

QUANTIFICATION OF RED WINE VOLATILE ORGANIC COMPOUNDS BY HEADSPACE SOLID-PHASE MICROEXTRACTION

Gulyaim Sagandykova

Meruyert Sergazina

Dr. Mereke Alimzhanova

¹ Center of Physical Chemical Methods of Research and Analysis, **Kazakhstan**

² Al Farabi Kazakh National University, **Kazakhstan**

ABSTRACT

Volatile organic compounds influences on quality and aroma of wine. However, these compounds (acids, esters, alcohols, aldehydes) in wines are in low concentration level. For their determination and quantification in wines, isolation or concentration step is required. The aim of this study was to investigate optimal parameters: extraction time and sample volume for quantification of 1-pentanol, iso-amylol, iso-butanol and ethyl hexanoate by standard addition approach in combination with solid-phase microextraction technique. Standard addition approach allows to determine concentrations of volatile organic compounds and control matrix effect of wine samples. The lowest values of relative standard deviations (0.71, 1.48, 3.27 and 1.85 % for iso-butanol, iso-amylol, ethyl hexanoate and 1-pentanol, respectively) were provided by extraction time and sample volume 1 min and 5 mL. Calibration plots by standard addition method are linear in the whole studied range, except ethyl hexanoate till concentration 1.00 mg/L. Determined concentrations with different parameters (extraction time and sample volume) are very close except 1-pentanol (RSD 68 %). Most optimal extraction conditions accounts for 1 min and 5 mL.

Keywords: VOCs, wine, solid-phase microextraction, standard addition, GC-MS

INTRODUCTION

Sensory properties of wines (taste, aroma and color) are important characteristics for consumers perception. At the same time, wine aroma is imparted by classes of volatile organic compounds (VOCs), mainly alcohols, esters, aldehydes and acids [1]. Wine aroma and subsequently, wine quality, is determined by fermentation and production process, ageing, grape variety and ripeness. Therefore, concentration of wine volatile organic compounds may be a criteria of wine quality [2].

Wines typically contain VOCs in low concentration levels (ng/L to mg/L) and subsequently, their quantification requires isolation or concentration step prior to analysis [3]. In addition, analysis of red wine VOCs can be complicated by its complex matrix that consists of large number of organic compounds - esters, aldehydes, acids, terpenes, alcohols, phenolic compounds. Solid-phase microextraction (SPME) is solvent-free extraction technique that allows to extract lots of compounds simultaneously during one analysis from complex matrixes [4]. Moreover, extraction process can be fully automated and answers requirements of green analytical chemistry. Solid-phase microextraction in headspace mode allows to protect fiber from molecular interferences and damage that can be caused by direct immersion of the fiber to liquid phase of the sample.

According to the Customs Union Regulation of Russia, Kazakhstan and Belarus [5], maximum permitted concentrations of alcohols (iso-amylol, iso-butanol) is 6 mg/L and esters - 13 mg/L in wines. For quantification of wine VOCs several approaches were used: external [3,6] and internal standard calibration [7]. However, external standard method does not allow to control matrix effect caused by complex wine composition. Application of internal standard approach may be complicated by selection of internal standard for target analytes. Approach applied more often in comparison with other methods is external standard calibration. However, lots of research papers describe only qualitative composition of VOCs of different wines [8,9]. Several research papers were focused on optimization of HS-SPME parameters for determination of wine VOCs [10].

The aim of this research was to develop method based on HS-SPME and GC-MS for quantitative analysis of 1-pentanol, iso-amylol, ethyl hexanoate and iso-butanol in red wines by standard addition method. Optimization parameters included sample volume and extraction time. It was investigated that most optimal conditions are extraction time 1 min and sample volume 5 mL. Developed method can be used for quantitative analysis of red wine volatile compounds for identification of wine quality.

EXPERIMENTAL

2.1.1. Reagents and chemicals

Standards of iso-butanol (99.5%), 1-pentanol (98%), iso-amylol (98%) and ethyl hexanoate (>98 %) were purchased from Sigma-Aldrich. Ethanol solution (>95 %) was used for preparation of solutions for standard addition method (Talgat, Kazakhstan).

