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МЕТОДИ ЗА ОПРЕДЕЛУВАЊЕ НА SO_2 И РЕДУЦИРАЧКИ ШЕЌЕРИ ВО ВИНА И АЛКОХОЛНИ ПИЈАЛАЦИ

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Апстракт: Во ова истражување беа оптимизирани и валидирани волуметриските методи за определување на SO_2 (слободен и вкупен) и редуцирачки шеќери во вина и алкохолни пијалаци. Линеарноста, точноста и прецизноста на методите беше потврдена со примена на стандардни раствори од SO_2 и редуцирачки шеќери (фруктоза и гликоза) подготвени во определен концентрациски опсег, како и со ниски, средни и високи концентрации. Дополнително, точноста на методите беше проверена со методата на стандардни додатоци. Повторливоста и репродуцибилноста на методите беа потврдени со повторени анализа на реални примероци од вина и алкохолни пијалаци. Резултатите од анализите потврдија дека двете методи се точни и прецизни и се соодветни за анализа на вина и алкохолни пријалааци.

Клучни зборови: *SO_2, редуцирачки шеќери, вино, алкохолни пијалаци, валидација.*

METHODS FOR DETERMINATION OF SO_2 AND REDUCING SUGARS IN WINES AND ALCOHOLIC BEVERAGES

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Abstract: In this study, volumetric methods for determination of SO_2 (free and total) and reducing sugars in wines and alcoholic beverages were optimized and validated. The linearity, accuracy and precision of the methods were confirmed using standard solutions of SO_2 and reducing sugars (fructose and glucose) prepared in appropriate concentration range, as well as with low, medium and high concentrations. Additionally, the accuracy of the methods was checked by standard additions. Repeatability and reproducibility of the methods was confirmed with repeated analyses of real samples, wines and

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alcoholic beverages. Obtained results for both methods presented satisfactory accuracy and precision, suggesting that these methods are appropriate for analysis of wines and alcoholic beverages.

Keywords: *SO_2, reducing sugars, wine, titration*

1. Introduction

Quality control of wines and alcoholic beverages is very important to be performed during the production as well as on the final product. One of the parameters responsible for the quality of wines is the content of SO_2. The use of SO_2 in winemaking is due to its ability as an effective antoxidant, preventing oxidation, antimicrobial agent, potential for bleaching the pigments and elimination of unpleasant odours. SO_2 can selectively act against the wild yeasts, which come from the grape skin or equipment in the winery, and stop their activity. Sulfur dioxide can be added in a form of a salt, potassium metabisulphate ($K_2S_2O_5$), which can be ionized in acid media, releasing gaseous SO_2. Only free SO_2 possesses antiseptic and antioxidant properties. Higher amounts of SO_2 negatively influence the wine quality (flavor and taste) (Ivanova-Petropulos & Mitrev, 2014). The content of SO_2 (free and total) is usually determined by iodine titration, according to the Ripper's method (Vahl & Converse, 1980), using standard solution of iodine in presence of stretch as an indicator and sulfuric acid. Before titration, solution of NaOH is used in order to release the bound SO_2.

The main carbohydrates in grapes and wine are glucose and fructose, usually called "reducing sugars". During the fermentation, reducing sugars are broken down by the action of the yeast, forming an alcohol (ethanol) and carbon dioxide. For determination of reducing sugars in must and wine, chemical methods usually are based on reduction-oxidation (redox) reactions between sugars and Fehling's solution, according to the Shorl's method. Fehling's solution contains copper (II) ions that can be reduced by some sugars to copper (I) ions (Ivanova-Petropulos & Mitrev, 2014).

The aim of this work is validation of volumetric methods for determination of SO_2 (free and total) and reducing sugars in wines and alcoholic beverages, and then, application of the methods on real samples.

2. Materials and methods

2.1. Reagents

Standard solution of SO_2 and standards of glucose and fructose were purchased from Sigma Aldrich (St. Louis, MO). All other reagents used were with analytical grade of purity.

2.2. Wines and alcoholic beverages

In total, ten different red and white wines from various varieties (vintage 2015) and three alcoholic beverages (yellow brandy, white brandy and mastika) (produced in 2015) were analyzed.

