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Short communication

SHORT COMMUNICATION

Development and validation of an LC–MS/MS method with a multiple reactions monitoring mode for the quantification of vanillin and syringaldehyde in plum brandies

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Abstract: An ultra-performance liquid chromatographic–triple quadrupole mass spectrometric (UPLC–QqQ–MS/MS) method with a multiple reactions monitoring mode (MRM) was developed and validated for the quantification of vanillin and syringaldehyde in plum brandy. The method showed good linearity (0.05 to 10 mg L^{-1}) and low limits of detection and quantification (the *LOD* and *LOQ* values were 11.6 and 38.2 μg L^{-1} for vanillin, and 12.7 and 42.0 μg L^{-1} for syringaldehyde, respectively). The overall intra-day and inter-day variations were less than 4.21 % and the overall recovery was over 93.0 %. The correlation coefficients (R^2) of the calibration curves were higher than 0.9999. In order to evaluate whether the method was suitable for use as a routine analytical tool, vanillin and syringaldehyde were determined in 31 samples of Serbian plum brandy.

Keywords: vanillin; syringaldehyde; plum brandy; LC–MS/MS.

INTRODUCTION

Plum tree (*Prunus domestica* L.) is a traditional plant in Serbian village households. Serbian plum brandy (Serbian Slivovitz) is the most popular Serbian alcoholic beverage obtained by distilling fermented fruit pulp. The production of aged brandy consists of: *i*) preparation of the raw material and fermentation, *ii*) distillation and *iii*) aging in oak wood casks when the organoleptic qualities of the distillate develop to produce the mature brandy.[1]

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Plum brandy, as well as other spirits, is aged in oak barrels for a period that can last for several years. Aging is the stage of the production process important for the extraction of phenolic compounds that enhance the sensory qualities, and the antioxidant capacity of a brandy, which may have health benefit if consumed in moderation.

The aromatic phenolic aldehydes, among others vanillin and syringaldehyde, formed by degradation of lignin from the oak barrels during coopering are extracted into spirit.[2] In a simulated experiment with four oak wood samples, the content of syringaldehyde was more than double the vanillin content.[3] Previous studies performed with several spirits showed ratio of syringaldehyde to vanillin was 1.4 to 2.5 when these aldehydes were produced by ageing of the spirit in oak barrels over a long period.[4] Thus, their concentrations could indicate the time of aging in oak casks. Since vanillin is an aldehyde that contributes the most to the aroma of alcoholic beverages and is easily obtained industrially, producers may be encouraged to add it to brandies, thereby simulating an aged character.[5] The syringaldehyde/vanillin ratio seems to be one of the characteristics of the original composition of a brandy, and may be considered as a marker to distinguish counterfeit alcoholic beverages from genuine ones.[4]

The undeniable appeal of oak aging makes the caution of the industry to change a tradition that is fundamental to the finished product understandable.[6] The analytical methods reported in the literature to quantify aromatic aldehydes in alcoholic beverages are HPLC with UV detection,[7,8] gas chromatography coupled with mass spectrometry (GC/MS),[9] derivative spectrophotometry[10] and high performance capillary electrophoresis with UV detection.[2]

The aim of the present study was to develop and validate a fast method for the quantification of vanillin and syringaldehyde in mature plum brandies of different origin using ultra-performance liquid chromatography (UPLC) combined with a triple quadrupole tandem mass spectrometry (QqQ–MS/MS) system operated in the multiple reaction-monitoring (MRM) mode. The advantage of UPCL over HPLC is higher resolution and speed of analysis owing to small particle sizes (2 μm compared to 5 μm), large surface areas, and application of high pressure to the solvent flow. UPLC is just a special variant of HPLC that affords better separation and very fast analysis.[11] The analytical procedure was tested on specimens of authentic Serbian plum brandy obtained directly from the producers.

EXPERIMENTAL

Chemicals and materials

Ultra high-purified water, acetonitrile HPLC grade, and formic acid (Merck Darmstadt, Germany) were used as solvents for the chromatography. Vanillin and syringaldehyde used as external standards were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Samples

Specimens of Serbian plum brandy produced from single or mixed plum varieties of the genus *Prunus* L. that were aged in oak wood casks (29 samples) and mulberry wood casks (2 samples), were filtered through 0.45μm pore size membrane filteres and injected directly without preliminary treatment such as concentration, extraction, *etc*.

