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Evaluation of a transfer technique for direct coupling of reversed-phase liquid chromatography with gas chromatography

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Abstract

A method that allows direct transfer of liquid chromatographic aqueous eluents into capillary gas chromatography, using a programmed temperature vaporizer as interface, was investigated. The method involved both the evaporative and nonevaporative modes of solvent elimination and allowed large fractions of methanol–water eluents to be transferred from LC to GC. The speed of sample transfer was 1800 μl/min and the internal diameter of the column used in the preseparation step was 4.6 mm. Working rules of the method were investigated to improve sensitivity for the direct analysis of trace compounds. Reproducibility was achieved and detection limits ranging from 0.1 to 1.5 μg/ml of ethyl esters covering a wide range of boiling points were obtained.

Keywords: Reversed-phase eluents; Large volume transfer; Coupling, LC–GC; Ethyl esters

1. Introduction

On-line coupling of high-performance liquid chromatography to capillary gas chromatography (HPLC–cGC) has become an important analytical technique mainly because the HPLC step provides far better resolution than conventional procedures of sample preparation. Also, the reliability of quantitative determinations is significantly enhanced as loss of sample or contamination during transfer is reduced or even virtually eliminated. Consequently, the use of direct HPLC–cGC improves the sensitivity achievable, makes the automation easy and considerably reduces the time required for the analysis [1]. However, a number of difficulties, mainly concerning the lack of water-resistant deactivation of uncoated precolumns, have hindered its more widespread use.

The first attempt at on-line HPLC–cGC coupling was described in 1980 [2] although only a few microlitres could then be introduced into the GC column. Use of conventional size LC columns at the present time often has the same disadvantage and even with microbore columns (I.D. 1 mm) the peak volume (in the range 10–500 μl) that has to be transferred to GC is usually large enough to cause difficulties. The earliest reported applications of coupled HPLC–cGC referred almost exclusively to micro-HPLC columns in which the peak volumes are so small that the whole fraction of interest can be easily transferred to the capillary column [3–6]. However, it is clear that the low sample capacity of micro-columns is a disadvantage for trace analysis.

As far as the HPLC operation mode is concerned, most of the reported applications on coupled HPLC–

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cGC have been obtained in the normal-phase LC mode as transfer of polar solvents, especially water, is far more difficult because of, e.g. large volume of vapour produced per unit volume of liquid, low evaporation rates, poor solvent effects and chemical aggressiveness which can destroy the deactivation of the GC column.

Although different transfer techniques (i.e., on-column HPLC–cGC transfer [3,7–9] and those involving the vaporization in packed beds and the intermediate trapping in a solid-phase [10–16]) have been developed in the last few years and a number of applications have already proven the capabilities of on-line HPLC–cGC (reviewed in [17–21]), there is still a need for methods to extend the application of HPLC–cGC.

Our previous research on HPLC–cGC focused on the use of typical reversed-phase eluents and proved its potential to perform the analysis of rather complex mixtures. However, some important aspects concerning the working rules of the method have not yet been considered. In this work, we present a preliminary study of the range of applicability of a transfer technique based on the use of a programmed temperature injector for RP-HPLC–cGC interfacing.

2. Experimental

2.1. Materials and columns

A test mixture consisting of 10 ethyl esters in methanol (concentrations ranged from 85 to 100 mg/l) was used. C_6 and C_{10} ethyl esters were purchased from Merck (Munich, Germany), C_7, C_9, C_{11}, C_{12}, C_{14}, C_{16} and C_{18} ethyl esters were obtained from PolyScience (Evanston, IL, USA) and C_8 ethyl ester was supplied by Fluka (Buchs, Switzerland). The water used was collected from a Milli-Q water purification system (Millipore, Milford, MA, USA) and methanol (HPLC grade) was provided by Lab-Scan (Dublin, Ireland).

The LC column was 5 cm×4.6 mm I.D. and was slurry packed using Vydac 214 TPB 10 (The Separations Group, Hesperia, CA, USA) as packing material according to a previously reported procedure [22]. The GC column was 25 m×0.25 mm coated with a 0.25-μm layer of SE-54 diphenyl–dimethylpolysiloxane (5:95, v/v) (SE-54, Sugelabor, Madrid, Spain).

