Simplex Optimization of the Direct Analysis of Free Sterols in Sunflower Oil by On-Line Coupled Reversed Phase Liquid Chromatography−Gas Chromatography

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Optimization of the direct analysis of the sterol fraction of a sunflower oil is carried out by means of the modified sequential Simplex method. Sensitivity achievable with the on-line coupling of reversed phase liquid chromatography and gas capillary chromatography (RPLC−GC) using a programmed temperature vaporizer (PTV) as interface is enhanced by optimization of some experimental conditions, namely, transferred volume, purge flow rate during transfer, interface temperature, and time during which the gas purges the interface once the transfer is completed. The optimization procedure allows volume fractions higher than 3000 μL of water−methanol eluents to be transferred into the GC system, 2000 μL/min being the speed of sample introduction. With this method, free sterol analysis of edible oils is performed by direct injection without the need for prior enrichment of the sample.

Keywords: *On-line coupled RPLC−GC, Simplex method, direct free sterol analysis, edible oil.*

1. INTRODUCTION

Over the past few years, on-line combination of high-performance liquid chromatography and high-resolution capillary gas chromatography (LC−GC) has become a prominent method for the analysis of complex mixtures. This is mainly due to the advantages of using a two-dimensional system which involves the combination of two different chromatographic techniques in such a way that the LC step enables the preseparation of the sample (thus replacing time-consuming conventional methods which are often a source of error and uncertainty), while the GC system provides the separating power required for the analysis. The possibility of performing a previous separation of the compounds to be analyzed into different classes and the subsequent selection of the specific fraction to be transferred into the GC allow the disposal of both the separation selectivity and the separation efficiency required to overcome the problems which usually pose the analysis of complex mixtures.

The potential of LC−GC has been previously demonstrated (Cortes, 1990; Grob, 1991, 1992, 1995; Vreuls et al., 1994), and a number of applications to real-life samples have been already reported (Mondello et al., 1994, 1996a,b). Most cases, however, refer to the use of normal phase in the LC step, because the eluents typically used make the transfer easier. In contrast, transfer to GC of polar solvents is rather difficult, mainly due to the large volume of vapor which is produced per unit volume of liquid and the poor wettability of retention gaps. Although these aspects have limited the applicable range of coupled reversed phase liquid chromatography to gas chromatography (RPLC−GC), this approach is clearly advantageous considering the range of compounds which can be analyzed and, especially, its adequacy for the analysis of aqueous samples.

Currently one of the main issues in on-line coupled RPLC−GC is the design of a suitable interface to efficiently allow the transfer of high fraction volumes of aqueous solvents. In this respect, several approaches have been proposed which demand the previous removal of the water (Vreuls et al., 1991; Staniewski et al., 1992; Mol et al., 1993; Goosens et al., 1994) although their performance is conditioned by a number of limitations, and, in some cases, results obtained do not correspond to expectations. Specifically, use of introduction rates adequate to couple LC columns having large internal diameters (i.e., 4.6 mm) and transfer of high volumes to make the analysis of the whole fraction of interest possible demand further investigation.

Previous experience on the introduction of large-volume samples for GC analysis by means of a temperature programmable (PTV) injector (Vogt et al., 1979; Schomburg, 1981; Poy et al., 1981) showed its usefulness in lowering detection limits, thus making the analysis of minor compounds easier (Villén et al., 1992; Señoráns et al., 1993; Staniewski and Rijks, 1993; Mol et al., 1995a,b). In addition, our previous results on the use of a PTV as an interface for the on-line coupled RPLC−GC analysis look promising (Señoráns et al., 1995a,b) although careful adjustment of experimental conditions is mandatory, especially to achieve the efficient transfer of high-volume fractions of aqueous eluents without introducing water into the GC system.