2.3. Determination of SO_2

Free SO_2. A volume of 50 mL wine or brandy was transferred to flask of 250 mL, followed by addition of 10 mL 25 % (*v/v*) solution of sulfuric acid (1+3) and 2-3 mL 1 % solution of stretch as an indicator. Sulfuric acid is added since the oxidation in acid conditions is more intensive. The prepared sample was titrated with a standard solution of iodine with concentration of 0.01 mol/L until the endpoint of titration (change of color to dark-blue for the wine and yellow for the brandy). The following equation was used for calculation of the content of free SO_2 (Ivanova-Petropulos & Mitrev, 2014):

$$\text{Free } SO_2/\text{mg/L} = V(I_2) \cdot c(I_2) \cdot M(SO_2) \cdot 1000/V(\text{wine})$$
$$\text{Free } SO_2/\text{mg/L} = V(I_2) \cdot 12.8$$

Total SO_2. A volume of 25 mL solution of 1 M NaOH was transferred to the flask of 250 mL, followed by addition of 50 mL sample (wine or brandy). The sample was mixed, flask was closed with a rubber stopper and left for 10 min in a dark place. Then, 10 mL of 25 % (*v/v*) solution of sulfuric acid (1+3) and 2-3 mL 1 % solution of stretch were added. The sample was titrated with a standard solution of iodine (0.01 mol/L) until the endpoint of titration (change of color to dark-blue). The following equation was used for calculation of the content of total SO_2 (Ivanova-Petropulos & Mitrev, 2014):

$$\text{Total } SO_2/\text{mg/L} = V(I_2) \cdot c(I_2) \cdot M(SO_2) \cdot 1000/V(\text{wine})$$
$$\text{Total } SO_2/\text{mg/L} = V(I_2) \cdot 12{,}8$$

The content of SO_2 (free or total) can be directly read out from Table 1, using the consumed volume of I_2 for titration of the sample.

2.4. Determination of reducing sugars

For determination of reducing sugars, 1 mL sample (wine or brandy) was transferred to 100 mL flask, followed by addition of distilled water to the mark. Then, 10 mL of the diluted sample was transferred to a flask (250 mL) that contained 10 mL Fehling I and 10 mL Fehling II solutions. The flask with the sample was heated on a moderate temperature until boiling temperature (or until appearance of 1-2 bubbles), followed with a change of color to red-brown (depending on the sugar content in the sample). After the heating, flask was cooled down (under tap water), and then, 10 mL of 20 % (*m*/*v*) solution of KI and 10 mL of 25 % (*v*/*v*) sulfuric acid were added to the flask. The flask was closed with a rubber stopper and left in a dark place to stand for 2-3 min. Then, a volume of 2-3 mL of 1 % (*m*/*v*) solution of stretch was added and the sample was titrated with 0.1 mol/L solution of $Na_2S_2O_3$ until change of the color from yellow-brown to milky-white. Previously, a blank sample should be prepared and titrated in a same way as the sample, using distilled water (20 mL). The total consumed volume of $Na_2S_2O_3$ was calculated as a difference between the volumes of $Na_2S_2O_3$ consumed for titration of the blank and sample:

$$V(Na_2S_2O_3) = V(Na_2S_2O_3)_{blank} - V(Na_2S_2O_3)_{sample})$$

and used for determination of the sugars content, using the Table 2 (Ivanova-Petropulos & Mitrev, 2014).

Table 1. Table for SO_2 (mg/L) in wine and alcoholic beverage

$V(I_2)$/ mL	0	1	2	3	4	5	6	7	8	9
0	0.00	1.28	2.26	3.84	5.12	6.40	7.68	8.96	10.24	11.52
1	12.80	14.08	15.36	16.64	17.92	19.20	20.48	21.76	23.04	24.32
2	25.60	26.88	28.16	29.44	30.72	32.00	33.28	34.56	35.84	37.12
3	38.40	39.68	40.96	42.24	43.52	44.80	46.08	47.36	48.64	49.92
4	51.20	52.48	53.76	55.04	56.32	57.60	58.88	60.16	61.44	62.72
5	64.00	65.28	66.56	67.84	69.12	70.40	71.68	72.96	74.24	75.52
6	76.80	78.08	79.36	80.64	81.92	83.20	84.48	85.76	87.04	88.32
7	89.60	90.88	92.16	93.44	94.72	96.00	97.28	98.56	99.84	101.12
8	102.40	103.68	104.96	106.24	107.52	108.80	110.08	111.36	112.64	113.92
9	115.20	116.48	117.76	119.04	120.32	121.60	122.88	124.16	125.44	126.72
10	128.00	129.28	130.56	131.84	133.12	134.40	135.68	136.96	138.24	139.52
11	140.80	142.08	143.36	144.64	145.92	147.20	148.48	149.76	151.04	152.32
12	153.60	154.88	156.16	157.44	158.72	160.00	161.28	162.56	163.84	165.12
13	166.40	167.68	168.96	170.24	171.52	172.80	174.08	175.36	176.64	177.92
14	179.20	180.48	181.76	183.04	184.32	185.60	186.88	188.16	189.44	190.72
15	192.00	193.28	194.56	195.84	197.21	198.40	199.68	200.96	202.24	203.52
16	204.80	206.08	207.36	208.64	209.92	211.20	212.48	213.76	215.04	216.32
17	217.60	218.88	220.16	221.74	222.72	224.00	225.28	226.56	227.84	229.12
18	230.40	231.68	232.96	234.24	235.52	236.80	237.08	238.36	239.64	240.92
19	243.20	244.48	245.76	247.04	248.32	249.60	250.88	252.16	253.44	254.72
20	256.00	257.28	258.56	259.84	261.12	262.40	263.68	264.96	266.24	267.52
21	268.80	270.08	271.36	272.64	273.92	257.20	276.48	277.76	279.04	280.32

