table 1 also includes the sum of the peak areas corresponding to volatile compounds in the chromatograms obtained under the specified extraction conditions. As can be seen, the highest were obtained at an extraction temperature of 60°C and extraction time 30 min. Increasing temperature has been reported to be a good way to improve recoveries (Zhang *et al.*, 1994). In the present work, 60°C and 30 min. were used to extract the standard mixture and to study the recovery and standard deviation of the method. However, to analyse the typical fruit flavour detected by the consumer, the extraction temperature must be similar to that of normal eating conditions. Consequently, the analysis of the real fruit samples was carried out at 30°C.

Table 1. Experiments for SPME conditions selection

Extraction temperature (°C)	Extraction time (min)	Sum areas (Area counts)
30	30	6765457
30	15	7559201
60	30	12411132
60	15	8855828

Subsequent experiments were done to evaluate the effect of an equilibration time prior to extraction, i.e. prior to the exposure of the fibre to the headspace of the sample, and the stirring of the sample with ultrasound during the extraction. Results indicated that there was almost no difference in the sum of the areas when a 30 min equilibration time was used (Total chromatogram area = 12.411.132) compared to the analysis performed without previous equilibration time (Total chromatogram area = 13.161.896). These results imply that the equilibrium between the solid phase and the headspace is established during the extraction time; therefore, an extra equilibration time is not required. Likewise, stirring did not improve the sum of the areas obtained. Therefore, neither equilibration time prior to extraction nor stirring, were utilised in the experimental conditions.

Standard mixture

A mixture of volatile compounds typically identified in fruits was prepared by adding the same amount of the following pure standards: propyl-acetate, 3-methyl-butanol, isoamyl-acetate, benzaldehyde, ethyl-hexanoate, isoamyl-butyrate, 1-octanol and 2-phenyl-ethanol of GC grade purchased from Merck (Darmstadt, Germany).

Standard mixture extraction

0.8 μl of the pure standard solution were placed into a 20 ml vial and capped with a plastic film. The fibre was exposed to the headspace under the conditions chosen as the best for volatile component extraction: sample temperature 60°C, extraction time 30 min.

Samples

Fresh raspberries, strawberries, blackberries, banana and mango were obtained commercially from a local store. 1 g of fruit was placed in a 20 ml vial and extracted for 30 min at 30°C.

Instrumental analysis

A Perkin–Elmer Model 8500 gas chromatograph equipped with a PTV injector and FID detector was used to perform the analysis. The system was coupled to a Model 2600 chromatography software system (Perkin–

Elmer Nelson Analytical). A 50 m×0.25 mm i.d. fused-silica capillary column (Chrompack, Middelburg, The Netherlands) coated with a 0.25 μm layer of CP-Sil-5 CB was used. Helium was the carrier gas. Thermal desorption of the compounds from the fibre coating took place in the GC injector at 200°C for 15 min in splitless mode for 5 min. The detector operated at 250°C. The oven temperature was programmed from 50°C (3 min constant temperature) to 250°C at 5°C min^{-1}. The final temperature was maintained for 17 min.

GC/MS analysis was carried out by coupling the gas chromatograph described above to a Perkin–Elmer ITD-50 ion trap detector (EI 70 eV). Capillary column and chromatographic program used as mentioned previously. Compounds were identified by comparison of the spectra with those in a general purpose library. Moreover, the identity of the components was confirmed by matching their mass spectrometric data with those obtained from the same equipment and corresponding to authentic reference compounds.

RESULTS AND DISCUSSION

Recovery of volatiles. Standard deviation

The standard mixture of 8 compounds typically found in fruit aroma was used to test both the recoveries and the repeatability obtained by using HS-SPME, as described in the Materials and Methods section. Recoveries were obtained by comparing the areas of the compounds extracted using HS-SPME with the areas obtained by direct injection of 0.8 μl of the pure standard mixture into the injector of the GC.

