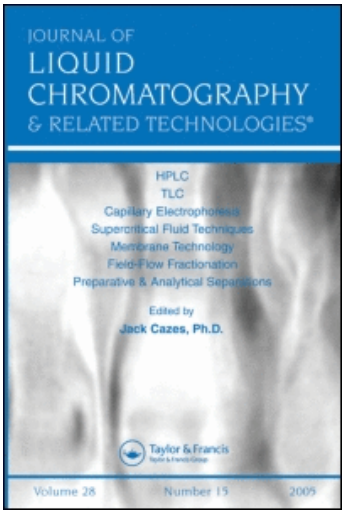

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Sorption of Flavonoids From Wine and Its Investigation by HPLC in the Reverse Phase

P. Špaček[a]; M. Jelínková[a]
[a] Institute of Macromolecular Chemistry Czechoslovak Academy of Sciences, Prague 6, Czechoslovakia

To cite this Article Špaček, P. and Jelínková, M.(1991) 'Sorption of Flavonoids From Wine and Its Investigation by HPLC in the Reverse Phase', Journal of Liquid Chromatography & Related Technologies, 14: 2, 237 — 251
To link to this Article: DOI: 10.1080/01483919108049611
URL: http://dx.doi.org/10.1080/01483919108049611

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(2), 237-251 (1991)

SORPTION OF FLAVONOIDS FROM WINE AND ITS INVESTIGATION BY HPLC IN THE REVERSE PHASE

P. ŠPAČEK AND M. JELÍNKOVÁ
Institute of Macromolecular Chemistry
Czechoslovak Academy of Sciences
CS-162 06 Prague 6, Czechoslovakia

ABSTRACT

A screening test of several commercially available sorbents was carried out with the main attention concentrated on flavonoids from wine. Of the sorbents studied the most advantageous properties are shown by Amidap, the sorption effects of which were quantitatively investigated by using a number of model compounds. The effect of the added sorbent on qualitative changes of several samples of natural wines was evaluated both gravimetrically and (predominantly) by the HPLC method in the reverse phase. The results of this study show that by applying a suitable amount of sorbent to wine it is possible to affect its flavour and colour properties (with the simultaneous tests by degustators).

INTRODUCTION

Sensory properties of wines (and also of the majority of the other juices of natural origin) are affected by the presence of many low-molecular weight

organic compounds, such as organic acids (e.g. tartaric, benzoic, cinnamic acid and their derivatives) or salts, and particularly by a numerous groups of compounds called flavonoids (1), i.e. compounds mostly of the phenolic character, consisting mainly of a skeleton composed of 15 carbon atoms. Although several hundreds of such compounds are known, a certain type of red wine contains about 50 and white wine, about 40 such phenolics (2). Their relative ratio and their total content play a decisive role not only in the colour of wine, but also in the determination of such properties as bitterness (the sensation perceived at the back of the tongue) or astringency (the "dry mouth" feeling produced by an interaction of phenolics with mouth proteins (3)).

Since the phenolics are mostly unstable, they undergo qualitative and quantitative changes during both ripening and ageing of wine, accompanied also by changes in colour and flavour properties (sometimes, sediment is also formed). In some cases their content may have a negative effect on the sensory properties of freshly produced wine (4). Hence, theoretically, the quality of wine could be affected by varying the concentration of organic compounds present in it, including flavonoids. Naturally, by using a suitable sorbent it would be advantageous to separate selectively only those compounds which have a negative effect on organoleptic properties of wine. This however is unrealistic, because on the one hand it is not exactly known which compounds should be removed, and on the other, because virtually no sorbent is able to separate selectively only certain components of a chemically very similar mixture (5). There is also another solution to the problem, namely, to find a sorbent capable of sorbing a whole group of phenolics, and thus of reducing the concentration of undesirable components, and also of producing a positive effect on sensory properties of wine. The decisive word regarding the result obtained should be that of a degustator.

The sorbent prodominantly used for purposes of this study was Amidap (6), which is relatively cheap, easy to prepare, has been successfully used in

Czechoslovakia for several years in the stabilization of beer (sorption of phenolics causing turbidity) and has been approved for use in the food industry. In this study its sorption efficiency has been investigated gravimetrically on the basis of mass balance, and chromatographically on the basis of changes in the area of peaks of compounds eluted in the range of retention times corresponding to the elution of commercially available model flavonoids; the dependence of the sorption of these model compounds on the amount of the added sorbent Amidap was also determined. Using red wine Oran (Algeria), we compared sorption effects of the sorbent Amidap with several other sorbents, which were available readily and in a major amount. Eventually, we evaluated the application of Amidap to several types of North American wines provided by courtesy of Dextran Product Ltd., Ontario, Canada.