Tenax TA (80–100 mesh, Chrompack, Middelburg, The Netherlands) was used as packing material in the glass-liner of the PTV (a silylated glass insert of 75 mm×1 mm I.D.×2 mm O.D. purchased from Gerstel, Mülheim/Ruhr, Germany). Prior to use, the Tenax TA was conditioned under a stream of helium at 350°C for 120 min.

2.2. Instrumentation

The analyses were carried out using a coupled HPLC–cGC equipment. For the HPLC system, a Hewlett-Packard Model 1050 chromatograph (Wilmington, DE, USA) equipped with a manual injection valve (Rheodyne, Model 7125; Cotati, CA, USA) having a 20-μl sample loop and an UV detector was used. All throughout the experimentation the column was maintained at 45°C and the UV detection was performed at 205 nm. Data acquisition from the UV detector was accomplished with HP CHEMSTATION (Hewlett-Packard).

The HPLC preseparation of the standard solution was carried out using a mixture of eluent A (methanol) and eluent B (water) at a flow-rate of 1800 μl/min. Initial conditions (eluent A–B, 55:45, v/v) were maintained for 0.30 min and followed by a linear gradient (to 90% A in 0.05 min), then eluent A–B (90:10, v/v) was held for 5 min and finally eluent A–B (100:0, v/v) was maintained until the end of the analysis. Before starting a new analysis, the Tenax TA was conditioned under a stream of helium at 350°C for 20 min. Working under the mentioned conditions, satisfactory blanks were obtained.

The GC system consisted of a Perkin-Elmer (Norwalk, CT, USA) Model 8500 instrument equipped with a programmed temperature injector (PTV) and a flame ionization detection system (FID). The equipment was coupled to a Model 2600 chromatography software (Perkin-Elmer Nelson Systems). The temperature of the GC oven was kept at 45°C for 10 min, then programmed to 100°C at 5°C/min and again increased at 8°C/min to 250°C. Throughout the experimentation an split ratio equal to 50:1 was used and the temperature of the FID was 250°C.

2.3. Transfer conditions

Upon elution (indicated by UV detection) of the beginning of the ethyl esters containing fraction, a 1800-μl volume was transferred to the GC just by switching a multiport valve (Rheodyne, Model 7060) from the waste position to the transfer position. The PTV injector of the GC system acts as the interface of the HPLC–cGC coupling and allows the transfer of the HPLC effluent to the GC through a line (60 cm×0.32 mm I.D. fused-silica tube) inserted into the septum of the PTV body. During transfer, a helium flow passes through the injector and once the transfer step was completed, both the initial PTV temperature and the helium flow-rate were kept constant during the purge time. In this work, different helium flow-rates (i.e., 0, 100, 300, 800, 1000 and 1500 ml/min) and purge times (0.32, 2, 4 and 6 min) were tested. Other variables affecting PTV operation during introduction of large sample volumes into cGC columns were optimised, according to our previous experience [23,24] as follows: initial PTV temperature, 21°C; packing material, Tenax TA (80–100 mesh); length of packing material, 4 cm.

Before starting the transfer procedure, the GC column end from the injector body was removed (to allow solvent elimination as a liquid) and it was reconnected after the purge time established for each analysis. Thermal desorption and transfer of the trapped material to the GC column was achieved by increasing the PTV at 14°C/s to 350°C. The final temperature was kept constant for 11 min.

3. Results and discussion

According to the model described by Staniewski and Rijks [25], the maximum speed of sample introduction which equals the solvent elimination rate when introducing large volume samples into cGC can be calculated as follows:

$$V_{\mathrm{inj,max}} = V_{\mathrm{el}} = \frac{Mp_{\mathrm{j}}}{\rho RT_o}\frac{p_o}{p_{\mathrm{i}}}V_{t,o} \qquad (1)$$

where $V_{\mathrm{inj,max}}$ is the maximum speed of sample introduction, V_{el} the solvent evaporation rate, M the molecular weight of the solvent, p_{j} the vapour pressure of the solvent, ρ the density of the solvent, R the gas constant, p_{i} the inlet pressure of the liner and $V_{\mathrm{t,o}}$ the total gas flow-rate at outlet conditions (T_{o} and p_{o}).