Table 2 shows the average recoveries obtained for the standard mixture with a minimum of 8 replicates. The recoveries achieved range from 10 to 45% (3-methylbutanol and ethyl-hexanoate, respectively). Obviously, the main advantages of the technique are speed and simplicity. As already suggested (Van der Kooi and Noij, 1994), since SPME is based in the distribution equilibrium of the analyte in the stationary phase and the sample, a 100% extraction efficiency can never be attained. The same authors obtained recoveries of volatile hydrocarbons ranging from 4 to 25%. Also, the non-quantitative character of the SPME sampling has been recognised (Yang and Peppard, 1994). This can be explained by the fact that this technique is a single-batch process and the amount of an analyte adsorbed on the fibre is determined by the adsorption kinetics and by the distribution coefficient of the solute between the fibre and the sample.

Even though the real recoveries obtainable by HS-SPME are relatively low, this technique is valuable for routine flavour analysis due to its reproducibility, especially for quick screening of the volatile composition (Yang and Peppard, 1994). Table 3 shows the relative standard deviation for the standard mixture in terms of absolute and normalised areas. Results of the normalisation with respect to an internal standard (benzaldehyde) are also presented in Table 3. As can be seen, the relative standard deviations are acceptable, the average for the absolute areas being around 11%, while for the normalised areas, values around 8% were obtained. Similar results have been reported for the extraction of pyrazines, constituents of a wide variety of food flavours, by using HS or liquid SPME (Ibáñez and Bernhard, 1996). In their paper, these authors obtained coefficients of variation of 5% or lower for the pyrazines produced in a model reaction when the SPME extraction time was consistent.

On the other hand, it is important to consider that, even if good variation coefficients can be achieved when standard mixtures are used, the results that can be obtained using SPME are also strongly dependent on the sample matrix.

Flavour analysis of fruits

The method was applied to the analysis of several fruits, such as raspberries, strawberries, blackberries, banana and mango. Figure 1 shows the chromatograms of the berries and Fig. 2 show the chromatograms of banana and mango. The identity of the peaks identified by GC/MS is included. All compounds determined in the present research have been previously identified by others authors as components of flavour of the fruits studied.

Table 2. Recoveries obtained for the standard mixture in the conditions described in the text

Compound	HS-SPME recovery (%)
Propyl-acetate	12.5
3-methyl butanol	10.7
Isoamyl acetate	27.7
Benzaldehyde	26.1
Ethyl hexanoate	45.3
Isoamyl butanoate	42.8
2-phenylethanol	44.4
1-octanol	15.1

Table 3. Relative standard deviation of HS-SPME for the standard mixture

Compound	RSD (%) Absolute areas	RSD (%) Normalized Areas	RSD (%) Normalized benzaldehyde
Propyl-acetate	14.5	15.8	14.1
3-methyl butanol	10.3	5.7	6.7
Isoamyl acetate	13.1	9.3	6.1
Benzaldehyde	8.6	4.6	—
Ethyl hexanoate	13.1	7.2	6.0
Isoamyl butanoate	14.1	6.7	8.9
2-phenylethanol	5.9	4.0	7.0
1-octanol	12.3	13.7	16.9

