Validation of a Method for Analysis of Aroma Compounds in Red Wine using Liquid–Liquid Extraction and GC–MS

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Abstract An analytical method for determination of volatile composition of wines using sample preparation by liquid–liquid extraction and gas chromatography coupled to mass spectrometry for separation and detection has been developed and validated. Extraction of volatile compounds was performed in dichloromethane, and 1-octanol was added as an internal standard. Kékfrankos red wine produced in Villány wine region in Hungary was used as a model wine for testing and validation of the method. The developed method allowed satisfactory determination of 33 volatile compounds in the wines. Compounds analyzed include alcohols, esters, lactones, fatty acids, furans, and nitrogen compounds. The calibration curves of the four reference compounds used (2-phenyl ethanol, ethyl nonanoate, butyrolactone, and tyrosol) were linear in all cases with correlation coefficients (R^2) ranging from 0.9951 to 0.9992. The accuracy of the method was checked with a standard addition method (recovery 92.2–103 %), showing good repeatability and reproducibility (RSD<10 %).

Keywords GC–MS · Volatile compounds · Wine · Method validation · Liquid–liquid extraction · Dichloromethane · Kékfrankos

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Introduction

Wine aroma, which is one of the most important characteristics for wine quality, represents a good balance of several hundred volatile compounds. Different groups of volatile compounds, such as alcohols, esters, aldehydes, lactones, terpenes, and phenols, have been identified in wines in a wide concentration range, affecting the wine aroma even present in low concentration. Among the volatiles, alcohols and esters are the main compounds present with the highest content in wines. Esters are important constituents of wine aroma, which possess high fruity nuances (Marais and Poll 1980).

Qualitative and quantitative characterizations of volatile compounds in wine are usually performed by GC–MS as one of the most sensitive techniques for analysis of aroma in different samples (Verzera et al. 2008; Andujar-Ortiz et al. 2009; Tang et al. 2011). In addition, the liquid–liquid extraction method using different organic solvents is a very suitable technique for extraction of a wide range of volatile components (Ortega-Heras et al. 2002; Andujar-

Ortiz et al. 2009). This technique shows high repeatability and possibility of carrying out simultaneous extractions, which is one of its advantages over other solvent-free techniques.

Kékfrankos is a grape variety grown in Hungary, in a number of wine regions such as Villány, Szekszárd, Sopron, and Eger. This variety is usually used for production of notorious red wine blend known as "Bull's Blood", and it is being used for production of a single varietal wine. The same cultivar is also grown in Austria and Germany where it is called Blaufränkisch and Lemberger, respectively, as well as in Croatia, Czech Republic, Slovakia, and Slovenia. However, the origin of this variety has not been determined yet, and therefore, it could be more accurately called "Middle European" variety. The wine from this variety has ruby red color, is rich in tannins, and possesses spicy character and aromas of cherry fruit, blackberries, and currants.

The Kékfrankos wine from Hungary has not been characterized with respect to the volatile composition. Therefore, the aim of the present work was twofold: to develop and validate the liquid–liquid extraction method followed by GC–MS analysis for characterization of the volatile composition of wine and to apply the validated method for characterization of the volatile components making the aroma profile of the local Kékfrankos red wines produced from the grape variety grown in the Villány wine region in Hungary.

Materials and Methods

Chemicals and Reagents

The reference standards, 2-phenyl ethanol, ethyl nonanoate, butyrolactone, tyrosol, and 1-octanol (used as an internal standard, IS) were supplied from Fluka (St. Louis, MO, USA). A commercial solution of 24 aliphatic hydrocarbons (C8–C32) in hexane from Supelco (Bellefonte, PA, USA) was used for calculation of the Linear Retention Indices (LRI). Dichloromethane, used for extraction of volatiles, was obtained from Merck (Darmstadt, Germany). The standard stock solutions were prepared by dissolving 10 mg of each reference compound, 2-phenyl ethanol, ethyl nonanoate, butyrolactone, and tyrosol, in 10 mL of dichloromethane.

Wine Samples

Five Kékfrankos red wines from different wineries located in the Villány wine region, Hungary, were purchased from the local market in Pécs, Hungary. The wine samples were from vintage, 2008, and they were kept at 4 °C before analysis.