On the other hand, characterization of minor components in edible oils and fats distinguishes between oils with different processing histories or of different quality. So far, mainly minor components have been studied in the unsaponifiable matter resulting after the removal

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Table 1. Experimental Variables, Base Levels, and Step Sizes Considered for the Simplex Optimization of the RPLC−GC Transfer

exptl variable	base level	step size	exptl variable	base level	step size
transferred vol, V (μL)	3000	900	interface temp, T (°C)	30	15
flow rate, F (mL/min)	700	210	purge time, t (min)	0.25	0.15

of triglycerides, but the official method (Official Journal of European Communities, 1988) not only involves a very tedious and time-consuming sample preparation step but also may cause the loss of important information due to the saponification reaction (Grob et al., 1990; Park et al., 1996). For that reason, different alternative procedures have been proposed to replace the pretreatment step required to analyze the sterol fraction, namely, the use of supercritical fluid extraction and on-line coupling LC−GC although so far almost exclusively normal phase LC has been employed (Grob and Lafranchi, 1989; Grob et al., 1989; Lanuzza et al., 1996; Biedermann et al., 1996). Our previous experience in the use of RPLC−GC for the analysis of free sterols in edible oils (Señorans et al., 1996) has demonstrated both the potential of the technique and the need to carefully adjust experimental conditions.

The primary objective of this work was focused on the elimination of error sources and problems associated with off-line sample preparation methods, while improving sensitivity and ease of operation in the analysis of minor components (i.e., free sterols) occurring in a real-life complex mixture. To this aim, some experimental variables affecting the on-line transfer of large-volume fractions of aqueous eluents from LC into a GC system were adjusted to establish the set of conditions which give the best results. Specifically, the sequential Simplex method (Spendley et al., 1962) was applied since previous work has demonstrated its ability to successfully optimize multifactor systems in chromatography (Blanch et al., 1993a,b).

To guarantee the applicability of the proposed method to the analysis of real-life samples, the complete optimization procedure was performed using exclusively sunflower oil.

2. EXPERIMENTAL PROCEDURES

2.1. Materials. A specially processed Tenax designed primarily as a trapping agent (Tenax TA, Chrompack, Middelburg, The Netherlands) was used as packing material in the PTV. This adsorbent material, the particle size (80−100 mesh), and the plug length (4 cm) were selected on the basis of our previous experience on RPLC−GC (Señoráns et al., 1995b).

Methanol (HPLC grade) was purchased from Lab Scan, and the water used was collected from a Milli-Q water purification system (Millipore, Milford, MA). The silylated glass inserts (75 mm × 1 mm i.d. × 2 mm o.d.) of the PTV injector were obtained from Gerstel (Mülheim/Ruhr, Germany).

2.2. Samples. The refined sunflower oil used throughout the optimization procedure was purchased in the local market. Prior to its analysis by RPLC−GC, the oil sample was filtered through a 0.22 μm Pro-X filter (Teknokroma, Madrid, Spain), and no further pretreatment of the sample was required.

2.3. Instrumentation. RPLC−GC was performed using on-line coupled equipment consisting of a Hewlett-Packard Model 1050 liquid chromatograph equipped with a manual injection valve (Rheodyne 7125) having a 20 μL loop and a UV detector and a Perkin-Elmer model 8310 gas chromatograph fitted with a PTV injector and a flame ionization detector (FID). The PTV acts as the interface for the system.

2.3.1. LC Conditions. LC preseparation was carried out on a 50 × 4.6 mm i.d. column slurry packed with 10 μm silica (C4, Vydac 214 TPB) according to a previously reported procedure (de Frutos et al., 1992). Initial composition of the eluent (methanol−water, 68:32 (v/v)) was kept for 12 s and subsequently followed by a linear gradient of up to 22% water (third minute). After elution of the sterol fraction, the gradient was reduced to 14% water from the seventh minute until the ninth minute and maintained there for 3 min. Afterward, the gradient was again reduced to 0% water within 4 min.

All analyses were carried out by maintaining the column temperature at 45 °C with the UV detector operated at 205 nm, 2000 μL/min being the flow rate used during transfer.