As the purpose of our investigation was the direct analysis of trace compounds by HPLC–cGC transfer of high-volume fractions (e.g., ~1 ml) of aqueous eluents it is clear that under our experimental conditions, volumes of vapour as high as 1 l/min or even more may be produced. In these circumstances, the heat for evaporation could reduce the interface temperature to such an extent that further evaporation of the HPLC eluent would not occur.

Nonevaporative sample introduction can also be applied in RP-HPLC–cGC although so far only relatively low values of both speed of sample introduction and transferred volumes have been reported [15], thus making the use of some HPLC columns difficult. For this transfer process, an adsorbent material placed in a packed bed acts as a solid-phase extraction (SPE) cartridge and the solvent passes through it as a liquid. The extraction efficiency depends on the affinity of the solutes for the packing material and the solubility of the components in the solvent.

In the present work, experimental values for some of the variables involved in RP-HPLC–cGC analysis were established on the basis of our previous experience [13,14,23,24] concerning the use of experimental designs for adjusting the controlling variables affecting large volume transfer from HPLC into GC. Specifically, in this study, the influence of both the purge flow (helium flow-rate during transfer) and the purge time (established once the transfer process is completed) was considered from the point of view of their contribution to both the evaporative and nonevaporative processes of solvent elimination.

Fig. 1 gives the recoveries obtained (calculated from a cold split injection into the GC of a 5-μl volume of the standard solution), from the absolute peak areas resulting from the RP-HPLC–cGC analysis of the standard mixture investigated when the purge time was kept constant (0.32 min). The highest recoveries were generally obtained when experimentation was performed at the fastest helium flow-rate while relative standard deviation values (R.S.D., $n=3$) varied from 9 to 21%. However,

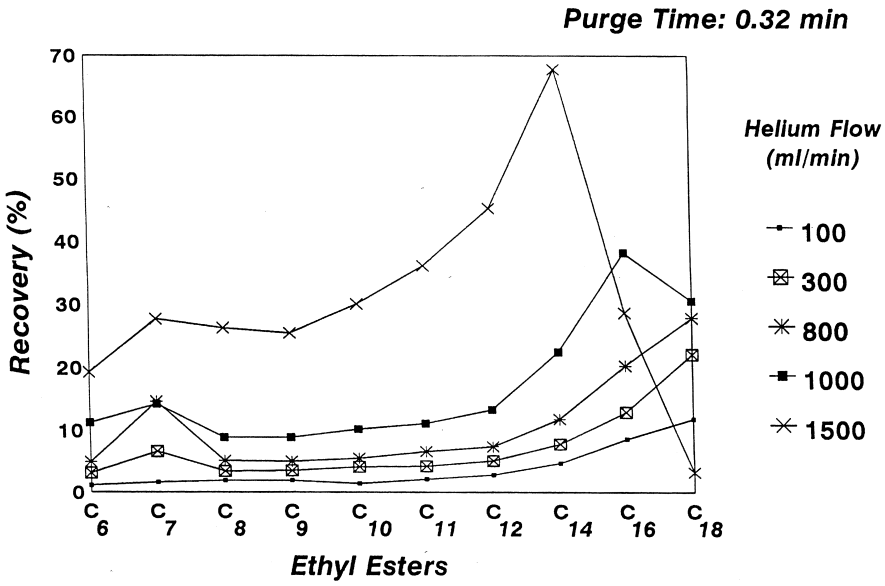

Fig. 1. Recoveries obtained at different helium flow-rates during RP-HPLC–cGC transfer of the fraction containing ethyl esters in methanol–water eluent (90:10, v/v). Purge time: 0.32 min.

unacceptable R.S.D. values (i.e., up to 70%) were obtained if no flow-rate was applied during transfer.

Experimental problems (e.g., extinction of the detector flame) were occasionally observed when either no flow-rate was applied during transfer or low purging times were considered, 100 ml/min being the purge flow-rate. Consequently, helium flow-rates higher than 100 ml/min should always be used to accelerate solvent evaporation (thus contributing to eliminating the remaining solvent in the PTV chamber) and also to protect both the GC column and the GC detector.