Liquid–Liquid Extraction

Liquid–liquid extraction was used for isolation of the volatile compounds from the wine samples. Thus, 50 mL of wine was spiked with 200 μL internal standard of 1-octanol in hexane with concentration of 500 μg/L, and the sample was placed in a glass-capped Erlenmeyer flask. A volume of 25 mL dichloromethane was added to the spiked wine sample, and the flask was placed in an ice bath, followed by continuous stirring of the mixture for 30 min on a magnetic stirrer. Then, the mixture was centrifuged at 3,000 rpm for 15 min at a temperature of 2–4 °C. Once the phases were separated, the dichloromethane layer was evaporated under a nitrogen stream to approximately 200 μL volume of the extract and then a volume of 1 μL was injected into the GC–MS system. All extractions were carried out in triplicate.

GC–MS Analysis

Analysis of wine volatile compounds was carried out using an Agilent 5975 Mass Spectrometer coupled to an Agilent 6890 N Gas Chromatograph (Agilent, Santa Clara, USA). The polar capillary column used for separation of the compounds was a Carbowax type from Agilent, with dimensions 30 m×0.25 mm ID and 0.25 μm film thickness. The working parameters were injector temperature of 240 °C, MS source of 230 °C, MS Quad of 150 °C, and transfer line of 280 °C. The initial temperature was 40 °C for 3 min and then rose to 180 °C at a rate of 3 °C/min. Then, the temperature was further raised to 260 °C with 20 °C/min and kept at 260 °C for 10 min. The carrier gas was He with a flow rate of 1.5 mL/min. Samples were injected using the splitless mode. A mass range of 50–400 m/z was recorded at one scan per second.

Calibration Curves

For quantification, five-point calibration curves were constructed for the following standard compounds: 2-phenyl ethanol (50–15,000 μg/L), ethyl nonanoate (50–20,000 μg/L), butyrolactone (100–20,000 μg/L), and tyrosol (100–5,000 μg/L), containing the internal standard (1-octanol). For that purpose, a synthetic wine model sample (12 % ethanol and 4 g/L tartaric acid with pH 3.4, adjusted by sodium hydroxide solution) was prepared, containing the known amounts of the standards, which was extracted and analyzed by GC–MS as above-mentioned. Extraction of volatile compounds was performed three times for each wine and then each extract was injected into the GC/MS.

In order to quantify the volatile compounds present in the wines, the relative peak area (in the total ion chromatogram)

of each analyte from the wine to the internal standard was compared to those obtained for the standards, taking into account the dilution of the samples. The concentration of the volatile compounds for which there was no pure reference was obtained using the calibration curve of one of the standard compounds with the most similar chemical structure, i.e., belonging to the same class of compounds.

Validation of the Method

Linearity

Linearity was tested in 3 days at five concentration levels of 2-phenyl ethanol, ethyl nonanoate, butyrolactone, and tyrosol, containing the internal standard, 1-octanol, in the wine samples.

Limit of Quantification (LOQ)

LOQ was determined by measuring the signals from the lowest concentration standards of 2-phenyl ethanol (50 μg/L), ethyl nonanoate (50 μg/L), butyrolactone (100 μg/L), and tyrosol (100 μg/L) in one wine (1) and calculating the standard error. For determination of LOQ, the wine sample spiked with the lowest concentration calibration standards was extracted three times in five analytical days, and errors were also calculated. The quantification of the volatile compounds present in the wine used for validation of the method was performed prior to standard addition.

Recovery

The recovery of the method was determined by analysis of the wine samples containing three concentration levels: 50, 200, and 500 μg/L, for each available standard (2-phenyl ethanol, ethyl nonanoate, butyrolactone, and tyrosol). The concentration of the internal standard, 1-octanol, was constant at 500 μg/L. Then, each wine sample for each concentration level of the standards was extracted three times during 1 day.

Repeatability and Reproducibility

Intraday repeatability and interday reproducibility have been studied using spiked wine (the amount of the volatiles that was previously determined) with known amounts of the standards. Thus, one wine sample containing known amounts of the added volatile compounds was extracted five subsequent times (in 1 day) applying the extraction procedure described above, and the extracts were analyzed by GC–MS in order to study the intraday repeatability. Furthermore, the spiked wine was extracted three times during three consecutive days in order to study the interday reproducibility. In order to avoid the

presence of oxygen in the wine analyzed during these 3 days, it was divided into three bottles of 250 mL and stored at 4 °C in darkness, closed with caps. Each bottle was opened before analysis.