EXPERIMENTAL

Apparatus and Methods

Chromatographic measurements were carried out by means of a liquid chromatograph made by Spectra Physics, consisting of a chromatograph type SP 8 100, UV/VIS detector type SP 8440 and a programmable integrator type SP 4200. A glass column in a metallic cartridge type CGC (7) (compact glass column), 150 mm long, inner diameter 3.3 mm (manufactured by Laboratory Instruments, Prague) packed with the sorbent Separon SIX C 18 (8) (spherical silicagel, particle diameter 5 μm, surface modified with the aliphatic chain C_{18}) was used in the separation; column efficiency was 49 000 or 55 000 theoretical plates per 1 m with benzene or toluene as the standard and methanol-water (7:3) as the mobile phase.

In the chromatographic analysis of wine samples and model compounds many modifications of the HPLC method and various types of gradients can

be used (9). In our case the following gradient was found useful (solvent A = methanol, solvent B = 5% acetic acid in water): 0-10 min linear gradient 2% A to 32% A; 10-40 min linear gradient 32% A to 100% A; 40-50 min isocratic elution at 100% A; 50-55 min linear gradient 100% A to 2% A; 55-70 isocratic elution at 2% A. Acetic acid was used due to the suppresion of ionization of the acid groups of compounds under separation present in the mixture (1) which should give rise to undesired nonspecific interactions with the sorbent. The gradient together with an example of separation of model flavonoids can be seen in Fig. 1.

The flow of the mobile phase in all experiments was 0.5 ml/min, the injected sample volume was 10 μl, the column was thermostated at 40°C, the maximal pressure gradient in the column was 15 MPa. A UV/VIS detector was employed at the wavelength 300 nm and sensitivity 0.64 AUFS. Integration of compounds eluted at retention times below 10 min and above 55 min was inhibited. On the basis of the chromatographic behaviour of model compounds it was decided that concentration changes of flavonoids in wine samples before and after the sorption, and of the fraction regenerated from the sorbent used would be compared, bearing in mind the sum of all integrated areas in the 10-55 min itervals.

Chemicals

Model compounds (used without further purification as supplied by the firm Sigma): Flavone, Quercetin (3,3′,4′,5,7-pentahydroxyflavone), Myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone), Rutin (glycosylated quercetin), Kaempferol (3,4′,5,7-tetrahydroxyflavone), Khellin (4,9-dimethoxy-7-methyl-5H-furo-3,2-g-1-benzopyrene-5-one), Riboflavin (vitamin B_2).

Sorbents: Amidap (prepared at the Polymer Department of the Institute of Chemical Technology) — porous block copolymer polyamide-

