Coumarins content in wine: application of HPLC, fluorescence spectrometry, and chemometric approach

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Abstract In this work, high performance liquid chromatography (HPLC) and fluorescence spectrometry methods for determination of natural coumarins in Tokaj wine were developed and compared. Molecularly imprinted solid phase extraction procedure was applied for sample preparation. The separation of esculin, coumarin, herniarin, 4-methylumbelliferone, scoparone, scopoletin was performed on core-shell C18 type of stationary phase (100 × 4.6 mm, 5 µm) with a gradient elution of mobile phase containing 1% aqueous solution of acetic acid and methanol, UV–VIS (280 nm for coumarin) and fluorescence detection (Ex 320 nm, Em 450 nm for other coumarins). The HPLC method was validated in term of linearity, limit of detection, limit of quantification, precision and accuracy. Fluorescence detection offers high sensitivity with limit of detection in the ng mL⁻¹ range. Scopoletine and 4-methylumbelliferone were identified and quantified in tested wines. Emission spectral data, synchronous fluorescence spectra of coumarins from fluorescence spectrometry and total concentrations of coumarins quantified by the HPLC method were used in the partial least squares regression. The linear regression between the concentrations predicted by the partial least squares model versus true values obtained by HPLC method confirmed good agreement between the two methods.

Keywords Wine · Coumarins · High performance liquid chromatography · Fluorescence spectrometry

Introduction

Aging of wine in wooden barrels is a traditional and standard procedure used in the enology for centuries to yield high-quality wines. It is carried out to enrich sensorial properties, improve or stabilize the colour, alternatively improve limpidity (Ribéreau-Gayon et al. 2006). Oak is the main wood used in making of barrels of wine production, but rarely the chestnut, cherry, acacia, and mulberry are also used. Many studies focused on (1) the composition and the effects of oak and non-oak woods on the characteristics of wine and other beverages, and (2) how to improve wine quality (Zhang et al. 2015; de Simon et al. 2014; Da Silva et al. 2009). Simple coumarins, compounds formed by a benzene and a pyrone ring with an oxygen atom in the α -position, are a group of the non-volatile phenolic compounds released from wood into the wine during the maturation. Their concentration in wine depends on the type of wood and the way it is seasoned. Umbelliferone, 4-methylumbelliferone, esculin and coumarin and scopoletin are identified in oak and other woods extracts (Da Silva et al. 2009; Puech and Moutounet 1988). Coumarins, mainly scopoletin, therefore can be considered as possible markers of storage of wines in oak barrels (Rentzsch et al. 2009). Coumarins can be presented in wines (and in grapes too) also as a result of cyclisation of the *cis*-2-hydroxycinnamic acid and its derivatives formed through isomerization and hydroxylation of the *trans*-2-hydroxycinnamic acid. Depending upon biosynthetic precursors, umbelliferone, esculetin, and scopoletin are formed (Garrido and Borges 2013). It is known, that

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coumarins are characterized by a great diversity, consequent not only from oxygenation degree of benzopyrene skeleton, but also they are capable exist in the free and conjugated forms. Both forms can affect organoleptic characteristics of wine, e.g. glycosylated forms of esculin and scopoline caused bitter taste and aglycone forms (esculetin, scopoletin) caused neutral or slightly acidic taste of wine (Ribéreau-Gayon et al. 2006; de Rosso et al. 2009; Canas 2017). In addition, coumarins exhibit many biological activities, such as antibacterial, nematocidal, phytotoxic, antifungal activity, etc., and they can have health protective effect (Borges et al. 2005).