Statistical Analysis

Statistical analysis included calculation of means and standard deviations for each of the 33 detected volatile compounds, which was performed by Excel 2007.

Results and Discussion

The method for determination of volatiles in wine was developed starting from selection of reference compounds based on literature data for the expected compounds, their grouping in the suitable groups: alcohols, esters, aldehydes, lactones, terpenes, and phenols and then deciding on the standards for calibration. For quantification of alcohols, 2-phenyl ethanol, the most important phenol-derived higher alcohol was used, ethyl nonanoate for the esters, and butyrolactone and tyrosol were used for analysis of these specific wine aroma compounds. Since the extraction step was essential for separation of the volatile fraction from the wine complex matrix, it was necessary to decide on the internal standard that would be suitable for the large and diverse group of target compounds. Since previous studies suggest that alcohols are the dominant fraction (Tao et al. 2008), 1-octanol was chosen as the most suitable because it is similar to the expected ones, but not present in wine. As for the liquid–liquid extraction procedure, the solvent dichloromethane and the volumes of the sample and extraction solvents were optimized following previous analogous studies (Andujar-Ortiz et al. 2009).

Method Validation

The linearity data of the analytical method are presented in Table 1. As can be seen from Table 1, the linearity is satisfactory in all cases with correlation coefficients (R^2) ranging from 0.9951 (tyrosol) to 0.9992 (2-phenyl ethanol).

Table 1 Linear regression data

Compound	Intercept	Slope	R^2	Range (μg/L)
2-Phenyl ethanol	−1.2582	0.0179	0.9992	50–15,000
Ethyl nonanoate	−0.0641	0.0138	0.9953	50–20,000
Butyrolactone	−0.1314	0.0009	0.9961	100–20,000
Tyrosol	−1.2787	0.0172	0.9951	100–5,000

Table 2 Limit of quantification of 2-phenyl ethanol, ethyl nonanoate, butyrolactone, and tyrosol

Analysis (days)	2-Phenyl ethanol		Ethyl nonanoate		Butyrolactone		Tyrosol	
	Found concentration (µg/L)	Relative error (%)	Found concentration (µg/L)	Relative error (%)	Found concentration (µg/L)	Relative error (%)	Found concentration (µg/L)	Relative error (%)
1	48.4	−3.2	51.6	3.2	104	4	98.3	−1.7
2	50.7	1.4	50.4	0.8	102	2	103	3
3	51.2	2.4	48.5	−3	98.6	−1.4	104	4
4	49.6	−0.8	53.2	6.4	103	3	103	3
5	52.4	4.8	52.4	4.8	102	2	97.6	−2.4
$\langle x \rangle$	50.5	0.92	51.2	2.44	102		101	
SD	1.53		1.84		2.03		2.99	
RSD	3.03		3.59		1.99		2.95	

The LOQ was determined for the four analytes: 2-phenyl ethanol, ethyl nonanoate, butyrolactone, and tyrosol by measuring the lowest concentration calibration standards in five analytical days and calculating the errors of the measurement. The results for the LOQ values were taken as the lowest concentrations because satisfactory values were obtained for the relative error for all four analytes (relative error <6.4 %). The results from these analyses are presented in Table 2.

The accuracy of the procedure was checked using the standard addition method. One wine sample, Kékfrankos (sample no. 1), was spiked with appropriate volumes of the standard solutions of 2-phenyl ethanol, ethyl nonanoate, butyrolactone, and tyrosol with concentrations of 50, 200, and 500 µg/L for each standard.