Table 2. Table for reducing sugars (g/L) in wine and alcoholic beverage

$V(Na_2S_2O_3)$/ mL	0	1	2	3	4	5	6	7	8	9
0	0.0	0.3	0.6	1.0	1.3	1.6	1.9	2.2	2.6	2.9
1	3.2	3.5	3.8	4.2	4.5	4.8	5.1	5.4	5.7	6.1
2	6.4	6.7	7.1	7.4	7.7	8.1	8.4	8.7	9.0	9.4
3	9.7	10.0	10.4	10.7	11.0	11.4	11.7	12.0	12.3	12.7
4	13.0	13.3	13.7	14.0	14.4	14.7	15.0	15.4	15.7	16.1
5	16.4	16.7	17.1	17.4	17.8	18.1	18.4	18.8	19.1	19.5
6	19.8	20.1	20.5	20.8	21.2	21.5	21.8	22.2	22.5	22.9
7	23.2	23.5	23.9	24.2	24.6	24.9	25.2	25.6	25.9	26.3
8	26.5	26.9	27.3	27.6	28.0	28.3	28.6	29.0	29.3	29.7
9	29.9	30.3	30.7	31.0	31.3	31.7	32.0	32.7	32.7	33.0
10	33.4	33.7	34.1	34.4	34.8	35.1	35.4	35.8	36.1	36.5
11	36.8	37.2	37.5	37.9	38.2	38.6	38.9	39.3	39.6	40.0
12	40.3	40.7	41.0	41.4	41.7	42.1	42.2	42.8	43.1	43.5
13	43.8	44.2	44.5	44.9	45.2	45.6	45.9	46.3	46.6	47.0
14	47.3	47.7	48.0	48.4	48.7	49.1	49.4	49.8	50.1	50.5
15	50.8	51.2	51.5	51.9	52.2	52.6	52.9	53.3	53.6	54.0
16	54.3	54.7	55.0	55.4	55.8	56.2	56.5	56.8	57.3	57.6
17	58.0	58.4	58.8	59.1	59.5	59.9	60.3	60.7	61.0	61.4
18	61.8	62.2	62.5	62.9	63.3	63.7	64.0	64.4	64.8	65.1
19	65.5	65.9	66.3	66.7	67.1	67.5	67.8	68.2	68.6	69.1
20	69.4	69.8	70.2	70.6	71.0	71.4	71.7	72.1	72.5	72.9
21	73.3	73.7	74.1	74.5	74.9	75.3	75.6	76.0	76.4	76.8
22	77.2	77.6	78.0	78.4	78.8	79.2	79.6	80.0	80.4	80.8
23	81.2	81.6	82.0	82.4	82.8	83.2	83.6	84.0	84.4	84.8
24	85.2	85.6	86.0	86.4	86.8	87.2	87.6	88.0	88.4	88.8
25	89.2	89.6	90.0	90.4	90.8	91.2	91.6	92.0	92.4	92.8

3. Results and discussion

3.1. Methods validation

Linearity, accuracy, precision, repeatability and reproducibility were checked for SO_2 and reducing sugars in wine and brandy using standard solutions of SO_2 and carbohydrates (glucose and fructose), respectively.

Linearity. The linearity data for determination of free SO_2, total SO_2 and reducing sugars are presented in Table 3. Linearity was satisfactory in all cases with correlation coefficients (R^2) of 0.999.