Chemical composition of wine depends not only on aging and technology conditions, but are influenced by many other factors, such as grape variety, cultivation and climate conditions. The evaluation and classification of wines require finding the specific markers and also development of analytical method for their determination. Many advanced instrumental analytical techniques, such as spectrometric, chromatographic, and electrophoretic, found application in wines analysis (de Villiers et al. 2012; Gomez and Silva 2016; Aleixandre-Tudo et al. 2017). Liquid and gas chromatography methods are suitable for quantification of non-volatile and volatile compounds from several chemical classes, phenolic compounds, carbohydrates, organic acids, biogenic amines, and other. The appropriate HPLC and UHPLC conditions for separations of coumarins and other phenolic compounds include octadecyl or phenyl-hexyl silica based analytical columns in reversed-phase separation mode and UV–VIS, fluorescence, electrochemical or MS detection (Jandera et al. 2005; Da Silva et al. 2009; Machyňáková and Hroboňová 2017a). Identification and quantification of trace levels of target analytes in complex samples (beverages, foods, biological and environmental samples) many times require use of cleaning and preconcentration steps before subsequent separation technique (Stauffer 2017). An innovative approach in sample preparation is based on application of selective sorbents. Solid phase extraction methods using molecularly imprinted polymeric sorbents (MIP SPE) were previously published for extraction of dibutyl phthalate, butyl benzyl phthalate, diethyl phthalate, dimethyl phthalate (Barciela-Alonso et al. 2017), catechin (Büyüktuncel et al. 2018), quercetin (Molinelli et al. 2002), ochratoxin A (Giovannoli et al. 2014), pesticides diazinon, quinalphos, chlorpyrifos (Sanagi et al. 2011), iprodione fungicide (Bitar et al. 2014), simple coumarins (Machyňáková et al. 2017) from wine samples.

Traditional spectrometric methods for determination of coumarins (e.g. fluorescence spectrometry, ultraviolet spectrometry, Raman spectrometry) are a rapid, easy-to-use and inexpensive compared with HPLC but also require the pretreatment of the sample, because the spectra of

coumarins and unknown fluorophores/chromophores in samples often overlap. Application of a chemometric approaches and spectral data is useful tool for simultaneous determination of compounds (Yang et al. 2011; Huang et al. 2017). By using more selective methods such as synchronous fluorescence spectrometry (SFS) and excitation-emission matrix fluorescence (EEM) spectrometry combined with mathematical separation of the analyte signal in the presence of interferences, coumarin, 4-hydroxycoumarin and dicoumarol were quantified in tea (Poláček et al. 2015), phenolic acids and scopoletin in brandies (Žiak et al. 2014), and umbelliferone and scopoletin in medical plant materials (Wang et al. 2017). The parameters of multivariate calibration model were determined from a calibration/validation sets containing a real samples with a known amount of standards added and subsequently the models were used for analysis of samples in the prediction step. In SFS, the best results were obtained by the partial least squares (PLS) method applied to SF spectra recorded at constant wavelength differences 90 and 100 nm (Poláček et al. 2015; Žiak et al. 2014). An alternative calibration approach based on the searching of the relationship between the spectral data and the amount of analytes previously quantified by a reference method also yielded good results, e.g., for amino acids and riboflavin in beer (Sikorska et al. 2008).

As the best knowledge, there are no published papers focused on analysis of wines in terms of separation and quantification of simple coumarins in the context of combination of chromatographic and fluorescence spectrometry data. Therefore, in this work, HPLC method with UV–VIS and fluorescence detectors was applied for quantification of coumarins in wines from Slovak Tokaj winery region. The analytes were cleaned by MIP SPE extraction procedure. Emission spectral data from fluorescence spectrometry and synchronous fluorescence spectra were used for PLS regression analysis. The concentrations predicted by the PLS model were compared with values obtained by HPLC method.

Materials and methods

Chemicals and samples

The target group of coumarins was: esculin (6,7-dihydroxycoumarin-6- β -D-glucoside, 98%), coumarin (99%), herniarin (7-methoxycoumarin, 98%), 4-methylumbelliferone (4-methyl-7-hydroxycoumarin, 98%), scoparone (6,7-dimethoxycoumarin, 98%), scopoletin (6-methoxy-7-hydroxycoumarin, 98%). The standards of coumarins were purchased from Sigma-Aldrich (St. Louis, USA). The solvents methanol, ethanol, (Merck, Darmstadt, Germany)

were of HPLC gradient grade purity. The chemicals for MIP synthesis included: umbelliferone (7-hydroxycoumarin, 99%; Sigma-Aldrich, St. Louis, USA), chloroform (HPLC grade purity), acetone (analytical grade purity), 2,2'-azobisisobutyronitrile (analytical grade purity), methacrylic acid (for synthesis grade purity), ethylene glycol dimethacrylate (for synthesis grade purity) were purchased from Merck (Darmstadt, Germany). Acetic acid (Microchem, Pezinok, Slovak Republic) was 99% purity. The deionized water (resistivity of 18.2 M Ω /cm) was obtained from a AquaMax ultra (series 370) water purification system.