The satisfactory results for the recovery ranging from 92.2 to 103 % (Table 3) confirmed that the method is accurate and convenient for quantitative analysis. Additionally, to confirm the accuracy of the method and to check repeatability, five replicated extractions on an actual wine sample (1) have been performed within 1 day. Every extract was injected three times into the gas chromatograph. The relative standard deviations (RSD) of the five replicate samples for each compound are presented in Table 4, showing satisfactory values for the RSD. Reproducibility was also checked with replicate samples analyzed in three different days (3 replicates×3 injections×3 days), and the RSD for each volatile compound was calculated (Table 4). Accordingly, the method showed good repeatability and reproducibility,

Table 3 Standard additions for checking the accuracy of the extraction procedure and the GC–MS method for determination of volatile compounds in wine samples (n=3)

Concentration in wine (µg/L)	Standard additions (µg/L)	Calculated (µg/L)	Experimentally found[a] (µg/L)	RSD (%)	Recovery (%)
	2-Phenyl ethanol				
14,170	50.0	14,220	14,372	3.18	101
	200	14,370	14,178	2.04	98.7
	500	14,670	14,516	8.29	99.0
	Ethyl nonanoate				
/	50.0	50.0	51.1	1.31	102
	200	200	206	1.23	103
	500	500	507	9.46	101
	Butyrolactone				
8,150	50.0	8,250	8,126	1.46	98.5
	200	8,400	8,561	3.69	102
	500	8,700	8,577	6.11	98.6
	Tyrosol				
1,480	50.0	1,530	1,577	3.01	91.7
	200	1,680	1,658	4.57	93.8
	500	1,980	1,975	2.03	92.2

[a] Values are average of three replicates

RSD relative standard deviation, (%)

Table 4 Results for repeatability and reproducibility data for each compound (numbers correspond to peak numbers in Fig. 1)

Number	Volatile compounds of Kékfrankos wine (1)	t_R/min	LRI	Repeatability (5 replicates)		Reproducibility (3 replicates×3 injections×3 days)	
				Mean concentration (µg/L)	RSD (%)	Mean concentration (µg/L)	RSD (%)
1	Ethyl propanoate	5.47	954.5	104	3.15	103.2	0.59
2	Ethyl isobutyrate	5.66	963.5	82.3	5.8	82.8	0.91
3	3-Heptanol	6.09	1,011.1	575	0.42	575	0.18
4	Ethyl butanoate	6.85	1,033.8	156	5.08	157	4.27
5	Isobuty alcohol	8.58	1,089.2	840	1.94	853	3.28
6	Isoamyl acetate	9.83	1,122.4	136	1.89	142	2.30
7	Butyl formate	10.59	1,140.9	111	2.20	109	2.24
8	1-Pentanol	13.24	1,209.7	11,600	9.50	11,561	1.63
9	Ethyl caproate	14.33	1,233.9	155	1.88	148	0.12
10	1-Butamine, N-nitro	16.65	1,343.5	1,353	7.22	1,368	1.41
11	1-Hexanol	19.02	1,351.8	743	0.25	745	0.78
12	E-3-Hexanol	19.34	1,361.5	567	0.07	565	0.04
13	Ethyl caprylate	22.85	1,434.3	199	1.81	186	1.34
14	Ethyl-3-hydroxybutanoate	25.04	1,514.8	117	3.93	120	2.52
15	2,3-Butanediol	26.26	1,539.4	280	8.48	285	0.87
16	1,3-Butylenglycol	27.72	1,577.2	598	0.49	599	0.48
17	Butyrolactone	30.35	1,618.3	8,150	1.07	8,162	2.08
18	Ethyl caprinate	30.79	1,638.3	90.3	10.0	87.5	4.36
19	Diethyl succinate	32.35	1,677.5	2,861	1.27	2,893	0.63
20	3-(Methylthio)-1-propanol	33.68	1,711.7	713	0.49	711	0.53
21	Phenyl ethyl acetate	36.93	1,810.4	81.5	4.26	82.3	2.35
22	N-(3-methylbutyl)acetamide	38.95	1,861.7	109	3.93	105.6	2.63
23	Benzyl alcohol	39.31	1,871.3	738	0.41	740	0.51
24	Phenyl ethanol	40.44	1,905.2	14,170	5.26	14,165	5.12
25	Diethyl malate	41.81	2,042.3	270	6.4	281	1.21
26	Octanoic acid	44.85	2,111.3	520	5.20	518	6.42
27	Acetyl glycine ethyl ester	46.11	2,153.1	89.5	4.04	90.3	2.78
28	Succinic acid, 2-hydroxy-3-methyl-diethylester	48.53	2,163.1	444	0.47	438	4.34
29	Vinyl guiacol	49.48	2,193.1	103	3.58	102	4.81
30	5-Oxotetrahydrofuran-2-caboxylic acid ethyl ester	50.44	2,233.3	402	1.34	396	3.68
31	Decanoic acid	51.92	2,336.5	201	1.62	210	0.27
32	Dihydrobenzo furan	52.64	2,398.2	1,500	3.43	1,513	1.87
33	Tyrosol	57.03	2,249.5	1,480	3.43	1,476	2.77