Figs. 2 and 3 show recoveries resulting from experiments performed at different purge times while maintaining the helium flow-rates at 100 and 1500 ml/min, respectively. Besides resulting in lower amounts of solvent in the PTV, additional purge time is not responsible for losses of material (although in some cases the less volatile compounds are almost completely lost). As far as the precision of the analysis is concerned, unacceptable R.S.D. values were obtained for some compounds (up to 82% for the ester of C_{18}) when using purge flow-rates as low as 100 ml/min, 6 min being the purge time. On the contrary, the use of high helium flow-rates during transfer (e.g., 1500 ml/min) and purge times of 4–6 min enabled us to obtain the best precision achievable in the RP-HPLC–cGC analysis (i.e., R.S.D. values lower, in general, than 10%).

From Figs. 1–3 and the R.S.D. values, it can be concluded that increasing both helium flow-rate during transfer and purge time leads to the most satisfactory results. An exception is the C_{18} ethyl ester. Apparently, not only the thermal desorption of C_{18} ethyl ester from Tenax may be difficult, but also its affinity for the packing material may be limited when working under specific experimental conditions.

However, the fact that higher purge flow gives better recoveries, should not be considered as an indication that the evaporative mode alone is responsible for the trapping, as transfer to GC of lower HPLC fractions did not yield equivalent results. Moreover, if the evaporative process were exclusively involved in the transfer, the evaporation rate of the solvent would restrict the applicability of the system. Eq. (1) shows that complete solvent elimination in the evaporative mode may be difficult or even impossible when large volumes of very polar solvents (e.g., methanol and water) have to be introduced into GC. Even significantly increasing the flow-rate in the liner, the increment of the solvent

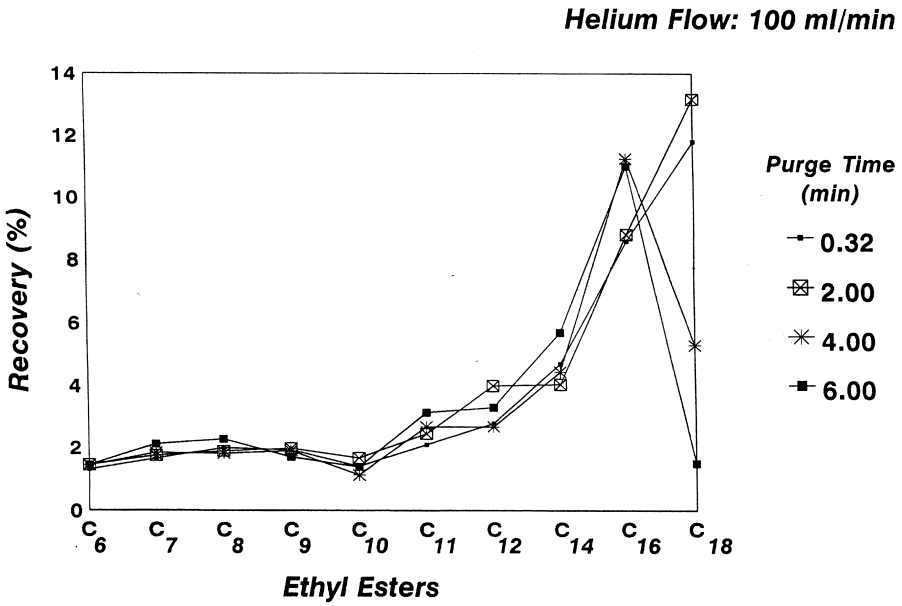

Fig. 2. Recoveries obtained at different purge times during RP-HPLC–cGC transfer of the fraction containing ethyl esters in methanol–water eluent (90:10, v/v). Helium flow-rate equal to 100 ml/min.