Analysed, bottled, wines used for investigations were produced by local wineries (Tokaj winery region, south-east of Slovakia). The Tokaj wines puttony nr. 6 (samples 1–6), nr. 5 (samples 7–11), nr. 4 (samples 12–15), and nr. 3 (samples 16, 17) are tested. The samples were kept at 8 °C and before analysis they were kept at room temperature at least for 60 min.

Preparation of standard solutions

To prepare stock solutions, the standards including esculin, coumarin, herniarin, 4-methylumbelliferone, scoparone, scopoletin were accurately weighed and dissolved in solvent (initial mobile phase) to reach concentration of 0.1 mg mL⁻¹. The mixed working solutions were prepared by diluting the stock solutions obtaining concentrations in the range of 0.5–100 μ g mL⁻¹ of coumarin (concentration levels: 0.5, 5.0, 25.0, 50.0, 75.0, 100.0 μ g mL⁻¹) and concentrations 0.5, 5.0, 10.0, 25.0, 50.0, 75.0, 100.0 ng mL⁻¹ of esculin, herniarin, 4-methylumbelliferone, scoparone and scopoletin.

Sample preparation

Before HPLC analysis, the analytes were extracted by solid phase extraction with lab-made MIP-umbelliferone sorbent. The MIP sorbent preparation was performed under following procedure (Machyňáková and Hroboňová 2017b): Umbelliferone (0.7 mmol), methacrylic acid (2.9 mmol), and chloroform (7.5 mL) were mixed in a glass tube for 15 min. Afterwards, ethylene glycol dimethacrylate (11.75 mmol) and 2,2'-azobisisobutyronitrile (0.18 mmol) were added. The final mixture was polymerized at 60 °C for 24 h. The resulting block of polymer was crushed, sieved through the 80 μ m sieve, and washed with acetone to remove fine particles. The Soxhlet extraction with mixture methanol/acetic acid (9/1, v/v; 48 h) was used to remove template.

The SPE cartridge (0.1 g of lab-made MIP-umbelliferone sorbent) was washed with 3 mL of methanol and 3 mL of 10% ethanol. The volume of 2 mL of sample was passed

and cartridge was washed with 3 mL of water. The analytes were eluted with 2 mL of methanol/acetic acid (9/1, v/v). Extract was filtered through a 0.45 μ m nylon membrane filter (AZ Chrom, Slovak Republic) and analysed by HPLC. For validation assay, the sample (evaluated for sample 17) was spiked with working standard solution of coumarins at concentration levels of 5.0 μ g mL⁻¹ for coumarin and 0.05, μ g mL⁻¹ for esculin, herniarin, 4-methylumbelliferone, scoparone, scopoletin. The spiked samples were conditioned for at least 60 min before being used for extraction procedure.

Fluorescence spectra of diluted samples (0.2% w/w in water) were recorded to minimize inner filter effect (Sádecká et al. 2018).