UPLC Analysis

The development and validation of chromatographic method and analysis of plum brandy samples was performed on a Waters Acquity UPLC system, equipped with a binary solvent manager, an autosampler, a column heater, a PDA detector, and interfaced to a tandem quadrupole detector. The separation column was an ACQUITY UPLC BEH C18 column (2.1 mm i.d.×50 mm, 1.7 μm particle size). The column heater was set at 30 °C and the mobile phase flow rate was maintained at 0.5 mL min^{-1}. The gradient (solvent A, 100 % acetonitrile, solvent B water/formic acid 99.8:0.2, *V*/*V*) was 0–3.0 min (16.0 % A), 3.0–8.0 min (98.0 % A). The injection volume was 1 μL. The DAD detector range was 190–600 nm.

QqQ–MS/MS–MRM

Identification of vanillin and syringaldehyde was performed by comparing the retention times and UV and mass spectra with those of the reference standards. Quantification of the identified compounds was performed using the external standard method. Calibration curves were established for vanillin and syringaldehyde. MS detection performed using a Waters TQD tandem quadrupole detector interfaced with an Acquity UPLC system *via* an ESI probe. The ESI source was operated in the positive ionization mode at 150 °C, with a desolvation temperature of 450 °C, a 700 L h^{-1} desolvation gas flow rate, and the capillary voltage was set at 3.5 kV. Argon was used as the collision gas at a flow rate of 0.10 mL min^{-1}. The collision energy used for vanillin was 16 eV and for syringaldehyde 10 eV. Based on the full-scan mass spectra of the aldehydes, the most abundant ions were selected and the mass spectrometer was set to monitor the transitions of the precursors to the product ions as *m*/*z* 153/93 for vanillin and *m*/*z* 183/155 for syringaldehyde. Data acquisition, peak integration and calibration were performed with Mass Lynx 4.0 software.

RESULTS AND DISCUSSION

UPLC method validation

The proposed method was validated for linearity, accuracy, precision and limits of detection and quantification.

Linearity. Calibration graphs were plotted based on linear regression analysis of the integrated peak areas *vs*. concentration (mg L^{-1}) in the standard solution at four different concentrations. The correlation coefficients obtained from the standard curves were for vanillin (0.99997) and syringaldehyde (0.99992). There was a strong linear correlation of the concentrations of the compounds with the peak areas.

Precision. Three different concentrations of vanillin and syringaldehyde (0.05, 0.5 and 1.0 mg L^{-1}) were analyzed in six independent series in triplicate during the same day (intraday precision) and over 3 consecutive days (interday precision). The relative standard deviation (*RSD*) was taken as a measure of

precision, and the results are given in Table I. The method showed a high degree of precision for both of the analyzed compounds.

TABLE I. Regression equations, linearity ranges, correlation coefficients, limits of detection (*LOD*), limits of quantification (*LOQ*), recoveries and relative standard deviations (*RSD*) for vanillin and syringaldehyde determination

Cmpd.	Regression equation	Linearity range mg L^{-1}	R^2	*LOD* µg L^{-1}	*LOQ* µg L^{-1}	Recovery %	Intra-day *RSD* / % (*n* = 6)	Inter-day *RSD* / % (*n* = 3)
Vanillin	$y = 6921900x + 184.24$	0.05–10	0.99997	11.6	38.2	99.0–103.2	0.59	1.23
Syringaldehyde	$y = 5254820x + 83.07$	0.05-10	0.99992	12.7	42.0	93.0–106.8	1.93	4.21

Limits of detection and quantification. The limit of detection (*LOD*) and limit of quantification (*LOQ*) were estimated from the signal-to-noise ratio. The *LOD* is defined as the lowest concentration resulting in a peak area of three times the baseline noise. The *LOQ* is defined as the lowest concentration that provides a signal-to-noise ratio higher than 10. The limits of detection and quantification observed for vanillin and syringaldehyde are listed in Table I.

Accuracy. The recovery (*R*) was used to evaluate the accuracy of the method. A known amount (*G*) of the considered compound (0.05, 0.5 and 1.0 mg L^{-1}) was added into a hydroalcoholic (40 vol. %) solution and the samples were injected under identical conditions in six replicate determinations (each concentration in triplicate). The values were calculated using the following equation:

$$R\ (\%) = \frac{G_{\text{determined}}}{G_{\text{added}}} \times 100$$

The proposed method afforded a recovery of 96.8–103.2 % for vanillin and 93.0–106.8 % for syringaldehyde (Table I). The accuracy was expressed as the percentage recovery.