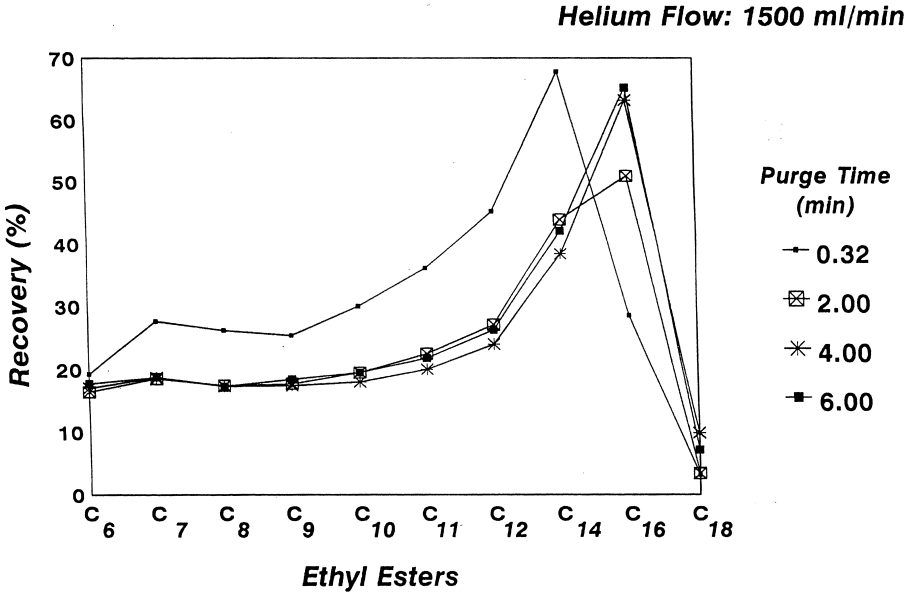

Fig. 3. Recoveries obtained at different purge times during RP-HPLC–cGC transfer of the fraction containing ethyl esters in methanol–water eluent (90:10, v/v). Helium flow-rate equal to 1500 ml/min.

evaporation rate predicted by Eq. (1) would not be high enough for the discharge of eluent vapours produced in our experiments.

The solvent (expressed as the average volume obtained from a minimum of three replicates) which passes as a liquid through the packed bed during the HPLC–cGC transfer (collected while the GC column is disconnected from the injector body) varied from 1.7 ml (helium flow-rate during transfer of 100 ml/min) to 1.5 ml (helium flow-rate of 1500 ml/min), the purge time being constant. However, increasing the purge time from 0.32 to 6 min does not have a significant influence on the volume collected at these different helium flow-rates. Increasing both the helium flow-rate during transfer and the purge time results in smaller solvent peaks. Therefore, better conditions for solvent elimination are achieved by using the highest helium flow-rate during transfer and a purge time of 4–6 min. If the nonevaporative mechanism were clearly predominant in the process, the application of a high helium flow-rate would strongly accelerate the passage of the liquid through the PTV, thus resulting in lower efficiency for the overall process. However, it was evident from our experimental work that low purge flow-rates make the analysis difficult or even impossible while a purge flow-rate as high as 1500 ml/min yields the best results.

Experimental evidence suggests that the separation method proposed in this work involves both the evaporative and nonevaporative modes of solvent elimination, with neither clearly predominant. The relative contributions of each mechanism cannot be easily estimated as recovery of compounds is strongly affected by their boiling points, chemical structures and retention in the packing material, thus making difficult the selection of experimental conditions for the analysis of some groups of compounds (e.g., those covering a wide range of boiling points such as the ethyl esters considered in this work). However, it should also be emphasized that although all compounds are partly or almost completely lost, the repeatability of the recoveries remains at a satisfactory level. With the method presented here, detection limits were calculated from the peaks giving a signal equal to ten times the detector baseline noise (determined from the width of the noisy baseline over a certain period of time).

Table 1
Detection limits (LOD) obtained for the on-line RP-HPLC–cGC transfer of a 1800-μl volume of methanol–water (90:10) eluent[a]

Ethyl ester	LOD[b] (μg/ml)
C_6	1.5
C_7	0.8
C_8	0.7
C_9	0.5
C_{10}	0.4
C_{11}	0.3
C_{12}	0.2
C_{14}	0.1
C_{16}	0.1
C_{18}	1.0

[a] Purge flow, 1500 ml/min; purge time, 6 min.
[b] Calculated as the amount of product giving a signal equal to ten times the background noise (determined from the width of the baseline).

As can be seen in Table 1, values obtained ranged from 0.1 (for C_{16} ethyl ester) to 1.5 μg/ml (C_6 ethyl ester). The highest detection limits were obtained for the most and the least volatile compounds (i.e., C_6 and C_{18} ethyl esters) where either effective trapping during HPLC–cGC transfer, or subsequent thermal desorption, may be especially difficult.