HPLC instrument and conditions

Separation of coumarins was carried out by the HPLC system (Agilent Technologies, Germany), consisting of a degasser (G1379B, series 1260), a binary pump (G1312B, series 1260), an autosampler (G1329A, series 1200), a column oven (G1316A, series 1200), diode array detector (G1315B, series 1100), and fluorescence detector (G1321A, series 1200). The mixture of coumarins was separated on Kinetex C18 (100 mm $\times$ 4.6 mm I.D., 5 μ m particle size) column (Chromservis SK s.r.o., Slovak republic). The mobile phase with flow rate 1.0 mL min⁻¹ consisted of the mixture of solvents (A) methanol/acetic acid 99/1 (v/v) and (B) 1% aqueous solution of acetic acid. The eluting gradient program was as follows: 0–12 min: a linear increase from 20 to 45% A, 12–12.5 min: a linear increase from 45 to 100% A, 12.5–14 min: 100% A, 14–14.5 min: a linear decrease from 100 to 20% A. The injection volume was 20 μ L and column temperature was maintained at 23 °C. Diode array detector was operated in the wavelength range of 190–400 nm. Monitoring wavelength was 280 nm for coumarin. Wavelength range of fluorescence detector was 300–500 nm and monitoring wavelengths were 320 nm (λ_{ex}) and 450 nm (λ_{em}) for esculin, herniarin, 4-methylumbelliferone, scoparone, scopoletin.

Fluorescence spectrometry instrument and conditions

Fluorescence spectra measurements were performed using a Perkin–Elmer LS 50 Luminescence Spectrometer equipped with the xenon lamp (Perkin–Elmer, USA). The excitation and emission slit width was 5 nm. Emission spectra were recorded in the 350–500 nm range using the excitation wavelength of 320 nm. Synchronous fluorescence spectra were recorded by simultaneously scanning the excitation and emission monochromators in the

Fig. 1 HPLC chromatograms standard mixture of coumarins (**a**) and Slovak Tokaj wines (**b** sample 2, **c** sample 4, **d** sample 7). Chromatographic conditions: Kinetex C18 (100 mm × 4.6 mm I.D., 5 μm particle size) column, gradient elution with methanol-1% acetic acid as mobile phase, flow rate 1.0 mL min⁻¹, column temperature 23 °C, Fluorescence detection at 330 nm (λ_{ex}) and 450 nm (λ_{em})

250–400 nm range, with a constant wavelength difference $\Delta\lambda = 80, 90$ and 100 nm between them.

Partial least squares regression analysis

PLS regression and related parameters employed in this work have been described previously (Poláček et al. 2015; Wold et al. 2001; Wise et al. 2006; Žiak et al. 2014). All calculations were done using the Microsoft Office Excel 2010, MATLAB version 7.0 (The MathWorks, Inc., USA, 2005) and PLS_Toolbox version 6.0 (Eigenvector Research, Inc., USA, 2010).

Results and discussion

HPLC separation and determination of coumarins

Coumarins profiles of wines were evaluated by HPLC with UV–VIS and fluorescence detection. The mixture of coumarins (esculin, coumarin, herniarin, 4-methylumbelliferone, scoparone, scopoletin) was efficiently separated using a core shell C18 type of stationary phase and binary elution gradient. The advantage of core shell type of stationary phase is lower backpressure than in the case sub-2 μm particles stationary phases and use of conventional HPLC equipment which operate at middle pressure (to 60 MPa) for analysis. Beside this, lower mass transfer time corresponds to less diffusion and sharper peaks. This leads to efficient separation of analytes in shorter analyses time. Several gradient profiles were tested and finally the separation of six target coumarins in analysis time to 15 min and resolution values higher than 1.5 were achieved using suitable chromatographic conditions (Fig. 1a; Table 1). The repeatability of retention times and areas expressed as the relative standard deviation percentage (RSD %) were lower than 0.5% and 4.0% ($n = 6$), respectively (the acceptance criterion was $\leq 5\%$). These results indicate good intra-assay precision.

For the all group of compounds under study, UV–VIS detection is suitable due to the presence of chromophore in molecule (λ_{max} of esculin, herniarin, 4-methylumbelliferone, scoparone, scopoletin are in the range 320–340 nm, λ_{max} of coumarin is 280 nm). When the fluorescence detection of esculin, herniarin, 4-methylumbelliferone,

Table 1 HPLC system suitability parameters for separation of coumarins^a and validation parameters of the method^b