Determination of vanillin and syringaldehyde in Serbian plum brandies

In order to evaluate whether the method is suitable for use as a routine analytical tool, vanillin and syringaldehyde were determined in 31 Serbian plum brandy samples and the results are given in Table II. The analyses were performed in triplicate and data are presented as mean ±*SD*. The concentrations of aldehydes were calculated by reference to the peak areas of the external standards. Checking for matrix effect was performed by addition of reference standards in the same concentration as obtained for brandy samples from the calibration curve. No matrix effect was found.[12] The chromatogram of plum brandy sample

5 is shown in Fig. 1, together with the extracted MRM chromatograms of vanillin and syringaldehyde.

TABLE II. Description of plum brandy analyzed and its quantification of vanillin (V) and syringaldehyde (S); hm. – homemade; in. – industrial, o.n.y.p. – old, no year of production

Plum variety	Type of barrel	Type / year of production	Age y	EtOH vol. %	V±*SD* mg L^{-1}	S±*SD* mg L^{-1}	S/V
Požegača + Crvena ranka	Oak	hm. / 1979	34	38.96	5.11±0.18	6.76±0.09	1.32
Požegača	Oak	hm. / 1983	30	37.62	5.10±0.08	6.25±0.13	1.23
Mixed varieties	Oak	in. / 1997	16	42.88	0.69±0.06	1.05±0.11	1.52
Mixed varieties	Oak	in. /1992	21	38.64	3.02±0.13	3.89±0.42	1.29
Mixed varieties	Oak	in. / 1995	16	44.60	2.68±0.06	2.71±0.04	1.01
Mixed varieties	Oak	hm. / o.n.y.p.	–	43.80	2.41±0.11	1.94±0.07	0.80
Požegača + Trnovača	Oak	hm. / 1991	22	45.56	1.23±0.04	1.46±0.13	1.19
Mixed varieties	Oak	hm. / 1992	21	43.17	1.37±0.09	2.03±0.14	1.48
Mixed varieties	Oak	in. / o.n.y.p.	–	43.74	0.12±0.003	0.07±0.01	0.57
Mixed varieties	Oak	in. / 1998	15	41.45	0.65±0.03	0.25±0.02	0.38
Mixed varieties	Oak	hm. / 1992	21	38.50	1.92±0.08	1.02±0.05	0.53
Požegača	Oak	hm. / 1951	62	40.92	3.46±0.03	5.21±0.34	1.51
Mixed varieties	Oak	hm. / 1998	15	38.59	1.65±0.05	0.66±0.03	0.40
Požegača	Oak	hm. / 1991	22	41.17	1.33±0.07	1.35±0.31	1.01
Mixed varieties	Mulberry	hm. / o.n.y.p.	–	45.44	0.82±0.08	0.95±0.04	1.16
Mixed varieties	Oak	hm. / 2003	10	41.20	0.36±0.01	0.34±0.01	0.94
Požegača	Oak	hm. / o.n.y.p.	–	42.08	1.04±0.02	1.17±0.04	1.13
Mixed varieties	Oak	hm. / 1990	23	43.10	3.16±0.30	2.86±0.06	0.91
Požegača	Oak	hm. / 1978	35	42.67	4.23±0.15	5.32±0.12	1.26
Mixed varieties	Oak	in. / 1992	21	44.47	0.23±0.007	0.25±0.003	1.08
Požegača	Oak	hm. / 1998	15	44.95	0.83±0.02	1.05±0.01	1.26
Požegača + Crvena ranka	Oak	hm. / 2003	10	52.3	0.67±0.07	0.58±0.06	0.87
Požegača + Crvena ranka	Oak	hm. / 1993	20	52.3	3.05±0.25	5.11±0.34	1.67
Požegača + Crvena ranka + Čačanska rodna + Stenli	Oak	hm. / 1994	19	50.7	4.23±0.28	10.61±0.67	2.51
Mixed varieties	Oak	hm. / 1999	14	49.7	1.76±0.23	4.06±0.52	2.31
Požegača	Oak	hm. / 1997	16	56.3	2.44±0.31	4.01±0.43	1.64
Požegača + Crvena ranka	Oak	hm. / 2003	10	44.6	0.30±0.02	0.66±0.03	2.18
Mixed varieties	Oak	hm. / 2006	7	53.3	4.27±0.21	8.89±0.32	2.08
Požegača + Crvena ranka + Čačanska rodna + Stenli	Oak	hm. / 1998	15	50.7	2.26±0.17	3.03±0.27	1.34
Mixed varieties	Oak	hm. / 1996	17	39.5	2.74±0.22	5.77±0.35	2.11
Mixed varieties	Oak	hm. / 2002	11	53.3	2.36±0.29	5.37±0.36	2.27