4. Conclusions

The method presented is the RP-HPLC–cGC transfer of high volume fractions at high speed. Operating conditions should be appropriately selected for each specific analysis based on the physicochemical properties of the solvent and the chemical structures of the compounds to be analyzed, but satisfactory precision can be obtained even for partially lost solutes covering a wide range of boiling points. Working in operating conditions involving high helium flow-rates during transfer (1500 ml/min) and purge times of 4–6 min, detection limits in the μg/ml range are obtained and recoveries from ~20 to 65% are achieved for C_6–C_{16} ethyl esters, R.S.D. values being $<10\%$. Limitations which should be considered are (a) the use of mobile phases with a large modifier content may involve losses of analytes caused by breakthrough from the packing material and (b) the need for effective removal of the

remaining solvent in the PTV chamber may require the careful optimization of some experimental values affecting the HPLC–cGC transfer. This preliminary study is far from comprehensive although it suggests the possibilities of the method proposed for on-line RP-HPLC–cGC coupling.

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References

[1] K. Grob, On-Line Coupled LC–GC, Hüthig, Heidelberg, 1991.
[2] R.E. Majors, J. Chromatogr. Sci. 18 (1980) 571.
[3] H.J. Cortes, C.D. Pfeiffer, B.E. Richter, J. High Resolut. Chromatogr. Chromatogr. Commun. 8 (1985) 469.
[4] H. Cortes, B. Richter, C. Pfeiffer, D. Jensen, J. Chromatogr. 349 (1985) 55.
[5] D. Duquet, C. Dewaele, M. Verzele, S. McKinley, J. High Resolut. Chromatogr. Chromatogr. Commun. 11 (1988) 824.
[6] H.J. Cortes, C.D. Pfeiffer, G.L. Jewett, B.E. Richter, J. Microcol. Sep. 1 (1989) 28.
[7] K. Grob, Z. Li, J. Chromatogr. 473 (1989) 423.
[8] H.J. Cortes, J. Chromatogr. 626 (1992) 3.
[9] E.C. Goosens, D. de Jong, G.J. de Jong, U.A.Th. Brinkman, J. Microcol. Sep. 6 (1994) 207.
[10] E. Noroozian, F.A. Maris, M.W.F. Nielen, R.W. Frei, G.J. de Jong, U.A.Th. Brinkman, J. High Resolut. Chromatogr. Chromatogr. Commun. 10 (1987) 17.
[11] J.J. Vreuls, V.P. Goudriaan, U.A.Th. Brinkman, G.J. de Jong, J. High Resolut. Chromatogr. 14 (1991) 475.
[12] H.G.J. Mol, J. Staniewski, H.G.M. Janssen, C.A. Cramers, R.T. Ghijsen, U.A.Th. Brinkman, J. Chromatogr. 630 (1993) 201.
[13] F.J. Señoráns, M. Herraiz, J. Tabera, J. High Resolut. Chromatogr. 18 (1995) 433.
[14] F.J. Señoráns, G. Reglero, M. Herraiz, J. Chromatogr. Sci. 33 (1995) 446.
[15] J.J. Vreuls, U.A.Th. Brinkman, G.J. de Jong, K. Grob, A. Artho, J. High Resolut. Chromatogr. 14 (1991) 455.
[16] A.-J. Bulterman, J.J. Vreuls, R.T. Ghijsen, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 16 (1993) 397.
[17] I.L. Davies, K.E. Markides, M.L. Lee, M.W. Raynor, K.D. Bartle, J. High Resolut. Chromatogr. 12 (1989) 193.
[18] M.-L. Riekkola, J. Chromatogr. 473 (1989) 315.
[19] H.J. Cortes, in: Multidimensional Chromatography Techniques and Applications. H.J. Cortes (Editor), Marcel Dekker, New York, 1990, p. 251.
[20] J.J. Vreuls, G.J. de Jong, R.T. Ghijsen, U.A.Th. Brinkman, J. AOAC Int. 77 (1994) 306.
[21] K. Grob, J. Chromatogr. A 703 (1995) 265.
[22] M. de Frutos, A. Cifuentes, L. Amigo, M. Ramos, J.C. Diez-Masa, Z. Lebensm. Unters. Forsch. 195 (1992) 326.
[23] J. Villén, F.J. Señoráns, M. Herraiz, G. Reglero, J. Tabera, J. Chromatogr. Sci. 30 (1992) 261.
[24] F.J. Señoráns, J. Tabera, J. Villén, M. Herraiz, G. Reglero, J. Chromatogr. 648 (1993) 407.
[25] J. Staniewski, J.A. Rijks, J. Chromatogr. 623 (1992) 105.