	Esculin	Scopoletin	Coumarin	Scoparone	4-methylumbelliferone	Herniarin
<i>System suitability parameters</i>						
t_R (min)	2.51	6.50	8.38	8.98	9.45	11.30
Repeatability RSD (%) t_R	0.31	0.50	0.28	0.31	0.39	0.30
A	2.10	2.01	1.67	3.88	3.72	3.80
Peak symmetry	1.42	1.25	1.20	1.38	1.11	1.29
Peak capacity	12.93	33.67	47.87	64.53	68.76	85.31
R_S	18.58	7.86	2.60	1.55	7.55	
<i>Validation parameters</i>						
Slope	131.1	1219.7	3650.8	82.8	306.3	25.5
Intercept	− 3.3	13.2	5.3	1.3	6.2	− 0.2
R^2	0.9993	0.9990	0.9995	0.9982	0.9987	0.9987
Linear calibration range (ng mL ^{−1})	3–100	3–100	500–100,000	10–100	0.5–100	12–100
LOD (ng mL ^{−1})	1.0	1.0	200	3.0	0.2	4.0
LOQ (ng mL ^{−1})	3.0	3.0	500	10.0	0.5	12.0
Precision (RSD %) ^c intra-day	2.1	2.5	2.8	3.0	3.2	2.9
inter-day	2.7	3.2	3.7	3.9	3.7	3.8
Recovery ± SD (%) ^d c1	70.1 ± 2.1	91.0 ± 3.0	90.0 ± 3.0	95.0 ± 1.6	90.8 ± 4.7	94.5 ± 3.0
c2	71.8 ± 1.6	95.5 ± 2.6	90.4 ± 3.6	93.2 ± 1.4	92.6 ± 3.1	95.3 ± 2.3
c3	72.1 ± 1.2	96.0 ± 3.5	92.3 ± 2.5	95.4 ± 1.1	95.4 ± 5.4	97.3 ± 2.4

^aSystem suitability test for coumarins mixture (concentration of esculin, scopoletin, scoparone, 4-methylumbelliferone, herniarin 50 ng mL^{−1}, concentration of coumarin 5.0 µg mL^{−1}); A—peak area

^bDAD detection for coumarin, FLD detection for other compounds

^cSix replicate measurements at one concentration level (50 ng mL^{−1} of esculin, scopoletin, scoparone, 4-methylumbelliferone, herniarin, 5.0 µg mL^{−1} of coumarin)

^dRecovery study for three concentration levels of esculin, scopoletin, scoparone, 4-methylumbelliferone, herniarin—minimal concentration level corresponded to LOQ of analyte (c1), 25 ng mL^{−1} (c2), 50 ng mL^{−1} (c3) and concentration levels of coumarin 0.5 µg mL^{−1} (c1), 5.0 µg mL^{−1} (c2), 50.0 µg mL^{−1} (c3)

scoparone, and scopoletin was carried out (taking advantage of their molecular emission properties) at monitoring excitation and emission wavelengths of 320 nm and 450 nm, the higher selectivity and sensitivity of the method were achieved [compared to HPLC–UV method the LODs were in the concentration range of µg mL^{−1} (Machyňáková and Hroboňová 2017a)]. Thus, HPLC method with diode array detector for quantification of coumarin and fluorescence detector for quantification of other coumarins in real sample was applied.

Solid phase extraction with lab-made sorbent prepared by molecularly imprinted technology was applied for sample preparation. MIP-umbelliferone, prepared and characterised in previous work, was selective not only for template molecule and also for group of other simple coumarins. Extraction procedure optimised previously and applied for the extraction of coumarins from macerate of plant sample was slightly modified for purpose of this study (Machyňáková and Hroboňová 2017b). The modification concerned the type of washing agent in conditioning

step, where ethanolic solution (10%) was used due to the higher compatibility with analysed samples. The change of type of conditioning solvent (previously water) results in higher extraction recovery of target coumarins from wine samples (> 86% for water, > 90% for 10% ethanolic solution; results without esculin). Due to the small content of coumarins in some of samples (sample 6, 13, 15), the preconcentration with factor 5 was used.

The quantitative analytical method was validated according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines (ICH Q2 R1, 2005) by assessing linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and results are summarised in Table 1.

