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Solid-phase extraction and high-performance liquid chromatographic separation of pigments of red wines

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Abstract

The adsorption and desorption capacities of 11 different solid-phase extraction sorbents were tested for the preconcenration of pigments of various Hungarian red wines. The concentrates were evaluated by multiwavelengh spectrophotometry combined with a spectral mapping technique (SPM) and by reversed-phase high-performance liquid chromatography. The highest (10-fold) concentration of pigments was achieved on octadecylsilica sorbent. It can be used five times without losing adsorption and desorption characteristics. SPM indicated that multiwavelength spectrophotometry can be employed for the differentiation of red wines. Comparison of the chromatograms of pigments with and without preconcentration showed that preconcentration makes possible the separation and detection of pigments present in low concentration in red wines.

Keywords: Wine; Food analysis; Solid-phase extraction; Pigments

1. Introduction

The colour of wines, especially that of red wines, is one of the most important properties used for the commercial evaluation and exerts a considerable influence on the marketability of the product. The quality of colour is determined by both the quality and quantity of pigments and by their composition and distribution. The exact knowledge of the amount and type of pigments of red wines may also facilitate the identification of origin and the detection of adulteration. Anthocyanins (substituted derivatives of the 2-phenolbenzopyrilium ion) are responsible for the colour of red wines. Until now more than 300 various anthocyanin derivatives were identified – their analysis is a serious challenge for chromatographers dealing with the separation of mixtures of natural pigments. Spectrophotometry has been successfully used for the determination of the total amount of anthocyanins [1]. Although spectrophotometry gives accurate information on the quantity of pigments it is unsuitable for the characterisation of the individual pigment fractions.

Various liquid chromatographic methods have been frequently employed for the separation and quantitative determination of anthocyanins. Thin-layer chromatographic methods have been recently reviewed [2,3]. High-performance liquid chromatography (HPLC) is the method of preference for anthocyanin analysis [4]. HPLC techniques were employed for the determination of anthocyanins in

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fruits [5], fruit juices [6], etc. As the retention characteristics of anthocyanin derivatives are highly different generally gradient elution is employed for their effective separation [7]. In order to improve the efficacy of pigment separation, anthocyanins were preconcentrated on a C_{18} solid-phase extraction (SPE) cartridge [8], or on a XAD-2 ion-exchange resin [9]. The application of HPLC to the determination of the anthocyanin profile of young red wines has also been reported [10]. Liquid chromatography has also been employed for the semipreparative [11] or preparative separation of anthocyanins [12].

Various multivariate mathematical–statistical methods have found application in the evaluation of chromatographic data of foods and food products. Thus, canonical correlation analysis was employed for the authenticity test of honeys of various origin [13], linear discriminant analysis for the determination of milk fat adulteration [14], principal component analysis for the authenticity test of edible oils [15], cluster analysis for classifying white wines [16], artificial neural network for the prediction of shelf-life of milk [17], and spectral mapping for the differentiation of paprika powders [18].

The objectives of the work were the classification of red wines according to their visible spectra by spectral mapping technique (SPM), and the comparison of the efficacy of various SPE sorbents and elution techniques for the purification and preconcentration of pigments of red wines for HPLC analysis. To the best of our knowledge the performance of various SPE sorbents in the concentration of pigments of red wines has never been studied in detail.

2. Experimental

Red wines from different wine producing regions of Hungary were included in the experiments. Their specifications are listed in Table 1. SPE sorbents used in the preliminary experiments are listed in Table 2. Cartridges filled with sorbents I–X were purchased from Waters (Milford, MA, USA), carbon black was prepared by Professor D. Berek (Polymer Institute of the Slovak Academy of Sciences, Bratislava, Slovak Republic) and the cartridge was filled in our laboratory. Each cartridge has an internal diameter of 10 mm and contained 500 mg of sorbent.