LRI linear retention index calculated using C10–C24 *n*-alkanes series, *RSD* relative standard deviation, (%)

and the values for RSD were <10 % (most of the values were <5 %).

Method Application

The method was then applied for analysis of the volatile composition of five wine samples from the Kékfrankos variety, and a total of 33 volatile compounds were identified and quantified in these red wines from the Villány region, Hungary, for the first time. The compounds, grouped in chemical classes, are presented in Table 5. A total ion chromatogram of one Kékfrankos wine is presented in Fig. 1.

Overall, 12 alcohols have been identified in the Kékfrankos wines (Table 5) with a total alcohol amount of 32.3, 14.4, 69.5, 0.59, and 4.3 mg/L for the five wines (1), (2) (3), (4), and (5), respectively. This volatile fraction was mainly composed of 2-phenyl ethanol, the most important phenol-derived higher alcohol (present from 11.7 to 43.7 % of the total alcohols), and 1-pentanol (26–57 % of the total alcohols) as the major components in the overall volatile content of the wines. The other important

Table 5 Concentrations of volatile compounds (microgram per liter) in the studied samples (1–5) of Kékfrankos wines (numbers correspond to peak numbers in Fig. 1)

Volatile compounds in Kékfrankos wines samples		(1) Concentration (µg/L)	(2) Concentration (µg/L)	(3) Concentration (µg/L)	(4) Concentration (µg/L)	(5) Concentration (µg/L)
Alcohols[a]						
11	1-Hexanol	743±0.25	1,578±5.63	676.70±1.72	29.15±4.27	43.84±5.52
8	1-Pentanol	11,600±9.50	80,232±5.53	39,895.3±4	161.77±0.24	1,125.38±1.81
16	1,3-Butylen glycol	598±0.49	2,503±6.49	1,176.10±2.20	4.34±0.38	32.39±1.97
15	2,3-Butanediol	280±8.48	147±29.88	/	/	9.85±20.52
3	3-Heptanol	575±0.42	346±27.1	329.23±3.16	6.01±1.77	5.12±1.42
20	3-(Methylthio)-1-propanol	713±0.49	723±3.93	452.34±3.47	15.76±2.01	16.00±2.23
23	Benzyl alcohol	738±0.41	1,079±4.14	8,019.63±165	85.11±8.69	900.98±99.21
12	E-3-Hexanol	567±0.07	240±5.12	197.84±8.28	3.90±1.80	7.35±3.82
5	Isobutyl alcohol	840±1.94	2,728±4.62	1,245.27±2.97	25.88±1.36	22.99±1.59
24	Phenyl ethanol	14,170±5.26	50,983±7.62	15,412.94±85.4	69.84±0.20	1,826.87±4.11
33	Tyrosol	1,480±3.43	3,248±9.21	1,794.19±6.14	180.11±8.28	310.51±9.34
29	Vinyl guiacol	103±3.58	209±16.84	323.98±143.4	14.46±6.75	33.30±12.47