2.1.1 Optimization of sample volume and extraction time

In previous research, it was investigated that concentration of wine volatile compounds (iso-butanol, 1-pentanol, isoamylol and ethyl hexanoate) can be calculated by standard addition method [11]. Parameters of HS-SPME for target analytes were optimized in previous research [12]. The goal of this experiment was to establish the optimal sample volume and extraction time for determination by SPME with standard addition. Independent variables of the experiment were sample volume and extraction time. The main working hypothesis of the experiment was: sample volume 5 mL and extraction time 1 min will be optimal. Four series of experimental samples having volumes 1.00 and 5.00 mL with seven standard solutions were placed in preliminary conditioned 20-mL crimp-top vials (HTA, Italy) with PTFE-Silicone septa. Series 1-2 were analyzed by SPME using extraction time 1 min, series 3-4 during 5 min at 28°C. Pre-incubation and desorption times accounted for 15 and 5 min, respectively. Samples were extracted by 100 µm PDMS fiber coating. Areas of the following peaks were selected as dependent variables in the course of the experiment: iso-butanol, iso-amylol, ethyl hexanoate and 1-pentanol and their RSDs were calculated. Peak areas of added VOCs were plotted versus their concentrations added.

2.1.2. Sample preparation

Experiment was carried out using red wine “Damskiy Ugodnik” (“Winnac” LLP, Almaty, Kazakhstan). Sample preparation steps included preparation of stock solution and spiking wine samples till 5, 10, 20, 50, 100 and 200 mg/L for iso-butanol; 5, 10, 20,

50 and 200 mg/L for 1-pentanol; 10, 20, 40, 100, 200 and 400 mg/L for isoamylol and 0.1, 0.2, 0.4, 1, 2 and 3.5 mg/L for ethyl hexanoate. Samples were prepared and analyzed in four replicates.

2.1.3. GC-MS conditions

Extraction and analysis were fully automated using HT280T autosampler (HTA, Italy) installed on 6890N/5975C (Agilent, USA) GC-MS system. Separation was achieved using a 30 m x 0.25 mm HP-Innowax (Agilent, USA) capillary column having film thickness of 0.25 μ m at a constant flow rate of carrier gas (helium grade "A") of 1 mL/min. Oven temperature was programmed from 40°C (10 min) to 240°C at a heating rate of 5°C/min. Detection was performed in scan mode (m/z 10-350). Temperatures of MS source, quadrupole and interface were 230, 150 and 240 °C, respectively. Instrument was controlled using MSD ChemStation software (Agilent, USA) ver. E.02.02.1431 Service Pack 1. Before the experiment, mass spectrometric detector was autotuned and gain factor was set to 1.0. After analysis, chromatograms were integrated, areas of selected VOCs and their RSDs were calculated. Peak areas of added VOCs were plotted versus their concentrations added.

RESULTS AND DISCUSSION

Obtained plots are linear in the whole studied ranges for all analytes, except ethyl hexanoate (Figure 1). After concentration of about 1.00 mg/L, calibration plot for ethyl hexanoate gets slightly curved. It can be caused by its displacement from fiber coating or decomposition that was observed in the previous experiments. Hypothesis on decomposition is confirmed by the fact that standard error of slope of ethyl hexanoate was much higher than that for other compounds studied for all series of experiment and reached 8% (Table 1).