Table 1
Specifications of red wines

No.	Commercial name	Origin	Year
1	Kékfrankos	Szekszárd	1996
2	Kékfrankos	Mátraalja	1996
3	Pinot Noir	Szekszárd	1996
4	Merlot	Dél-Balaton	1996
5	Cabernet Sauvignon	Villány	1996
6	Kékfrankos	Balatonboglár	1996
7	Cabernet Franc	Tihany	1996

Cartridges were preconditioned successively with 3 ml acetone, 3 ml methanol and 3 ml of distilled water. Thereafter red wines were loaded into cartridges with a flow-rate of 0.2 ml/min until the pigments appeared at the vent. It can be assumed that the removal of ethanol from the samples of red wine may increase the loading capacity of the SPE cartridges. However, the removal of ethanol is an additional step in the analysis, it is time-consuming and the pigments can decompose during the process. Cartridges were flushed with 5 ml of bidistilled water to remove unwanted impurities. Then, cartridges showing considerable loading capacity (ca. 5 ml of red wine) were eluted with acetonitrile, methanol, tetrahydrofuran and dioxane each of them containing 10% (v/v) conc. formic acid. When the removal of pigments was insufficient the concentration of formic acid was increased in the eluent as the total desorp-

Table 2
SPE sorbents used for the preconcentration of pigments of red wines and their sample capacity

Sorbent		Sample capacity
No.	Type	(ml red wine)
I	Silica	<1
II	Octadecylsilica	10
III	Cyanopropyl	<1
IV	Alumina (acidic)	<1
V	Alumina (basic)	<1
VI	Alumina (neutral)	<1
VII	Diol	<1
VIII	Aminopropyl	8
IX	Florisil[a]	<1
X	Accell Plus QMA[b]	5
XI	Carbon black	5

[a] Polar, highly active, weakly basic adsorbent.
[b] Silica-based hydrophilic, strong anion exchanger with large pore size.

tion was achieved. The efficiency of desorption was checked by measuring the visible spectra of the eluates. Pigments were eluted with 2×1 ml of eluents. The absorption characteristics of the eluted pigments were determined in a 1-mm cuvette by a Jasco V-570 UV–Vis–near IR spectrophotometer (Jasco, Edo, Japan). Their absorbances were determined at 340, 397, 455, 513, 570, 627, 685 and 743 nm after appropriate dilution. In order to explore the possibility of reusing the octadecylsilica cartridge the whole preconcentration process was carried out five times and the eluates were evaluated by multiwavelength spectrophotometry as described above. SPM was applied to the separation of the overall amount of pigments (absorbance values measured at different wavelengths) and their distribution according to wavelengths [19]. SPM divides the information into two matrices. The first one is a vector containing the values proportional to the overall amount of pigments (so-called potency values). The second one is a matrix containing the information related to the selectivity (distribution of pigments without taking into consideration their concentration). SPM calculations were carried out twice: absorbance values measured at eight different wavelengths were the variables and the red wines the observations (SPM calculated the overall concentrations of pigments and their distribution among the red wines); red wines were the variables and absorbance values measured at eight different wavelengths the observations (SPM calculated the overall absorbance of red wines at different wavelengths and their distribution according to the wavelength). As the visual evaluation of multidimensional matrices of spectral maps is difficult the dimensionality of spectral maps was reduced to two by the nonlinear mapping technique [20].

Reversed-phase (RP) HPLC was employed for the comparison of the distribution of pigment fractions with and without SPE pretreatment. The system consists of an ISCO Model 2360 pump, an ISCO Model 2350 gradient programmer (both Isco, Lincoln, NE, USA), a Waters 991 photodiode array detector (Millipore, Milford, MA, USA), an NEC PowerMate SX/16 computer (Nec Technologies, Boxborough, MA, USA) with a Waters photodiode array (PDA) program (Millipore), and a Valco injector with 20-μl sample loop (Valco, Houston, TX, USA). Separations were performed on a 250×4 mm I.D. column packed in our laboratory with octadecylsilica stationary phase (LiChroSpher, particle size, 5 μm, Merck, Darmstadt, Germany) by means of a Shandon analytical HPLC packing pump (Shandon, Pittsburgh, PA, USA). The gradient elution steps are detailed in Table 3. The flow-rate was 1 ml min^{-1} and the detection wavelength varied between 320 and 600 nm including the absorption maxima of pigments. The column was not thermostated, developments were performed at room temperature (22±2°C). Each measurement was run in triplicate and the relative standard deviation (RSD) of retention time and peak areas was calculated.

Table 3
Gradient table for the reversed-phase high-performance liquid chromatographic separation of pigment fractions in red wines[a]

Time (min)	A (%, v/v)	B (%, v/v)
0	100	0
20.00	75	25
45.00	75	25
85.00	50	50
150.00	50	50

[a] Eluent A=bidistilled water–conc. formic acid (9:1, v/v); eluent B=bidistilled water–acetonitrile–conc. formic acid (6:3:1). For chromatographic conditions see Experimental.