2.3.2. LC−GC Transfer. After elution (indicated by UV detection) the sterol-containing fraction was transferred to the GC by means of a multi port valve placed between the detector of the HPLC system and the PTV injector of the GC (Señoráns et al., 1995a).

The LC−GC transfer is performed while both the PTV temperature and the helium flow rate which passes the injector at the values established for each experimental run in the optimization procedure, are maintained as detailed below. Further variables to be optimized were the transferred volume fraction and the purge time (i.e., time during which both the PTV temperature and the helium flow rate were kept constant once the transfer step is completed). The purge time is required to eliminate the remaining solvent from the glass liner before transfer of the compounds to the GC column.

Discharge of the large volume of vapor resulting from the aqueous eluent during LC−GC transfer is promoted by removing the GC column end from the injector body. After completion of the transfer step, temperature programming of the GC column (see below) was started and the PTV injector was heated at 14 °C/s to 350 °C. This temperature was held for 6 min to efficiently eliminate the remaining solvent from the glass liner.

2.3.3. GC Analysis. GC separations were performed on a 5% phenyldimethylpolysiloxane fused silica column (25 m × 0.250 mm i.d., 0.25 μm film thickness) with helium as the carrier gas. The GC oven was programmed from 130 to 265 °C at 20 °C/min and subsequently at 3 °C/min to 300 °C. The FID temperature was maintained at 320 °C, and the 2600 Chromatography software (Perkin-Elmer Nelson Systems) was employed for data collection.

2.4. Simplex Optimization of Transfer Conditions. Table 1 shows the four variables which were optimized for on-line LC−GC transfer of aqueous eluents as well as their base levels (the starting physical values of the variables) and their step sizes (the physical values which correspond to a mathematical unit of each variable). The selection of the variables to be optimized, as well as their base levels and step sizes, was based on our previous experience concerning the LC−GC transfer of high-volume fractions of water-containing eluents (Señoráns et al., 1995a,b, 1996).

RESULTS AND DISCUSSION

Table 2 shows the experimental values tested throughout the modified Simplex optimization procedure of the RPLC−GC transfer of a selected fraction preseparated from the direct injection of a sunflower oil, as described under the Experimental Section.

To evaluate the effect of changes in the four variables on the overall performance of the process, the optimization criterion to be used was selected in such a way that both the sensitivity achievable (expressed as the sum of the integrated peak areas transferred in each experi-

Table 2. Experimental Runs and Results for the Simplex Optimization of the RPLC−GC Transfer Performed from the Direct Injection of a Sunflower Oil

vertex no.	simplex no.	retained vertices	rejected vertices	exptl variable levels[a]						
				V	*F*	*T*	*t*	y_1[b]	y_2[b]	response[c]
1				3000	700	30	0.25	311 840	324 629	110.0451
2				3833	746	33	0.28	204 905	257 935	107.1166
3				3197	894	33	0.28	243 049	261 224	108.0158
4				3197	746	44	0.28	231 320	187 509	106.2773
5				3197	746	33	0.39	228 575	284 607	108.0292
6	1	1, 2, 3, 5	4	3416	797	21	0.32	368 935	547 108	112.7215
7[d]	2	1, 2, 3, 5	4	3526	823	10	0.34			
8	3	1, 3, 5, 6	2	2572	823	25	0.34	254 389	295 178	108.7088
9	4	1, 5, 6, 8	3	2896	639	22	0.36	332 006	249 769	109.0133
10	5	1, 6, 8, 9	5	2745	733	16	0.25			
11[e]	6	1, 6, 8, 9	5	3084	743	29	0.35	397 480	348 071	111.3717
12	7	1, 6, 9, 11	8	3626	616	25	0.31	405 793	378 617	111.8546
13	8	1, 6, 11, 12	9	3667	790	31	0.25	360 975	327 543	110.7070
14	9	6, 11, 12, 13	1	3897	773	23	0.36	422 655	393 986	112.2040
15	10	6, 11, 12, 14	13	3344	675	18	0.42			
16[e]	11	6, 11, 12, 14	13	3587	761	28	0.29	372 395	355 563	111.2146
17	12	6, 11, 12, 14	16	3425	704	21	0.38	383 797	372 627	111.5519
18	13	6, 12, 14, 17	11	4099	702	16	0.33			
19[e]	14	6, 12, 14, 17	11	3338	733	26	0.35	376 913	393 148	111.7041
20	15	6, 12, 14, 19	17	3714	756	26	0.29	394 641	400 874	111.9916