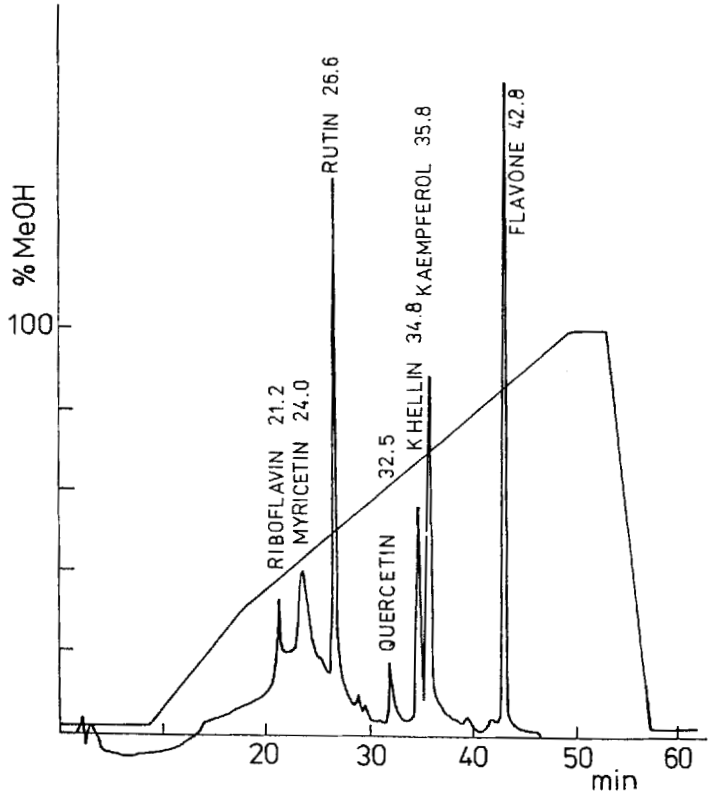

FIGURE 1. Chromatogram of model compounds and solvent gradient

6-polyoxyethylene, specific surface area 5.2 m^2/g, particle size 100-500 μm; Amberlite XAD 4 (Serva, Heidelberg, FRG) - macroporous copolymer styrene-divinylbenzene, specific surface area 750 m^2/g, particle size 300-1000 μm; G-60 (prepared at the Institute of Macromolecular Chemistry, lot No GMA 427) - macroporous copolymer glycidylmethacrylate-ethylenedimethacrylate (6:4), specific surface area 60 m^2/g, particle size 200-300 μm; Separon HEMA 1000 (Laboratory Instruments, Prague) macroporous copolymer 2-hydroxyethylmethacrylate - ethylenedimethacrylate, specific surface area 26.2 m^2/g, particle size 125-200 μm; DEAHP cellulose (pre-

pared at the Institute of Macromolecular Chemistry - lot No 5.1913/1980), bead cellulose bearing groups $-OCH_2-CH(OH)-CH_2-N(CH_2CH_3)_2$, content of groups 1.08 mmol/g, particle size 135-440 μm, water regain 5.3 g H_2O/g. All sorbents were washed with water before use, decanted and (with the exception of cellulose) dried.

Wines: Oran - red wine from Algeria with a high content of colour and flavour components, Niagara (white), Catawba (red), Concorde (red); the last three types supplied by courtesy of the firm Dextran Products.

Procedures: Sorption of model compounds: Model compounds were dissolved in a mixture methanol - water - acetic acid 20:75:5 (by volume) in an amount 0.1 mg/ml or 0.05 mg/ml (in the case of kaempherol and quercetin). The sorbent Amidap was added to the solution in various amounts (cf Fig. 2) and the suspesion was stirred by means of a magnetic stirrer for 20 min, after it had been verified experimentally in advance that prolonged stirring does not affect the integrated area of peaks of these compounds. After sedimentation of the sorbent the clear supernatant was injected into the chromatograph.

Sorption of phenolics in wine: Into a weighed round flask, 250 ml in volume, 100 ml of wine was poured and evaporated to dryness in a vacuum rotary evaporator at 30°C. The weight of the evaporated residue was determined, after which the residue was dissolved in a water-methanol mixture (1:1), 5 ml in volume (in the case of Oran) or 10 ml (in the case of the other wines). The solution thus obtained was directly employed in chromatography. Into another 100 ml fraction of wine 1 g of the dry sorbent was added, the suspension was stirred with a magnetic stirrer at room temperature for two hours. After that, the sorbent was removed by filtration and the filtrate was treated in the same way as the original wine. The sorbent removed by filtration was transferred into 400 ml of methanol, the suspension was stirred at room temperature for 24 h and filtered. The filtrate was evaporated in vacuo to dryness, weighed, dissolved in 10 ml of a methanol-water mixture (1:1), and chromatographed.