Calculations were carried out on an IBM AT computer. Software for SPM and nonlinear mapping were prepared by Dr. B. Bordás (Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary).

3. Results and discussion

The loading capacities of sorbents for red wines are listed in Table 2. Compared to the data in the literature the loading capacities are surprisingly low. This fact can be tentatively explained by the supposition that the concentration of pigments in red wines is relatively high and the large pigment molecules occupy the absorption centres of sorbents very rapidly. Moreover, the presence of ethanol (about 11%, v/v) as an eluent in the samples may facilitate the removal of pigments from sorbents. We assume that the phenomena observed are the combined result of the effects outlined above.

Large differences were found among the sample capacities of sorbents emphasising the importance of the right selection of sorbent for the preconcentration of these type of pigments. Sorbents exposing hydrophobic (octadecylsilica), hydrophilic (Accell Plus QMA) and mixed retention mechanisms (aminopropyl, carbon black) equally retained pigments. These results indicate that both hydrophobic and hydrophilic interactive forces can be involved in the binding of anthocyanins to the stationary phase. Desorption of pigment was difficult from the aminopropyl, Accell Plus QMA and carbon black support, it needed methanol solution containing more than 50% (v/v) of conc. formic acid. Desorption was easy from octadecylsilica sorbent using methanol–conc. formic acid solution (9:1, v/v). The retention behaviour (absorption and desorption characteristics) of pigments of various red wines was identical, therefore, a method developed for the preconcentration of pigments from one red wine can be successfully applied to other red wines as well. The second fraction of 1 ml of eluent did not show any absorbance in the visible region highlighting the good desorption efficacy of eluent. As octadecylsilica sorbent showed the maximal retention capacity and less eluent strength for pigment desorption, it was selected for sample preparation. Using the ''*t*'' test no significant difference was found between the absorption values of the concentrated pigments in the five consecutive preconcentration procedures which means that the cartridge can be reused five times without losing its adsorption and desorption characteristics.

The overall absorbance (potency) values calculated by SPM are compiled in Table 4. Potency values related to the amount of pigments of individual red wines do not show high deviations indicating that the amount of pigment determined by spectrophotometric methods is not suitable for the classification of wines. Differences between the absorbances measured at different wavelengths are considerably higher than those between the absorbance of red wines. Interestingly, red wines show marked absorbance even in the yellow region suggesting that red wine also can contain yellow pigment characteristics of white wines.

The two-dimensional nonlinear selectivity map of red wines is shown in Fig. 1. Red wines are widely distributed on the map and do not form clear-cut clusters either according to the type of grape or according to the origin. This finding indicates that both factors mentioned above exert a marked influence on the pigment fractions and the strength of their impact on the composition of pigments is commensurable. The distribution of pigments further indicates that the measurement of absorbance at different wavelengths and the application of SPM for the evaluation of the data matrix consisting of absorbance data may help the identification of the type and origin of red wines. We are well aware that

Table 4
Overall absorption capacity of red wines taking into consideration of their absorbance simultaneously at each wavelength (related to the amount of pigments) and their overall absorbance at different wavelengths taking into consideration each red wine sample simultaneously[a]

Wine No.	Potency (arbitrary units)	Wavelength (nm)	Potency (arbitrary units)
1	2.96	340	7.78
2	2.60	397	0.63
3	3.32	455	1.87
4	2.76	513	2.05
5	2.63	570	2.81
6	3.17	627	2.75
7	2.71	685	2.42
		743	1.23

[a] Results of spectral mapping technique.

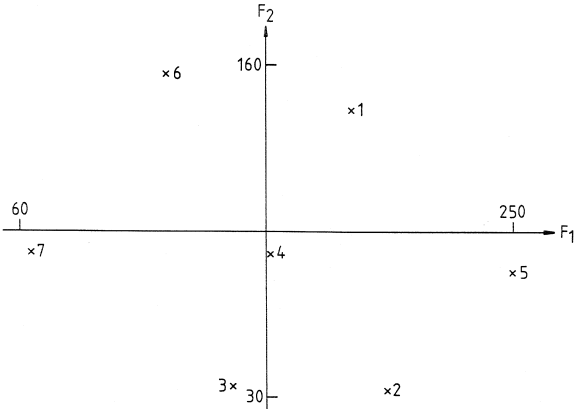

Fig. 1. Similarities and dissimilarities between the red wines according to the selectivity of their absorbance at eight different wavelengths. Two-dimensional nonlinear selectivity map. No. of iterations: 76; maximum error: $3.53 \cdot 10^{-2}$. Numbers refer to red wines in Table 1.