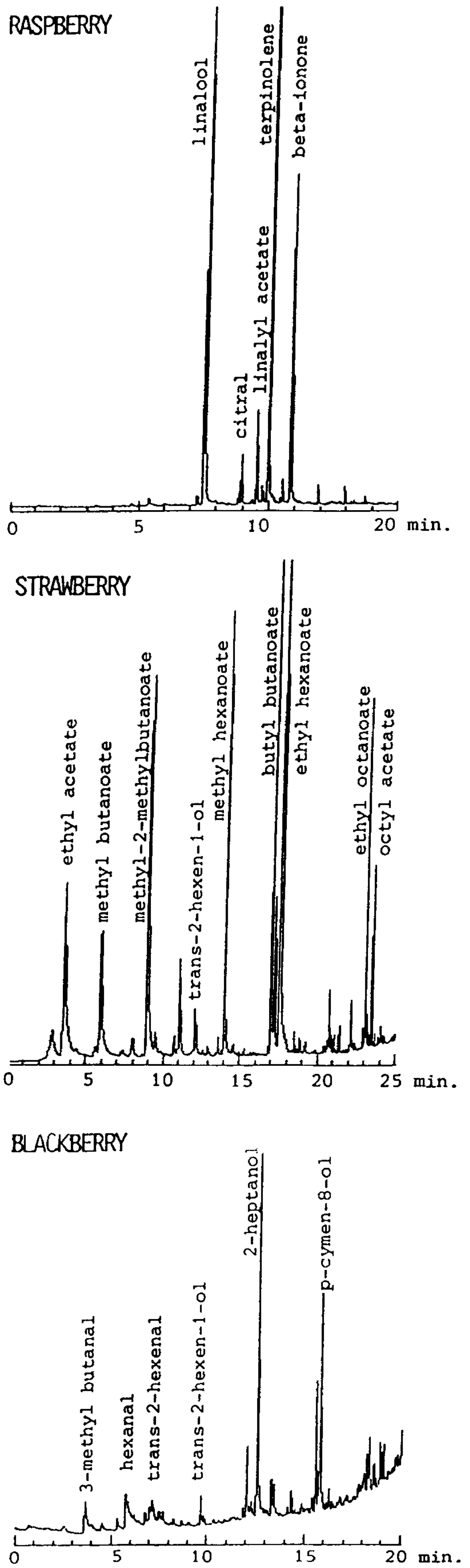

Fig. 1. Gas-chromatographic patterns of the HS-SPME extracts of strawberry, raspberry and blackberry.

Raspberry
With the exception of β-ionone, components of the raspberry HS-SPME extract have been reported as representative of differences between aromas of wild and cultivated raspberries (Honkanen *et al.*, 1980).

Strawberry
HS-SPME patterns have a higher number of peaks than the other berries studied. The strawberry chromatogram includes several esters and trans-2-hexen-1-ol. Among the esters identified, methyl butanoate and butyl butanoate have been positively correlated with the overall quality and strawberry odour (Shamaila *et al.*, 1992). Octyl acetate have been related by the same authors with undesirable attributes due to changes of strawberries stored under modified atmosphere packaging. Also, the importance of C6 compounds such as trans-2-hexen-1-ol and low boiling esters as discriminants between strawberries subjected to deep-freezing processes has been reported (Douillard and Guichard, 1990).

Blackberry
2-Heptanol and p-cymen-8-ol have been identified as the most important volatile components of blackberry flavour and their contribution has been described, respectively, as fruity-herbaceous and flowery-spicy (Georgilopoulos and Gallois, 1987). Carbonyl compounds have been reported as contributors to the overall aroma of blackberry.

Banana
Esters represent the more numerous compounds in banana. HS-SPME extracts of banana include isoamyl acetate. It has been confirmed, by sniffing GC, that this ester is the main aroma constituent of banana (Pérez *et al.*, 1993).

Mango
Terpenic compounds included in the HS-SPME extract of mango have been previously identified by Koulibaly *et al.* (1992). These authors reported the value of the mentioned compounds for classifying different cultivars of mango. Results obtained by applying HS-SPME to fruit flavour analysis showed that the patterns obtained include the most representative volatile components of fresh fruit or volatiles originating during processing or storage.

At present several studies are being conducted in our laboratory to detect the changes in flavour of different fruits due to both freezing and thawing. The principal objective of these studies is to contribute to the knowledge of the changes (chemical and physical) produced during food processing in order to select the varieties and the process conditions that minimise these changes. In this respect, the SPME technique is a very useful tool. Results will be published in the near future.