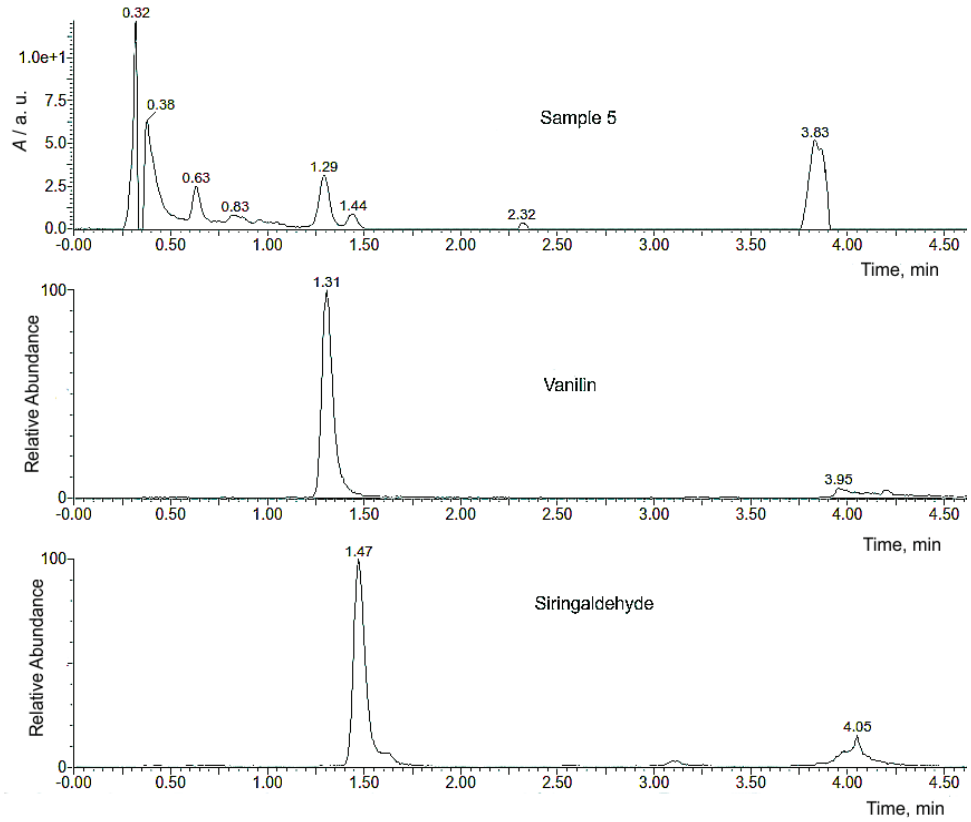

Fig. 1. DAD chromatogram of a plum brandy and extracted MRM chromatograms of vanillin and syringaldehyde, a) HPLC–UV–DAD chromatogram of plum brandy (Sample 5), b and c) extracted MRM chromatograms of vanillin and syringaldehyde in the ES+ mode, respectively.

CONCLUSIONS

This novel UPLC–QqQ–MS/MS–MRM method is proposed for the simple and reproducible detection and quantification of vanillin and syringaldehyde in one rapid chromatographic analysis. The short analytical run time (about five min per run) allows the use of a low amount of solvent and hence, the small amount of waste generation makes the method well suited for routine analysis. The method is useful for the identification of counterfeit brandy, which is easy to recognize by the ratios of syringaldehyde to vanillin.

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ИЗВОД

РАЗВОЈ И ВАЛИДАЦИЈА LC–MS/MS МЕТОДЕ У MRM РЕЖИМУ ЗА КВАНТИФИКАЦИЈУ ВАНИЛИНА И СИРИНГАЛДЕХИДА У РАКИЈАМА ШЉИВОВИЦАМА

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Развијена је и валидована UPLC–QqQ–MS/MS метода у MRM режиму за квантификацију ванилина и сирингалдехида у ракији шљивовици. Метода показује добру линеарност (0,05–10 mg L^{-1}) и низак лимит детекције и квантификације (*LOD* и *LOQ* за ванилин су 11,6 и 38,2 μg L^{-1}, а за сирингалдехид 12,7 и 42,0 μg L^{-1}). Варијације резултата у току једног и више дана су мање од 4,21 %, а *Recovery* преко 93,0 %. Корелациони коефицијент (R^2) калибрационих кривих је већи од 0,9999. У циљу процене погодности коришћења методе као рутинске, одређен је садржај ванилина и сирнгалдехида у тридесет и једном узорку српске шљивовице.

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