	Total alcohols (µg/L)	**32,407±30.3**	**144,016±126**	**69,522±425**	**596±35.7**	**4,335±164**
Esters[b]						
30	5-Oxotetrahydrofuran-2-caboxylic acid ethyl ester	402±1.34	763±6.92	631.75±49.67	30.85±4.88	59.66±6.29
27	Acetyl glycine ethyl ester	89.5±4.04	2,065±8.01	979.08±36.24	106.77±16.54	24.42±2.75
7	Butyl formate	111±2.20	48.7±32.17	16.99±41.19	/	15.77±21.13
25	Diethyl malate	270±2.1	/	35.26±3.31	21.58±4.46	/
19	Diethyl succinate	2,861±1.27	8,105±9.82	17,420.90±0.86	47.67±0.58	1,189.64±4.36
14	Ethyl-3-hydroxybutanoate	117±3.93	216±116.96	36.48±79.66	12.57±8.76	9.42±4.15
4	Ethyl butanoate	156±5.08	438±11.62	985.67±147.26	17.98±10.76	13.73±6.01
18	Ethyl caprinate	90.3±10.0	119±18.66	92.36±9.42	/	19.94±10.67
9	Ethyl caproate	155±1.88	284±5.46	146.30±6.16	20.46±22.75	16.37±11.69
13	Ethyl caprylate	199±1.81	509±4.06	128.32±6.84	7.30±9.52	5.10±2.28
2	Ethyl isobutyrate	82.3±5.8	252±19.14	84.48±23.61	7.96±14.53	15.13±14.46
1	Ethyl propanoate	104±3.15	924±7.69	416.84±2.61	21.22±5.09	13.23±2.43
6	Isoamyl acetate	136±1.89	335±5.23	/	19.27±24.91	14.57±31.00
21	Phenyl ethyl acetate	81.5±4.26	4,270±3.42	1,86.59±101.70	26.61±2.89	36.47±6.92
28	Succinic acid, 2-hydroxy-3-methyl-diethylester	444±0.47	1,089±8.82	899.16±82.09	26.90±4.64	67.85±4.55
	Total esters (µg/L)	**5,298±49.2**	**19,417±258**	**22,060±588**	**367±180**	**1,500±129**
Fatty acids[a]						
31	Decanoic acid	201±1.62	453±4.66	268.93±57.28	19.38±4.81	169.62±22.43
26	Octanoic acid	520±5.20	868±2.91	978.15±4.45	63.75±32.80	29.00±7.37
	Total acids (µg/L)	721±6.82	1,321±7.57	1,247±61.7	83.1±37.6	198±29.8
Furans[a]						
32	Dihydrobenzo furan	**1,500±3.43**	**641±22.5**	**220±38.9**	**39.7±10.6**	**457±111**
Lactones						
17	Butyrolactone	**8,150±1.07**	19,542±5.05	14,360±1.27	277±1.42	839±4.37
Other compounds[a]						
10	1-Butamine, N-nitro	1,353±7.22	6,307±7.01	3,905±0.99	57.0±1.56	164.1±3.32
22	N-(3-methylbutyl)acetamide	109±3.93	5,524±6.92	229±191	23.0±2.17	1,015±90.6
	Total other compounds (µg/L)	**1,462±11.2**	**11,831±13.9**	**4,133±192**	**80.1±3.73**	**1,179±93.8**
	Total volatiles (µg/L)	**49,786±101**	**79,206±330**	**59,061±1,111**	**1,317±263**	**5,448±439**

[a] Expressed in equivalents of phenyl ethanol

[b] Expressed in equivalents of ethyl nonanoate

Concentration in microgram per liter±RSD (relative standard deviation, (%))