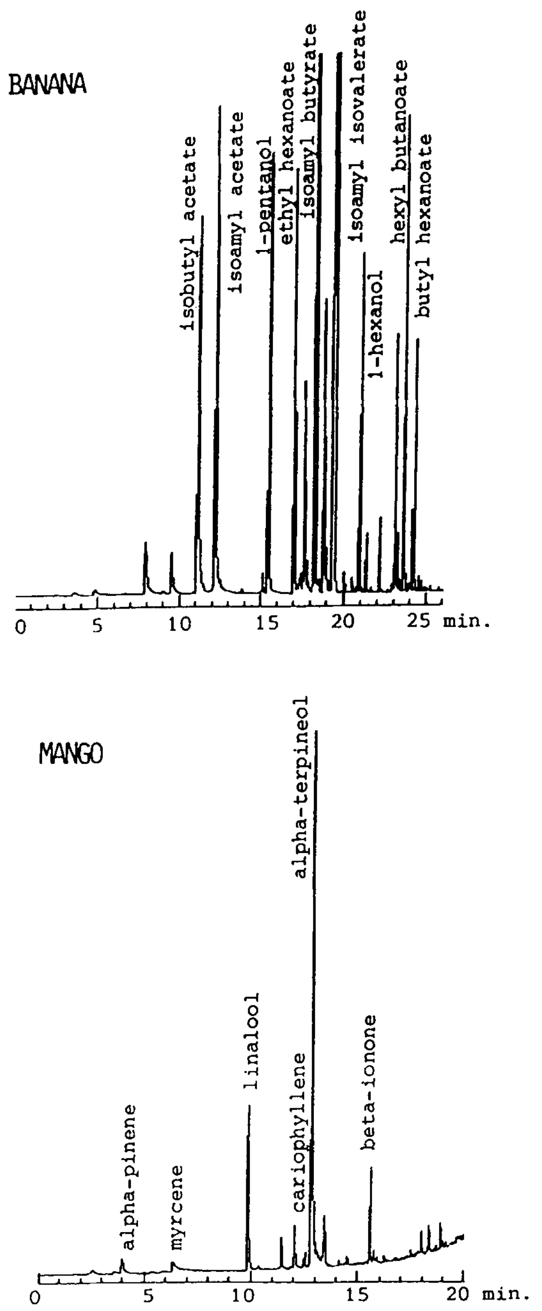

Fig. 2. Gas-chromatographic patterns of the HS-SPME extracts of banana and mango.

ACKNOWLEDGEMENTS

This work was made possible by financial assistance from the Comisión Interministerial de Ciencia y Tecnología Project ALI95-0105.

REFERENCES

Arthur, C. L. and Pawliszyn, J. (1990) Solid-phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **62**, 2145–2148.

Blanch, G. P., Reglero, G., Herraiz, M. and Tabera, J. (1991) A comparison of different extraction methods for the volatile components of grape juice. *J. Chromatogr. Sci.* **29**, 11–15.

Boresjsza-Wysocky, W., Goers, S. K., McArdle, R. N. and Hrazdina, G. (1992) p-Hydroxyphenyl-butan-2-one levels in raspberries determined by chromatographic and organoleptic methods. *J. Agric. Food Chem.* **40**, 1176–1177.

Douillard, C. and Guichard, E. (1990) The aroma of strawberry (*Fragaria ananassa*): Characterization of some cultivars and influence of freezing. *J. Sci. Food Agric.* **50**, 517–531.

Georgilopoulos, D. N. and Gallois, A. N. (1987) Aroma compounds of fresh blackberries (*Rubus lactiata L.*). *Z. Lebensm. Unters. Forsch.* **184**, 374–380.

Hawthorne, S., Miller, D., Pawliszyn, J. and Arthur, C. (1992) Solventless determination of caffeine in beverages using solid-phase microextraction with fused silica fibers. *J. Chromatogr.* **603**, 185–191.

Honkanen, E., Pyysalo, T. and Hirvi, T. (1980) The aroma of Finnish wild raspberries. *Rubus idaeus, L. Z. Lebensm. Unters. Forsch.* **180**, 180–182.