[a] Variables and units as in Table 1. [b] Sum of the integrated peak areas obtained from the two noise factor levels considered. [c] Taguchi signal-to-noise ratio as in eq 1. [d] Obtained with expansion coefficient equal to 2. [e] Obtained with contraction coefficient equal to −0.5.

ment, excluding the solvent peak) and the precision of the analysis were considered. In addition, it was also intended to lower the eventual effect of some of those factors whose control is either difficult or undesired (noise factors) so that satisfactory robustness of the method could be finally achieved. To this aim, each experiment was carried out at two different levels corresponding to a noise factor previously established and the so-called "Taguchi signal-to-noise ratio" (*S*/*N*) (Taguchi, 1987; Peace, 1993) was used as the system response according to the following equation ("larger-the-better" case):

$$\frac{S}{N} = -10 \log\left[\frac{(1/y_1^2) + (1/y_2^2)}{2}\right] \qquad (1)$$

where *S*/*N* is expressed in decibels and y_1 and y_2 account for the sum of integrated peak areas obtained at the two noise factor levels under which the experimentation is performed. The *S*/*N* ratio must always be maximized, no matter whether the "larger-the-better" or the "smaller-the-better" case is used. In the present work, the possible variation (±12 s) of the elution time corresponding to the volume fraction to be transferred from LC into GC was considered as a noise factor.

Table 2 includes values obtained for y_1 and y_2 in each experimental run as well as a column headed as response which summarizes the *S*/*N* data obtained by applying eq 1.

Experimental conditions defining the initial simplex (experiments 1−5 in Table 2) were established from the matrix of mathematical coordinates proposed by Spendley et al. (1962) and the base levels and step sizes considered for each variable according to Table 1. Subsequently, the initial experimental design was moved in the direction given by the rules of movement of the modified Simplex method (Nedler and Mead, 1965; Deming and Morgan, 1973) as it allows operations of expansion and contraction in the searching progress and, consequently, enables us to overcome several limitations of the sequential Simplex method of Spendley mainly related with its lack of provision for acceleration.

The assessment of the responses obtained in the initial simplex allows the rejection of the worst value. As in the present work the Taguchi signal-to-noise ratio is used as response, the vertex giving the lowest response value (i.e., vertex 4) was rejected, and, consequently, vertices 1, 2, 3, and 5 were maintained. The second simplex was formed by addition of a new vertex resulting from the mirror image of the rejected vertex. Subsequently, the procedure was repeated to move from one simplex into another in such a way that considered vertices were successively either rejected or maintained, as shown in Table 2.

It should be underlined the fact that the self-directing nature of the modified Simplex method may bring about a movement outside the experimental range previously established as more convenient or adequate to instrumental requirements. If such boundary violation is observed, the consequences of its acceptance or not should be carefully evaluated. In the present work, a minimum value (20 °C) was initially considered as admissible for the interface temperature simply for the sake of the ease of the operation since this temperature can be easily set with the commercial configuration of the equipment. Consequently, values lower than 20 °C (as in vertices 7, 10, 15, and 18) were considered as unacceptable, and the simplex was forced to move back inside the boundaries by rejecting the corresponding vertex without experimentation.