The method linearity was evaluated using calibration mixed standard solutions. The calibration graph of each analyte was obtained by plotting mean peak area versus the corresponding concentration. The correlation coefficients in the range of 0.9982–0.9995 show on good linearity of

the method. The LOD and LOQ values were calculated as signal to noise ratio of 3 and 10, respectively. The LODs were found to be 200 ng mL^{-1} for coumarin detected by UV–VIS detector and in the range $0.2\text{--}4.0 \text{ ng mL}^{-1}$ for other coumarins under study detected by fluorescence detector. The LOQs are in the range $0.5\text{--}12.0 \text{ ng mL}^{-1}$ for esculin, herniarin, 4-methylumbelliferone, scoparone, scopoletin and 500 ng mL^{-1} for coumarin. The intra-day and inter-day precisions (expressed as RSD % between six replicate measurements conducted in 1 day and three consecutive days) are in the ranges 2.1–3.2% and 2.7–3.9%, respectively. Accuracy of the method was determined as the recovery for spiked sample (six preparations, triplicate injection) at three concentration level of coumarins. The recovery values listed in Table 1 are higher than 90% for scopoletin, coumarin, scoparone, 4-methylumbelliferone, and herniarin. The glucoside moiety in molecule of esculin caused incompatibility with imprinted cavity and thus lower selectivity of MIP based sorbent results to lower recovery value (70%).

The developed method with MIP SPE and HPLC was applied for quantification of coumarins in wines. The target compounds were identified through comparison of retention times, UV absorption and fluorescence spectra. In tested wines from Tokaj region of Slovakia, two of coumarins, scopoletin and 4-methylumbelliferone were identified. Other of coumarins were not detected and identified in samples at concentration levels over LODs. Figure 1 shows the chromatograms of the two wines analysed. The data in Table 2 shows the concentrations of coumarins (quantified at the ng mL^{-1} concentration levels), calculated using the calibration curves. Generally, higher concentrations were found for 4-methylumbelliferone compared to scopoletin. These compounds could affect sensory attribute of wine, as was presented in introduction. The large variation in the concentration of the target analytes in tested samples might be because of the differences in aging and type of wood barrels used for wine production depending on the wine producer.

Obtained results are in agreement with results previously published for wines from different regions. Coumarins identified in grapes and wines are from the group of the simple coumarins (Garrido and Borges 2013). In Moravian white wines 4-hydroxycoumarin at concentration levels of $1.48\text{--}4.84 \text{ ng mL}^{-1}$ was quantified (Jandera et al. 2005) and in France wines stored in oak barrels, esculetin, umbelliferone, scopoletin, and 4-methylumbelliferone were quantified ($0.4\text{--}400 \text{ ng mL}^{-1}$) (Salagoity-Auguste et al. 1987). Coumarin and scopoletin (concentrations $0.1\text{--}11 \text{ } \mu\text{g mL}^{-1}$ of coumarin and $7\text{--}110 \text{ ng mL}^{-1}$ of scopoletin) were identified and quantified as ones of major extractive compounds in American and European oak and Brazilian woods used in barrel aging of wine (Da Silva

Table 2 Coumarins content in the investigated wines

Sample nr.	Puttony ^a	Year	Compounds concentration (ng mL^{-1})	
			Scopoletin	4-methylumbelliferone
1	6	1986	n.d.	6.2 ± 0.3
2		1989	36.7 ± 1.3	15.1 ± 0.6
3		1993	n.d.	32.6 ± 1.3
4		1999	n.d.	54.6 ± 2.1
5		2003	18.4 ± 0.5	n.d.
6	5	2006	4.4 ± 0.2	17.1 ± 0.8
7		1989	n.d.	170.6 ± 7.9
8		1990	n.d.	101.9 ± 5.0
9		2003	6.7 ± 0.2	n.d.
10		2003	n.d.	18.6 ± 0.4
11	4	2004	21.4 ± 1.1	n.d.
12		1995	n.d.	17.9 ± 0.8
13		2002	3.6 ± 0.2	21.5 ± 1.0
14		2004	16.7 ± 0.7	12.0 ± 0.6
15		2016	0.2 ± 0.1	n.d.
16	3	1999	n.d.	n.d.
17		2003	n.d.	n.d.