Bold numbers indicate the total amount of each group of volatile compounds

Fig. 1 GC/MS chromatogram of the volatile compounds in Kékfrankos wine (sample 1). Peak numbers refer to the compounds listed in Tables 4 and 5
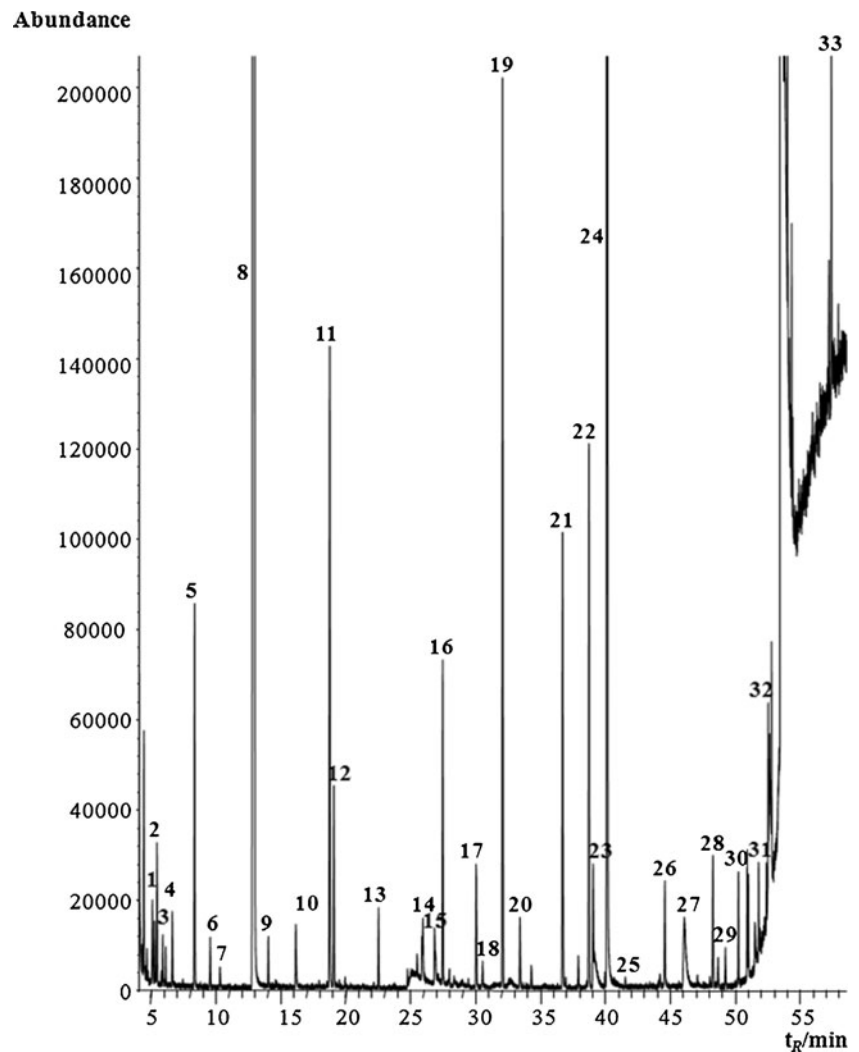
alcohol, detected in a considerable amount in the Kékfrankos wines, was tyrosol, comprising 2–7 % of the total alcohols in four wines, except for wine (4) which contained 30 % tyrosol of the total alcohols in the wine. Isobutyl alcohol was also found in the wine, which is formed during the catabolic pathway from its corresponding amino acid, valine (Li et al. 2008). In addition, all Kékfrankos wines presented higher content of alcohols compared to Cabernet Sauvignon wines from Changli County (China) (Tao et al. 2008), while three wines, (1), (2), and (3), had a higher amount of alcohols compared to Tempranillo wines (Castro-Vázquez et al. 2011), but a lower amount compared to Albarello and Brancellao wines (Cortés and Díaz, 2011) as well as from the varieties Ribolla Gialla and Malvasia Istriana (Bavčar et al. 2011).

Another important family of aroma compounds in wine are the esters. In our study, 15 esters were identified and quantified in the analyzed wines (Table 5), present from 0.36 to 22 mg/L total content of esters. The main contributor to this class of volatile compounds was diethyl succinate which comprised 13–54 % of the total amount of esters found in wines (1), (2), and (4), and 79 % in wines (3) and (5).

Diethyl succinate possesses fruity aromatic impact, affecting the overall wine aroma characteristics. The acetate esters, such as phenylethyl acetate and isoamyl acetate, possessing sensory impact described as "banana and apple", were also detected in the wine (except in wine (3) which did not contain isoamyl acetate). Overall, Kékfrankos wines possessed a similar amount of total esters in comparison to other red wines (Qian et al. 2009, Bavčar et al. 2011, Castro-Vázquez et al. 2011).

Butyrolactone was the only compound that was detected from this group, found in a relatively high concentration (15–25 % from all volatiles) (Table 5). Its content depends on grape variety, maceration, and aging (Bueno et al. 2003). Volatile components belonging to the group of fatty acids, such as octanoic and decanoic acids, were also detected and quantified in the wine (Table 5).

Conclusion

A method for analysis of volatiles in wine using liquid–liquid extraction with dichloromethane and GC–MS for

separation and detection has been developed and validated. The method of validation confirmed its satisfactory linearity, LOQ, accuracy, repeatability, and reproducibility. It was then used for determination of 33 volatile compounds in the red Kékfrankos wines produced in Hungary.

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