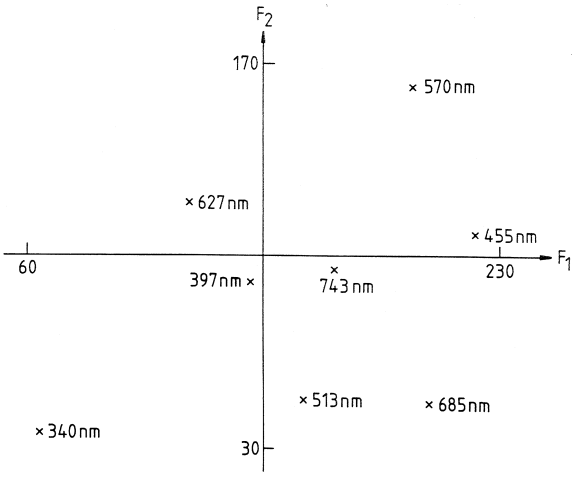

Fig. 2. Similarities and dissimilarities between the selectivities of wavelength taking into consideration simultaneously the absorbance of each red wine. Two-dimensional nonlinear selectivity map. No. of iterations: 161; maximum error: $3.21 \cdot 10^{-2}$.

the number of wines included in the experiments is not sufficient to draw more concrete conclusions. However, we think that the method offers an interesting possibility and should be studied more in detail in the future.

The two-dimensional selectivity map of the individual wavelengths is shown in Fig. 2. The wavelengths are well separated from each other on the map. This result indicates that the information content of each wavelength is different, therefore, their inclusion as separate variables in SPM calculations is justified.

The RSDs of the retention times and peak areas were 0.8–1.5% and 2.4–3.6%, respectively. The RSDs of retention times are low indicating the good stability and reproducibility of the HPLC system. However, the RSDs of peak areas are higher than those generally accepted for HPLC. This discrepancy can be tentatively explained by the fact that the

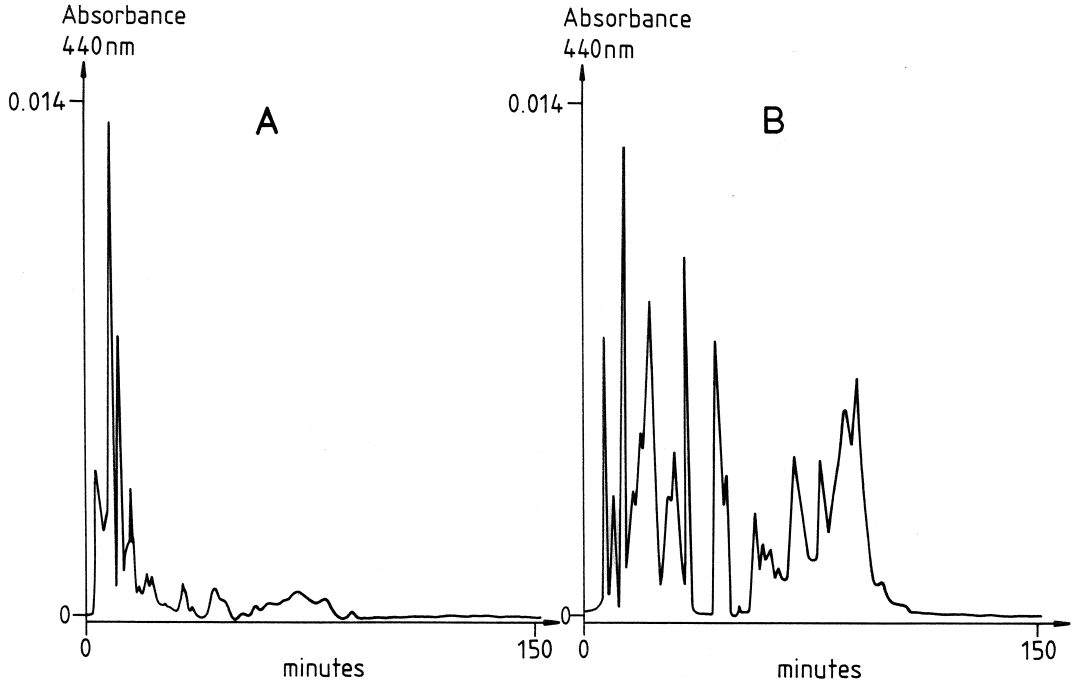

Fig. 3. RP-HPLC chromatogram of red wine 1 without preconcentration (A) and with preconcentration (B) at 440 nm. For chromatographic conditions see Experimental. Number refers to the red wine in Table 1.