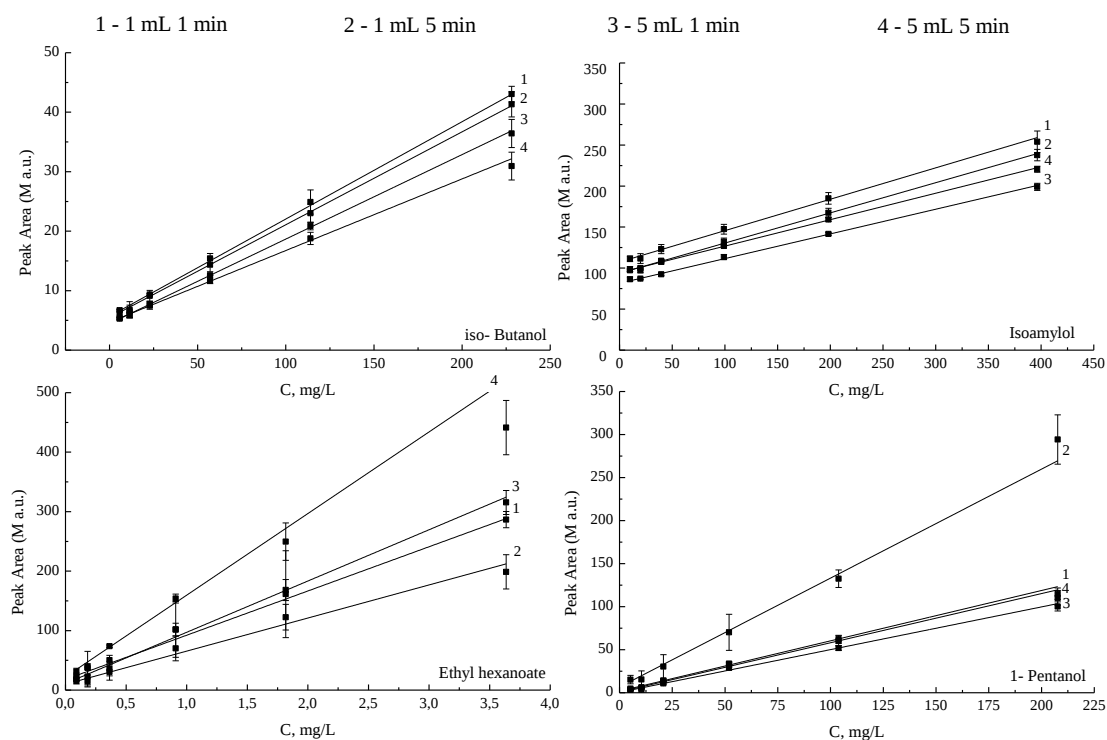


Fig. 1. Effect of amount of standard solution added on SPME-GC-MS response of selected red wine VOCs

Obtained plots were used to calculate concentrations of analytes. Concentrations calculated for different extraction conditions are very close (Table 1) for all analytes except 1-pentanol. High RSD (68%) observed for 1-pentanol can be caused by its very low concentration in sample in comparison with standard addition - lowest addition was 4.5 mg/L, close to the highest replicate 5.21 mg/L. For ethyl hexanoate RSD was 26%, probably due to its poor stability.

For iso-butanol, calculated concentrations at sample volume 1.00 mL were close to the average value. At sample volume 5.00 mL, they were outside the range of standard deviation. For iso-amylol, calculated concentrations at sets of sample volume and extraction time 1.00 mL and 1 min, 5.00 mL and 5 min were outside the standard deviation range. From this point of consideration, best set of sample volume and extraction time are 1.00 mL and 5 min.

Table 1. Linearity coefficients of the calibration plots of iso-butanol, 1-pentanol, iso-amylol and ethyl hexanoate by standard addition method

Compound	Sample volume, mL	Extraction time, min	Slope	SD of slope	Intercept	R ²	C, mg/L	C _{mean} , (mg/L)	SD (RSD)
iso-butanol	1	1	0.164	0.0034	5.68	0.9991	34.6	35.0	3.1 (8.8%)
		5	0.156	0.0017	5.46	0.9998	35.0		
	5	1	0.142	6.6E-4	4.47	0.9999	31.5		
		5	0.120	0.0022	4.70	0.9993	39.0		
iso-amylol	1	1	0.368	0.012	93.7	0.9979	255	275	16 (5.9%)
		5	0.383	0.011	107	0.9983	279		
	5	1	0.301	0.0053	81.5	0.9994	270		
		5	0.322	0.0038	94.5	0.9997	293		
ethyl hexanoate	1	1	55.6	3.3	9.88	0.9929	0.178	0.177	0.047 (26%)
		5	74.4	2.2	17.8	0.9983	0.239		
	5	1	86.2	3.3	11.0	0.9970	0.128		
		5	137.2	11	22.4	0.9874	0.163		
1-pentanol	1	1	1.266	0.055	6.60	0.9963	5.21	2.74	1.87 (68%)
		5	0.571	0.017	0.941	0.9983	1.65		
	5	1	0.496	0.013	0.494	0.9987	0.996		
		5	0.585	0.033	1.816	0.9938	3.10		

Comparison of RSDs of slope factors of calibration plots (Figure 2) demonstrates that minimum values are achieved at sample volume 5 mL and extraction time 1 min. Slightly higher RSDs are observed at extraction time 5 min and sample volume 1 or 5 mL.