Taking into account response values obtained (Table 2), the optimized experimental variables for the direct RPLC−GC analysis of sunflower oil were finally considered to be those of vertex 6 (i.e., transferred volume fraction, 3416 μL; helium flow rate which passes the injector, 797 mL/min; interface temperature, 21 °C; purge time, 0.32 min). A similar (though lower) response (i.e., the second best response) was obtained under experimental conditions defining vertex 14 which involves the transfer from LC to GC of a higher fraction (3897 μL vs 3416 μL), although, in this case, it did not enable us to improve the sensitivity achievable in the analysis, probably due to the fact that the increase of the volume fraction brings about the transfer of LC eluent.

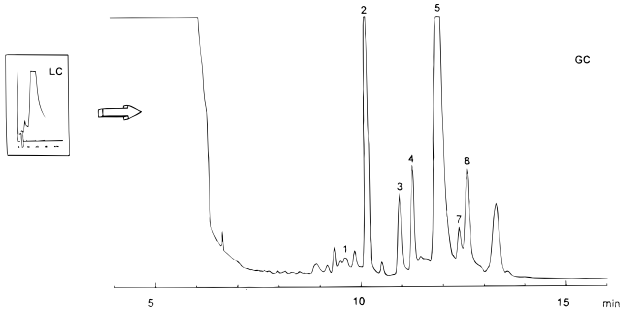

Figure 1. Liquid chromatogram of sunflower oil and gas chromatogram obtained after transfer of the indicated fraction (3000 μL of methanol−water eluent, 78:22) under the initial experimental conditions of the optimization procedure (vertex 1 in Table 2). Identification peak number: 1, γ-tocopherol; 2, α-tocopherol; 3, campesterol; 4, stigmasterol; 5, β-sitoterol; 6, Δ^5-avenasterol; 7, Δ^7-stigmasterol; 8, Δ^7-avenasterol. (HPLC flow rate, 2000 μL/min).

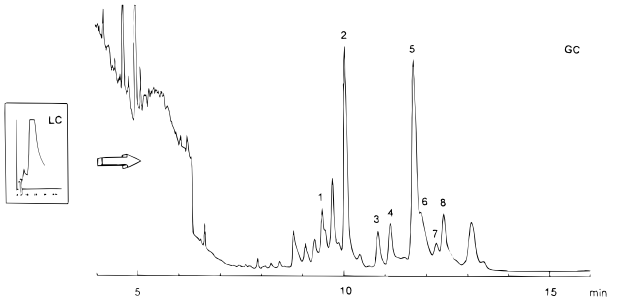

Figure 2. Liquid chromatogram of sunflower oil and gas chromatogram obtained after transfer of the indicated fraction (3197 μL of methanol−water eluent, 78:22) under the experimental conditions giving the worst response in the optimization procedure (vertex 4 in Table 2). Identification peak number as in Figure 1. (HPLC flow rate, 2000 μL/min).

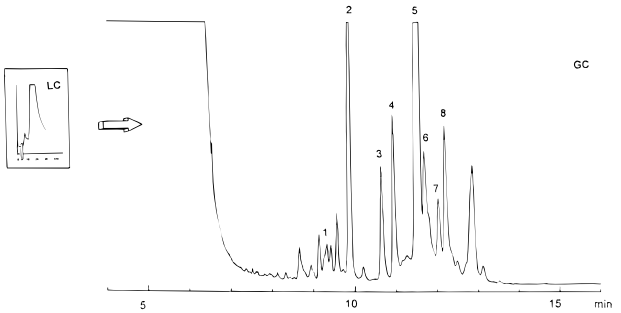

Figure 3. Liquid chromatogram of sunflower oil and gas chromatogram obtained after transfer of the indicated fraction (3416 μL of methanol−water eluent, 78:22) under the experimental conditions giving the best response in the optimization procedure (vertex 6 in Table 2). Identification peak number as in Figure 1. (HPLC flow rate, 2000 μL/min).