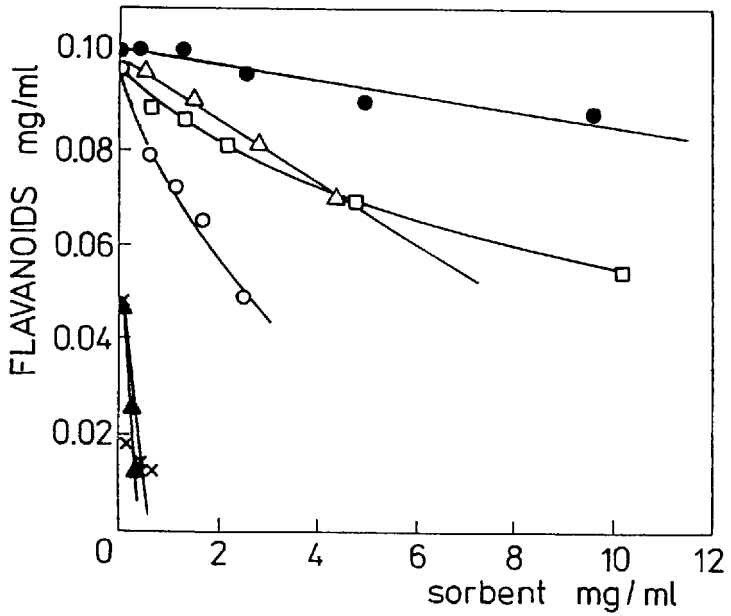

FIGURE 2. Dependence of the amount of phenolics sorbed from a model solution having the concentration 0.1 mg/ml or 0.05 mg/ml on the amount of the sorbent Amidap added: Rutin □, riboflavin ○, flavone △, khellin ●, kaempherol ▲, quercetin ×

RESULTS AND DISCUSSION

Sorption of Model Compounds

Although it is not clear whether the model compounds used are a representative sample of phenolics in natural wines, they were used as a good model to suggest a quantitative chromatographic method, especially in the optimization of the gradient of the mobile phase (cf Fig. 1). To be able to estimate the possibility of using the sorbent Amidap in the sorption of this type of compounds, the consumption of the individual components from so-

lution after the addition of various quantities of the sorbent was measured. Changes in the concentration of the individual model compounds were determined chromatographically from relative changes in the integrated areas of relevant peaks (cf Fig. 2).

Sorption of Compounds from Natural Wines

Screening of the sorption properties of the following sorbents: Amidap, Amberlite XAD 4, Separon HEMA 1000, G-60 and DEAHP cellulose was carried out using the red wine Oran. The results obtained by weighing the dry residue of the original wine and of the wine after the addition of the individual sorbents and, finally, of the dry residues from the extraction of the sorbents are summarized in Table 1. The data show that the largest fraction of nonvolatile compounds is sorbed by the weakly basic anion exchanger based on bead cellulose. Obviously, what we have in this case is binding of predominantly acid compounds to the sorbent by the ionic bond which cannot be disrupted by the standard washing of the sorbent with methanol, so that more than 13% of compounds remain irreversibly sorbed. Similarly, with the sorbent G-60, where more than 11% of compounds remain irreversibly sorbed, the epoxy group probably reacts with components of the wine. In the case of nonionogenic sorbents, such as Amberlite, Separon and Amidap there is only an insignificant fraction of compounds irreversibly sorbed on the sorbent, not exceeding 4% of their total amount.

In view of the fact that sorption of those compounds which can participate in the formation of flavour properties of wine is of the greatest interest, it is more advantageous to investigate predominantly changes in their concentration by using the chromatographic method and by integrating the area of peaks situated in the range of the elution times 10-55 min. It may be assumed that the predominant majority of compounds derived from organic acids and

TABLE 1

Effect of Sorbents on the Amount of Separated Nonvolatile Components of Wine Oran and the Total Mass Balance Determined from Weight Changes

Sorbent	Content of nonvolatile components in wine					Difference from original wine[a]	
	After sorption		Desorbed		Total		
	g	%	g	%	g	g	%
None	2.672	100			2.672		
AMIDAP	2.536	94.9	0.174	6.5	2.710	+0.038	+1.4
Amberlite XAD 4	2.482	92.9	0.118	4.4	2.600	−0.072	−2.7
Separon HEMA 1000	2.356	88.2	0.216	8.1	2.572	−0.100	−3.7
G-60	2.123	79.5	0.236	8.8	2.359	−0.313	−11.7
DEAHP Cellulose	2.046	76.5	0.273	10.0	2.319	−0.363	−13.5

[a] Losses arising by irreversible sorption on washing with methanol

TABLE 2

Effect of Sorbents on the Amount of Separated Nonvolatile Components of Wine Oran Determined by Integration of the Areas of Chromatographic Peaks with Retention between 10 and 55 min

Sorbent	Content of nonvolatile components in wine		Difference from original wine
	After sorption	Desorbed	
	%	%	%
None	100		
AMIDAP	40.8	35.9	−23.3
Amberlite XAD 4	75.4	29.8	+4.4
Separon HEMA 1000	58.7	33.2	−8.3
G-60	50.3	22.3	−27.5
DEAHP Cellulose	33.0	6.1	−60.9

their salts will be eluted at the very beginning of the chromatogram, and that by eliminating them it is possible to increase the sensitivity of the method used (compared with weighing of the dry residues mentioned above). The results are summarized in Table 2, an example of the effect of the sorbent Amidap on wine Oran can be seen in Fig. 3. Data in Table 2 show that in agreement with the preceding results weakly basic cellulose retains the greatest fraction of irreversibly sorbed compounds; only 6% of the compounds is desorbed and more than 60% is retained after washing with methanol. As regards the other tested sorbents, in the field of chromatographic peaks corresponding to the elution of sensorically interesting compounds the best results are obtained with the sorbent Amidap, which retains virtually 60% of components of this type of compounds present in wine, while with the other sorbents a similar range of sorption can be reached only by using them in a higher amount.