Ibáñez, E. and Bernhard, R. A. (1996) Solid-phase microextraction (SPME) of pyrazines in model reaction systems. *J. Sci. Food Agric.* **72**, 91–96.

Kok, M. F., Yong, F. M. and Lim, G. (1987) Rapid extraction method for reproducible analysis of aroma volatiles. *J. Agric. Food Chem.* **35**, 779–781.

Koulibaly, A., Sakho, M. and Crouzet, J. (1992) Variability of free and bound volatile terpenic compounds in mango. *Lebensm. Wiss. U. Technol.* **25**, 374–379.

Macku, C. and Jennings, W. G. (1987) Production of volatiles by ripening bananas. *J. Agric. Food Chem.* **35**, 845–848.

Macku, C. and Shibamoto, T. (1991) Application of simultaneous purging and solvent extraction technique for flavour monitoring of natural products. *Food Chem.* **42**, 121–127.

Miszczak, A., Forney, Ch.F. and Prange, R. K. (1995) Development of aroma volatiles and color during postharvest ripening of "Kent" strawberries. *J. Amer. Soc. Hort. Sci.* **120**, 650–655.

Pelusio, F., Nilsson, T., Montanarella, L., Tilio, R., Larsen, B., Facchetti, S. and Madsen, J. (1995) Headspace solid-phase microextraction analysis of volatile organic sulfur compounds in black and white truffle aroma. *J. Agric. Food Chem.* **43**, 2138–2143.

Pérez, A. G., Sanz, C., Rios, J. J. and Olias, J. M. (1993) Comparison of Main Aroma Constituents of Apple, Banana and Strawberry. *Rev. Esp. Cien. Tec. Ali.* **33**, 665–667.

Rizzolo, A., Polesello, A. and Polesello, S. (1992) Use of headspace capillary GC to study the development of volatile compounds in fresh fruits. *J. of High Resol. Chromatogr.* **15**, 472–477.

Robertson, G. W., Griffiths, D. W., Woodford, J. A. T. and Birch, A. N. E. (1995) Changes in the chemical composition of volatiles released by the flowers and fruits of the red raspberry (*rubus idaeus*) cultivar glen prosen. *Phytochem.* **38**, 1175–1179.

Shamaila, M., Powrie, W. D. and Skura, B. J. (1992) Analysis of volatile compounds from strawberry fruit stored under modified atmosphere packaging (MAP). *J. Food Sci.* **5**, 1173–1176.

Shirey, R. E. (1994) Rapid analysis of environmental samples using solid-phase microextraction (SPME) and narrow bore capillary columns. *Proceedings of the Sixteenth International Symposium on Capillary Chromatography*, pp. 27–30. Riva del Garda, Italy, Sept.

Ueda, Y., Tsuda, A., Bai, J. H., Fujishita, N. and Chanchin, K. (1992) Characteristic pattern of aroma ester formation from banana, melon and strawberry with reference to the substrate specificity of ester sinthetase and alcohol contents in pulp. *J. Jap. Soc. Food Sci. Technol.* **39**, 183–187.

Van der Kooi, M. M. E. and Noij, Th. H. M. (1994) Evaluation of solid-phase microextraction for the analysis of various priority pollutants in water. *Proceedings of the Sixteenth International Symposium on Capillary Chromatography*, pp. 1087–98. Riva del Garda, Italy, Sept. 27–30.

Yang, X. and Peppard, T. (1994) Solid-phase microextraction for flavor analysis. *J. Agric. Food Chem.* **42**, 1925–1930.

Young, H., Perea, C. O. and Paterson, V. J. (1992) Identification of E-hex-3-enal as an important contributor to the off-flavour aroma in kiwifruit juice. *J. Sci. Food Agric.* **58**, 519–522.

Zhang, Z., Yang, M. J. and Pawliszyn, J. (1994) Solid-phase microextraction. *Anal. Chem.* **66**, 844A–853A.