Figures 1−4 show both the liquid chromatogram resulting from the preseparation obtained from sunflower oil under conditions described under Experimental Procedures and the gas chromatogram collected after transfer of the indicated fraction. Differences between these figures refer to values considered for the experimental variables optimized. Concretely, Figure 1 corresponds to initial conditions considered in the optimization procedure (vertex 1 in Table 2). Figures 2 and 3 depict the worst and the best GC chromatograms, respectively, obtained in the optimization, while Figure 4 results from conditions yielding the second best response. As can be seen, the sum of the integrated

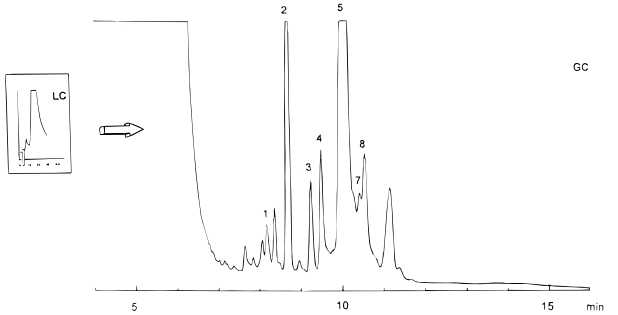

Figure 4. Liquid chromatogram of sunflower oil and gas chromatogram obtained after transfer of the indicated fraction (3897 μL of methanol−water eluent, 78:22) under the experimental conditions giving the second best response in the optimization procedure (vertex 14 in Table 2). Identification peak number as in Figure 1. (HPLC flow rate, 2000 μL/min.)

peak areas obtained at the highest level of the noise factor (y_2 value) in the optimized conditions (vertex 6 in Table 2) is nearly three times higher than that resulting from experimental conditions defining vertex 4. Generally speaking, it can be stated that the optimization procedure presented in this work allows a clear improvement (approximately 40%) of the sensitivity achievable in the analysis performed under the optimized conditions with respect to that corresponding to the initial conditions. It is worth remarking that relative standard deviation (RSD) obtained (from four replicates) for the sum of the peak areas transferred under the experimental conditions giving the best response is 3.9%, while a value equal to 2.0% was obtained under conditions defining vertex 14. These low values obtained for RSD suggest the adequate stability of the system over an extended period of time. In fact, the lifetime of the liquid and gas chromatographic columns used for direct analysis of oil samples has been found to be of several months.

It should also be mentioned that although the optimization procedure presented in this work exclusively refers to free sterols, the transferred LC fraction also contains other compounds (e.g., tocopherols). Evidently, this aspect can be a further advantage of the proposed method providing that not only free sterols but also other compounds are to be analyzed.

Summarizing, the RPLC−GC method proposed here allows the reliable and rapid analysis (the overall procedure takes less than 25 min) of the sterol fraction of a sunflower oil without requiring prior enrichment of the sample, thus avoiding the risk of artifact formation which involves the preparation of the unsaponifiable matter according to the official method.

LITERATURE CITED

Biedermann, M.; Grob, K.; Mariani, C.; Schmidt, J. P. Detection of desterolized sunflower oil in olive oil through isomerized Δ7-sterols. *Z. Lebensm. Unters. Forsch.* **1996**, *202*, 199−204.

Blanch, G. P.; Tabera, J.; Herraiz, M.; Reglero, G. Preconcentration of volatile components of foods: optimization of the steam distillation−solvent extraction at normal pressure. *J. Chromatogr.* **1993a**, *628*, 261−8.

Blanch, G. P.; Herraiz, M.; Reglero, G.; Tabera, J. Preconcentration of samples by steam distillation−solvent extraction at low temperature. *J. Chromatogr. A* **1993b**, *655*, 141−49.

Cortes, H. J. In *Multidimensional Chromatography. Techniques and Applications;* Cortes, H. J., Ed.; Dekker: New York, 1990; p 251.

de Frutos, M.; Cifuentes, A.; Amigo, L.; Ramos, M.; Diez-Masa, J. C. Rapid analysis of whey proteins from different animal species by reversed-phase high-performance liquid chromatography. *Z. Lebensm. Unters. -Forsch.* **1992**, *195*, 326−31.