n.d. not detect

^aPuttony nr. is based on the content of sugar and sugar-free extract in the mature wine

et al. 2009). Compared with previous reported HPLC methods, the presented method is characterized with (1) shorter analysis time (15 min versus ~ 60 min (Jandera et al. 2005), ~ 35 min (Salagoity-Auguste et al. 1987) and ~ 70 min (Da Silva et al. 2009); however these methods are focused on separation of wide group of phenolic compounds, while present method is focused only on simple coumarins); (2) a lower LOQ (7 ng mL^{-1} for scopoletin (Da Silva et al. 2009); and (3) higher recoveries [65–97% (Salagoity-Auguste et al. 1987); 87.5 (Izquierdo et al. 2000)]. The presented offline SPE method is the alternative to online SPE coupled to HPLC (Machyňáková et al. 2017). Although the online coupling extraction and separation techniques appears to be the most suitable approach, some limitations were observed: (1) the optimisation of separation conditions in accordance with the type and volume of washing and eluting solvents for SPE were needed; (2) the type of matrix solvent also influenced efficiency of extraction; (3) in the case of more complex samples, not sufficient purification of the extracts may be observed.

Fluorescence spectrometry and chemometry

The results of quantification of coumarins in wines obtained by HPLC method were compared with values predicted by the PLS model on the base data from

fluorescence spectrometry. Emission spectral data from fluorescence spectrometry were obtained at excitation wavelength $\lambda_{\text{ex}} = 320$ nm and synchronous fluorescence spectra were recorded at $\Delta\lambda = 80, 90$ and 100 nm. The values of $\Delta\lambda$ resulted from our previous works (Poláček et al. 2015; Žiak et al. 2014) as well as from the total synchronous fluorescence spectra (Fig. 2a–c) showing the fluorescence of standards at $\Delta\lambda > 70$ nm. The spectra were used as the independent variable (X-block) data and sum of concentrations of scopoletin and 4-methylumbelliferone quantified by the HPLC method were used as the dependent variable (Y-block) data in the PLS regression. After excitation at $\lambda_{\text{ex}} = 320$ nm, maximum fluorescence intensity was observed at 448, 448 and 438 nm for scopoletin, 4-methylumbelliferone and Tokaj wine, respectively (Fig. 2d). Raman scattering appeared as a band at 359 nm, and fluorescence of both scopoletin and 4-methylumbelliferone was observed at a wavelength higher than 400 nm, and therefore, only the emission intensities obtained from 400 to 500 nm were used in the PLS. One synchronous fluorescence maximum at $\lambda_{\text{ex}} = 332$ nm ($\Delta\lambda = 100$ nm) was observed for both scopoletin and 4-methylumbelliferone, while two maxima appeared in the synchronous

fluorescence spectrum of the Tokaj wine (Fig. 2a–c). The first band with maximum at $\lambda_{\text{ex}} = 270$ nm ($\Delta\lambda = 80$ nm) corresponded to phenolic compounds and catechins, the second band with maximum fluorescence at $\lambda_{\text{ex}} = 325$ nm ($\Delta\lambda = 100$ nm) was due to coumarins overlapped with p-coumaric acid and other fluorophores (Sádecká et al. 2018). The synchronous fluorescence intensities recorded from λ_{ex} of 300 to 400 nm, corresponding to the second band of Tokaj wine spectrum, were used in the PLS. PLS regression was calculated for emission spectral data obtained at excitation wavelength $\lambda_{\text{ex}} = 320$ nm and synchronous fluorescence spectra recorded at $\Delta\lambda = 80, 90$ and 100 nm. The spectral data were used as the independent variable (X-block) data and total concentrations of coumarins quantified by the HPLC method were used as the dependent variable (Y-block) data in the PLS regression. Data was pre-treated using the autoscaling method. The models were validated using leave-one-out cross-validation. The root mean-square error of calibration (RMSEC), the root mean-square error of cross-validation (RMSECV), the coefficient of determination of calibration ($R^2\text{Cal}$) and the coefficient of determination of cross-validation ($R^2\text{CV}$) between the HPLC and the predicted values were used as