baseline separation of pigments was not achieved in each instance which adversely influenced the interation process. It can be assumed that the peak symmetry and peak shape can be considerably improved by the modification of the HPLC method (use of column with higher theoretical plate number, thermostation of the column, buffering of the mobile phase, etc.) and the improved HPLC method can be used for the future investigation of the composition of pigments of red wines.

The HPLC chromatograms of red wine 1 (Kékfrankos, Szekszárd, 1996) at 440 and 570 nm without preconcentration and with preconcentration are shown in Figs 3 and 4. Chromatograms clearly show that preconcentration enhances the sensitivity of detection and increases the number of pigment fractions. Chromatograms entirely support our previous conclusions that even red wines contain a considerable amount of pigments absorbing at 440 nm (characteristic for yellow pigments).

It can be concluded from the data that the pigments of red wines can be readily preconcentrated on SPE cartridges containing octadecylsilica sorbent. The preconcentration step makes possible the separation and detection of pigment fractions present in low concentrations in red wines by HPLC. Multiwavelengh spectrophotometry and HPLC seem to be a valuable tool for the analysis of pigments and may facilitate the identification of red wines and the detection of adulteration.

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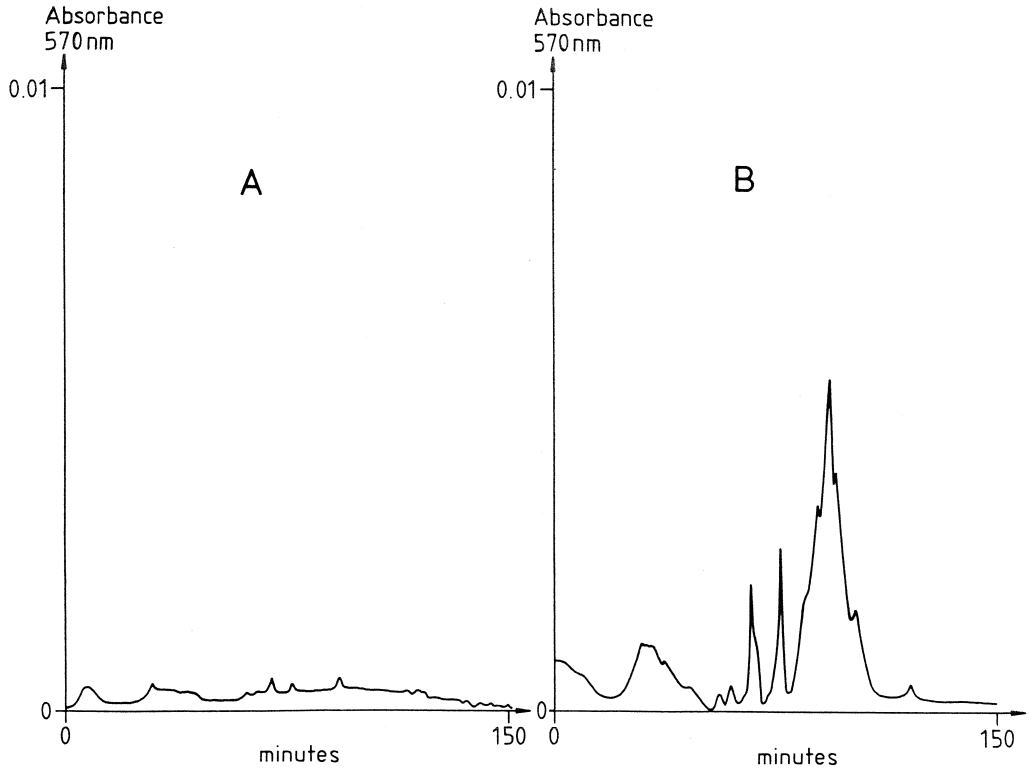

Fig. 4. RP-HPLC chromatogram of red wine 1 without preconcentration (A) and with preconcentration (B) at 570 nm. For chromatographic conditions see Experimental. Number refers to the red wine in Table 1.

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