Deming, S. N.; Morgan, S. L. Simplex optimization of variables in analytical chemistry. *Anal. Chem.* **1973**, *45*, 278A−83A.

Goosens, E. C.; de Jong, D.; de Jong, G. J.; Brinkman, U. A. Th. Reversed-phase liquid chromatography coupled on-line with capillary gas chromatography. II. Use of a solvent vapor exit to increase introduction volumes and introduction rates into the gas chromatograph. *J. Microcolumn Sep.* **1994**, *6*, 207−15.

Grob, K. *On-Line Coupled LC-GC*; Hüthig: Heidelberg, Germany, 1991.

Grob, K. Hypenated high-performance liquid chromatography-capillary gas chromatography. *J. Chromatogr.* **1992**, *626*, 25−32.

Grob, K. Development of the transfer techniques for on-line high-performance liquid chromatography-capillary gas chromatography. *J. Chromatogr.* **1995**, *703*, 265−76.

Grob, K.; Lanfranchi, M. Reproducibility of results from LC−GC of sterols and wax esters in olive oil. *J. High Resolut. Chromatogr.* **1989**, *12*, 624−26.

Grob, K.; Lanfranchi, M.; Mariani, C. Determination of free and esterified sterols and of wax esters in oils and fats by coupled liquid chromatography-gas chromatography. *J. Chromatogr.* **1989**, *471*, 397−405.

Grob, K.; Lanfranchi, M.; Mariani, C. Evaluation of olive oil through the fatty alcohols, the sterols and their esters by coupled LC-GC. *J. Am. Oil Chem. Soc.* **1990**, *67*, 626−34.

Lanuzza, F.; Micali, G.; Calabrò, G. On-line HPLC−HRGC coupling and simultaneous transfer of two different LC fractions. Determination of aliphatic alcohols and sterols in olive oil. *J. High Resolut. Chromatogr.* **1996**, *19*, 444−8.

Mol, H. G.; Staniewski, J.; Janssen, H. G.; Cramers, C. A.; Ghijsen, R. T.; Brinkman, U. A. Th. Trace level analysis of micropollutants in aqueous samples using gas chromatography with on-line sample enrichment and large volume injection. *J. Chromatogr.* **1993**, *630*, 201−12.

Mol, H. G. J.; Janssen, H. G.; Cramers, C. A.; Brinkman, U. A. Th. Large volume sample introduction using temperature programmable injectors: Implications of liner diameter. *J. High Resolut. Chromatogr.* **1995a**, *18*, 19−27.

Mol, H. G. J.; Hendriks, P. J. M.; Janssen, H. G.; Cramers, C. A.; Brinkman, U. A. Th. Large volume injection in capillary GC using PTV injectors: Comparison of inertness of packing materials. *J. High Resolut. Chromatogr.* **1995b**, *18*, 124−8.

Mondello, L.; Bartle, K. D.; Dugo, P.; Gans, P..; Dugo, G. Automated LC-GC: A powerful method for essential oils analysis. Part IV. Coupled LC-GC-MS (ITD) for bergamot oil analysis. *J. Microcolumn Sep.* **1994**, *6*, 237−44.

Mondello, L.; Dugo, G.; Bartle, K. D. On-line microbore high performance liquid chromatography-capillary gas chromatography for food and water analysis. A review. *J. Microcolumn Sep.* **1996a**, *8*, 275−310.

Mondello, L.; Dugo, P.; Dugo, G.; Bartle, K. D. On-line HPLC-HRGC-MS for the analysis of natural complex mixtures. *J. Chromatogr. Sci.* **1996b**, *34*, 174−81.

Nedler, J. A.; Mead, R. A simplex method for function minimization. *Comput. J.* **1965**, *7*, 308−13.