Fig. 2 Total synchronous fluorescence spectrum of **a** 4-methylumbelliferone, **b** scopoletin and **c** Tokaj wine (sample 7) and **d** emission spectra of scopoletine, 4-methylumbelliferone, and Tokaj wine (sample 7) obtained at excitation wavelength of 320 nm

Table 3 Statistical characteristics of the PLS methods using the emission and synchronous fluorescence spectral data sets

Type of spectra	Emission	Synchronous fluorescence		
		$\Delta\lambda = 80$ nm	$\Delta\lambda = 90$ nm	$\Delta\lambda = 100$ nm
Setting	$\lambda_{\text{ex}} = 320$ nm	300–400 nm	300–400 nm	300–400 nm
Wavelength range used in PLS	400–500 nm			
Explained variance/% X-block	99.4	99.3	99.7	99.5
Explained variance/% Y-block	99.2	94.5	99.5	97.9
Number of latent variables	3	3	4	4
RMSEC	3.7	10.1	1.7	3.7
RMSECV	7.1	13.1	2.2	5.6
$R^2\text{Cal}$	0.992	0.945	0.996	0.979
$R^2\text{CV}$	0.971	0.902	0.994	0.951

RMSEC root mean square error in calibration, *RMSECV* root mean square error in cross-validation, $R^2\text{Cal}$ coefficient of determination in calibration, $R^2\text{CV}$ coefficient of determination in cross-validation

Fig. 3 Values of total coumarin calculated using the synchronous fluorescence spectra ($\Delta\lambda = 90$ nm) combined with PLS regression versus the values obtained by the HPLC method

characteristics for comparing the models. The number of latent variables (LV) was selected according to the rule to only choose additional factors when the RMSECV improves by at least 2%. Three LVs allowed to explain more than 99% of the coumarin variability, being based on either emission spectra ($\lambda_{\text{ex}} = 320$ nm) or synchronous fluorescence spectra at $\Delta\lambda = 80$ nm, whereas four LVs needed for the synchronous fluorescence spectra at $\Delta\lambda$ of 90 and 100 nm (Table 3). The PLS processing of the synchronous fluorescence spectra at $\Delta\lambda = 90$ nm led to minimum RMSEC and RMSECV values (1.2 and 2.2) and the highest $R^2\text{Cal}$ and $R^2\text{CV}$ (0.996 and 0.995). Values of the parameters in cross-validation were very similar to those in calibration, indicating that the model is stable. Figure 3 shows a linear regression between the concentrations predicted by the PLS model versus true values obtained by HPLC method confirming good agreement between the two methods. The advantage of the fluorescence spectrometric method is rapidity and simplicity, with

the drawback of determining only the total amount of coumarins (in the case of these work the sum of concentrations of scopoletin and 4-methylumbelliferone).

Conclusion

Offline MIP SPE and HPLC method with UV–VIS and fluorescence detectors were applied for analysis of wines from Slovak Tokaj winery region. The suitable HPLC conditions for separation of mixture of six coumarins, esculin, scopoletin, coumarin, scoparone, 4-methylumbelliferone, herniarin included Kinetex core shell C18 type of stationary phase and gradient elution with increasing concentration of methanol in mobile phase containing 1% aqueous solution of acetic acid. The method was validated with good sensitivity, accuracy and precision. In tested wines, two of coumarins (scopoletin and 4-methylumbelliferone) were identified and quantified at the ng mL^{-1} concentration levels. Differences in type and concentration of the identified coumarins of aged wines have been observed as results of the differences in aging and type of wood barrels used for wine production depending on the wine producer. Emission spectral data and synchronous fluorescence spectra of coumarins from fluorescence spectrometry and total concentrations of coumarins quantified by the HPLC method were used in the PLS regression. The linear regression between the concentrations predicted by the PLS model versus true values obtained by HPLC method confirm good agreement between these two methods. The developed method can find application for quantification of coumarins in other types of beverages or other complex matrices.

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Compliance with ethical standards

Conflict of interest Katarína Hroboňová declares that she has no conflict of interest. Jana Sádecká declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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