Basing on the results obtained with the model wine Oran, where Amidap seems to be the best sorbent, the latter was used to assess its influence on the sorption of phenolics from North American wines Concorde and Catawba

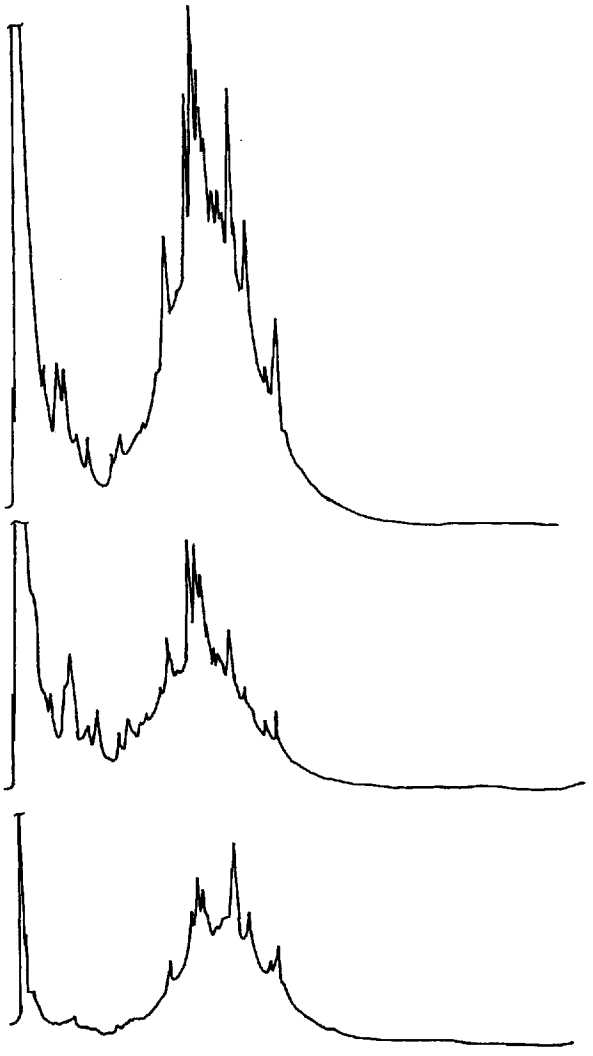

FIGURE 3. Chromatograms of wine Oran: original (upper part), after treatment by sorbent Amidap (middle part) and desorbed compounds (lower part)

TABLE 3

Effect of Sorbent AMIDAP on the Amount of Separated Nonvolatile Components and Total Mass Balance Determined from Weight Changes

Wine	Content of nonvolatile components					Difference from original wine	
	Original	After sorption		Desorbed			
	g	g	% orig	g	% orig	g	%
Concorde	3.093	2.303	74.5	0.410	13.3	−0.38	−12.2
Catawba	3.000	2.572	85.7	0.283	9.6	−0.15	−4.7
Niagara	2.324	2.244	96.6	0.273	11.7	+0.19	+8.3

TABLE 4

Effect of Sorbent AMIDAP on the Amount of Separated Nonvolatile Components Determined by Integration of the Area of Chromatographic peaks with Retention between 10 and 55 min

Wine	Content of nonvolatile components in wine		Difference from original wine
	After sorption	Desorbed	
	%	%	%
Concorde	14.3	17.9	−67.8
Catawba	30.4	18.4	−51.2
Niagara	47.5	35.8	−16.7