Official Journal of the European Communities, *Off. J. Eur. Communities* **1988** (Jan 29), L22, 58 (EEC Regulation 183/93 of Jan 29, 1993).

Park, P. W.; Guardiola, F.; Park, S. H.; Addis, P. B. Kinetic evaluation of 3,*β*-hidroxycholest-5-en-7-one (7-Ketocholesterol) stability during saponification. *J. Am. Oil Chem. Soc.* **1996**, *73*, 623−29.

Peace, G. S. *Taguchi Methods. A Hands-On Approach to Quality Engineering*; Addison-Wesley: Reading, MA, 1993.

Poy, F.; Visani, S.; Terrosi, F. Automatic injection in high-resolution gas chromatography: A programmed temperature vaporizer as a general purpose injection system. *J. Chromatogr.* **1981**, *217*, 81−90.

Schomburg, G. Sampling systems in capillary chromatography. In *Proceedings of the 4th International Symposium on Capillary Chromatography, Hindelang;* Kaiser, R. E., Ed.; Hüthig: Heidelberg, Germany, 1981; pp 371−4.

Señoráns, F. J.; Tabera, J.; Villén, J.; Herraiz, M.; Reglero, G. Variables affecting the introduction of large sample volumes in capillary gas chromatography using a programmed-temperature vaporizer. *J. Chromatogr.* **1993**, *648*, 407−14.

Señoráns, F. J.; Reglero, G.; Herraiz, M. Use of a programmed temperature injector for on-line reversed phase liquid chromatography-capillary gas chromatography. *J. Chromatogr. Sci.* **1995a**, *33*, 446−50.

Señoráns, F. J.; Herraiz, M.; Tabera, J. On-line reversed phase liquid chromatography-capillary gas chromatography using a programmed temperature vaporizer as interface. *J. High Resolut. Chromatogr.* **1995b**, *18*, 433−38.

Señoráns, F. J.; Tabera, J.; Herraiz, M. Rapid separation of free sterols in edible oils by on-line coupled reversed phase liquid chromatography−gas chromatography. *J. Agric. Food Chem.* **1996**, *44*, 3189−92.

Spendley, W.; Hext, G. R.; Himsworth, F. R. Sequential application of simplex design in optimization and evolutionary operation. *Technometrics* **1962**, *4*, 441.

Staniewski, J.; Rijks, J. A. Potential and limitations of differently designed programmed temperature injector liners for large volume sample introduction in capillary GC. *J. High Resolut. Chromatogr.* **1993**, *16*, 182−7.

Staniewski, J.; Janssen, H. G.; Cramers, C. A..; Rijks, J. A. Programmed-temperature injector for large-volume sample introduction in capillary gas chromatography and for liquid chromatography-gas chromatography interfacing. *J. Microcolumn Sep.* **1992**, *4*, 331−8.

Taguchi, G. *System of Experimental Design. Engineering Methods to Optimize Quality and Minimize Cost*; Kraus International Publications: White Plans, NY, 1987.

Villén, J.; Señoráns, F. J.; Herraiz, M.; Reglero, G.; Tabera, J. Experimental design optimization of large volume sampling in a programmed temperature vaporizer. *J. Chromatogr. Sci.* **1992**, *30*, 261−6.

Vogt, W.; Jacob, K.; Ohnesorge, A.; Obwexer, H. W. Capillary gas chromatographic injection system for large sample volumes. *J. Chromatogr.* **1979**, *186*, 197−205.

Vreuls, J. J.; Brinkman, U. A. Th.; de Jong, G. J.; Grob, K.; Artho, A. On-line solid-phase extraction-thermal desorption for introduction of large volumes of aqueous samples into a gas chromatograph. *J. High Resolut. Chromatogr.* **1991**, *14*, 455−9.

Vreuls, J. J.; de Jong, G. J.; Ghijsen, R. T.; Brinkman, U. A. Th. Liquid chromatography coupled on-line with gas chromatography: State of the art. *J. AOAC Int.* **1994**, *77*, 306−27.

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