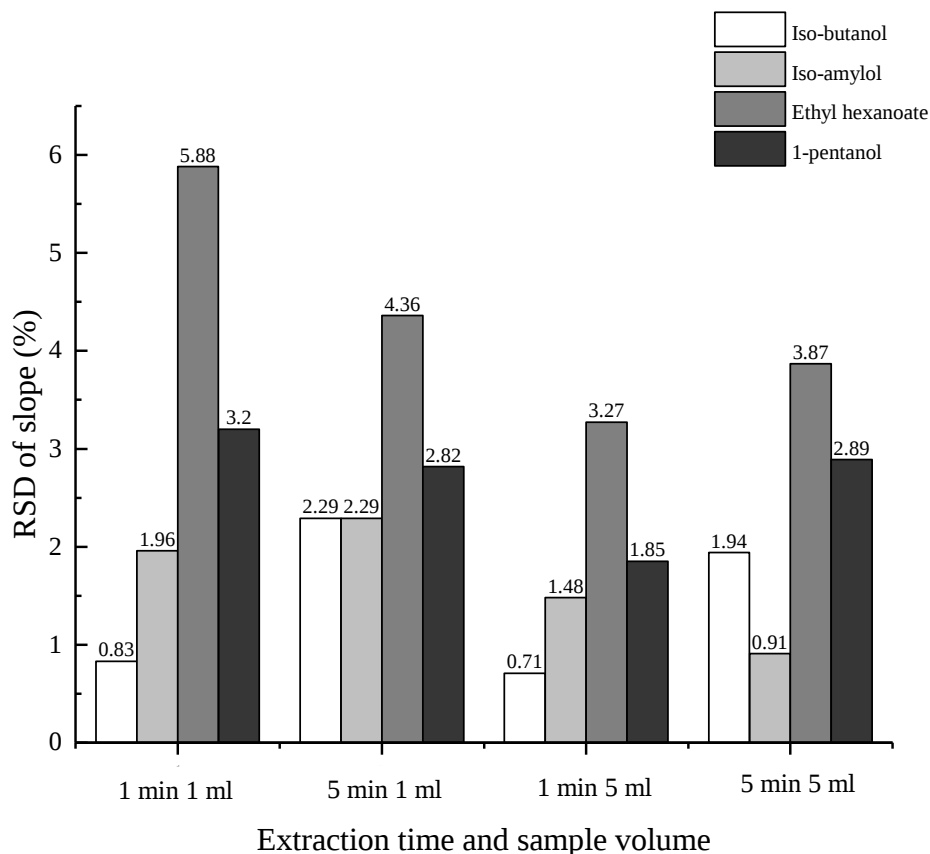


Fig. 2. Comparison of RSD of slope different volume of wine and extraction time

Obtained plots are linear in the whole studied ranges for all analytes, except ethyl hexanoate concentration of which 1.00 mg/L, calibration plot gets curved. Concentrations calculated for different extraction conditions are very close for all analytes except 1-pentanol, for which high RSD (68%) can be caused by its very low concentration in wine. Standard deviations are much higher at sample volume 1 mL and extraction time 1 min compared to other conditions. Most optimal conditions are extraction time 1 min and sample volume 5 mL.

CONCLUSION

Quantification of red wine volatile organic compounds was performed by standard addition method, comparing with existing methodologies with application of external and internal standard calibration approaches. Sample volume and extraction time were optimized for determination of red wine VOCs by HS-SPME and standard addition method. Relative standard deviations were compared in different extraction conditions: 1 min 1 mL; 5 min 1 mL; 1 min 5 mL; 5 min 5 mL. Concentrations of iso-butanol, iso-amylol, ethyl hexanoate and 1-pentanol were calculated and compared for different extraction conditions. Concentrations calculated for different extraction conditions are very close for all analytes except 1-pentanol, for which high RSD (68%) can be caused by its very low concentration. It was investigated that lower values of RSDs are achieved at sample volume 5 mL and extraction time 1 min. Standard deviations are much higher at sample volume 1 mL and extraction time 1 min compared to other conditions. Most optimal conditions are extraction time 1 min and sample volume 5 mL.

Plots obtained by standard addition method are linear in the whole studied range of concentrations. Method can be used in future for quantification of iso-butanol, iso-amylol, 1-pentanol and ethyl hexanoate in red wines by SPME.

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