(both red) and Niagara (white). The results of experiments based on weighing dry residues are summarized in Table 3 (obtained similarly to data in Table 1), data obtained on the basis of integration of the area of chromatographic peaks in the range of elution of flavonoids are summarized in Table 4 (obtained under the same conditions as data in Table 2). By comparing the results in both Tables we can see that, e.g., in the case of wine Concorde about 25% of all compounds determined gravimetrically is sorbed, which corresponds to more than 85% of the sorption of compounds determined chromatographically in the range of the retention times of flavonoids. It seems, therefore, that just those components of wine which play the decisive role in its flavour are sorbed preferentially; a similar reasoning holds also in the case of the

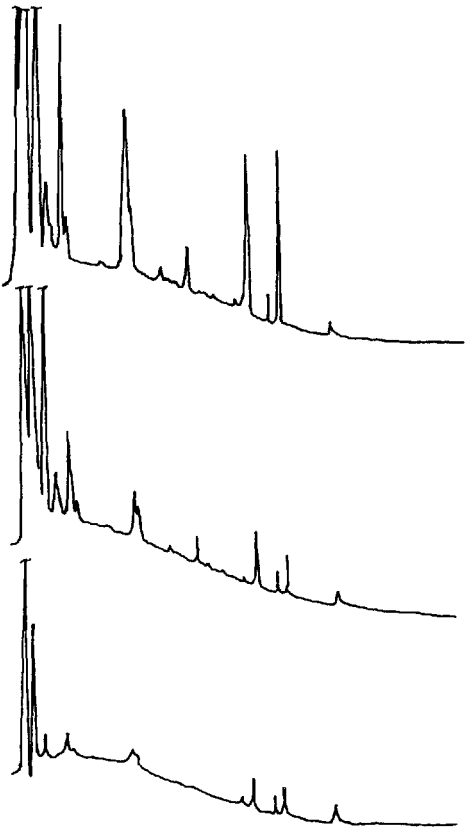

FIGURE 4. Chromatograms of wine Catawba: original (upper part), after treatment by sorbent Amidap (middle part) and desorbed compounds (lower part)

remaining types of tested wine. Fig. 4 shows a chromatogram which documents the effect of the sorbent on the composition of phenolics in the wine Catawba: we can see that the wine contains a comparatively small number of compounds attractive with respect to their flavour or colour (cf Fig. 3 for wine Oran). Hence, it is quite likely that in such cases there is only a very restricted possibility to improve the flavour of wine by sorption; moreover,

the sorbent should be added with great caution and in small doses (exactly determined on the basis of the degustator's reference). A comparatively large fraction of undesorbed compounds (the last column in Table 4) may be due to the greater error of the method at a very low concentration of relevant compounds, and also to the fact that a simple decantation of the sorbent inmethanol (chosen for the sake of simplicity for the sorbent's application inpractice) is not sufficient for a quantitative desorption of small quantities of the compounds sorbed.

CONCLUSIONS

We have shown in this study that a suitable chosen sorbent makes possible a relatively selective sorption of phenolics from wine. The amount of compounds sorbed may be regulated very easily by the amount of the sorbent added; this can be controlled either by measuring changes in the weight of the dry residue (which is not very conclusive, since wine contains many compounds not interesting with respect to its flavour and colour), or by a chromatographic determination of the eluated components within the corresponding range of the retention times.

A screening test of commonly used sorbents suggests that Amidap might be a suitable sorbent: it is readily available, has been approved for use in the food industry and is already successfully applied for the removal of compounds causing turbidity in beer. It should be mentioned, however, that results of this study are not decisive for the adjustment of flavour properties of wine, but ought to be supplemented by adding the results of a reliable sensory test based on an expertise carried out by a team of degustators.

Acknowledgment

Support of this research by Polydex Pharmaceuticals Ltd, Toronto, Canada is gratefully acknowledged.

REFERENCES

1 Salagoity-Auguste M.H. and Bertrand A., J. Sci. Food Agric., 35, 1241, 1984

2 Wulf L.W. and Nagel C.W., J. Chromatogr., 116, 271, 1976

3 Reville E., Alonso E. and Estrella M.I., Chromatografia, 22, 157, 1986

4 Barroso C.G., Torrijos R.C. and Perez-Bustamante J.A., Chromatografia, 17, 249, 1983

5 Castele K.V., Geiger H. and van Sumere Ch.F., J. Chromatogr., 240, 81, 1982

6 Veruovič et al CS-PV 7581-78

7 Špaček P. et al CS Patent 223 732

8 Vozka S. et al US Patent 4,104,363

9 Saigle D.J. and Conkerton E.J., J. Liquid Chromatogr., 11, 309, 1988