Table 3. Intercept, slope and correlation coefficients (R^2)

Compound	Intercept	Slope	R^2	Range
Free SO_2	0.4305	0.9933	0.9999	0-500 (mg/L)
Total SO_2	0.3510	0.9957	0.9999	0-500 (mg/L)
Reducing sugars	0.0611	0.9985	0.9998	0-100 (g/L)

Reducing sugars: glucose+fructose

Accuracy and precision. The intra-day and inter-day accuracy and precision were determined with titration of standard solutions of SO_2 and reducing sugars with low (5 mg/L), medium (25 mg/L) and high concentration (50 mg/L), as presented in Table 4. For determination of intra-day accuracy and precision, freshly prepared solutions were used, analyzed immediately, in 10 repetitions during one day. Inter-day accuracy and precision were determined with titration of the standard solutions during 10 consecutive days.

The accuracy was expressed with a relative error of the determined concentration compared with the true (nominal) value. Satisfactory results are considered when relative error is lower than 20 % for low concentrations and lower than 10 % for high concentrations.

Precision was expressed as a relative standard deviation (*RSD*). Satisfactory results are considered when *RSD* is lower than 20 % for low concentrations and lower than 10 % for high concentrations.

The relative errors for inter-day and intra-day accuracy for SO_2 ranged between -0.8 to 6 % and -10.4 to -2.4, while for the reducing sugars were -0.8 to -4 and -2.8 to 1.2, respectively (Table 4). These results confirmed that the suggested are accurate and convenient for quantitative analysis of SO_2 (free and total) and reducing sugars.

Table 4. Intra- and Inter- day accuracy and precision data for standard solutions of SO_2 and reducing sugars (n=10)

	SO_2						**Reducing sugars**					
Sample	**5 mg/L**		**25 mg/L**		**50 mg/L**		**5 mg/L**		**25 mg/L**		**50 mg/L**	
	Found (%)	e_R	Found (%)	e_R	Found (%)	e_R	Found (%)	e_R	Found (%)	e_R	Found (%)	e_R
	Inter- day accuracy and precision											
<*x*>	4.70	6.0	25.2	-0.8	50.3	-0.6	5.2	-4	25.2	-0.8	51.9	-2.2
SD	0.75		0.74		0.73		0.17		0.35		0.74	
***RSD* (%)**	15.8		2.93		1.46		3.33		1.39		1.44	
	Intra- day accuracy and precision											
<*x*>	5.52	-10.4	26.0	-4.0	51.2	-2.4	5.1	-2	25.7	-2.8	49.4	1.2
SD	0.73		0.74		1.28		0.30		0.72		0.56	
***RSD* (%)**	13.1		2.84		2.5		5.88		2.81		1.11	

Labels: <*x*> - average value of ten repetitions, SD – standard deviation, *RSD* – relative standard deviation

Additionally, the accuracy of the methods was checked using standard addition method. Samples, including red wine, white wine and brandy, previously analyzed, were spiked with appropriate volumes of the standard solutions of SO_2 and reducing sugars (glucose and fructose) with concentration of 5, 10 and 50 mg/L for each standard. Obtained results for the recovery were satisfactory recovery ranging from 94.8–102% (Table 5), confirming that methods are accurate and convenient for quantitative analysis.

Table 5. Results from the standard additions method for checking the accuracy of the volumetric methods for determination of SO_2 (free and total) and reducing sugars in wine and brandy (n = 3)

	γ (Free SO_2)			**γ (Total SO_2)**			**γ (Reducing sugars)**		
White wine Standard addition	Calculated /mg/L	Found /mg/L	Recovery, %	Calculated /mg/L	Found /mg/L	Recovery, %	Calculated /mg/L	Found /mg/L	Recovery, %
I	40.85	39.68	97.1	97.16	96.0	98.8	6.60	6.40	96.9
II	45.85	46.08	100.5	102.1	99.84	97.7	11.6	11.0	94.8
III	85.85	84.48	98.5	142.2	140.1	99.0	51.6	50.8	98.4
Red wine									
I	19.08	20.48	107	63.88	62.72	98.2	7.20	7.40	102.7
II	24.08	24.31	101	68.88	70.4	102	12.2	12.0	98.3
III	64.08	62.72	97.8	108.8	106.2	97.6	52.2	52.6	100.7
Brandy									
I	6.28	6.4	101.9	8.84	8.96	101.3	8.50	8.40	98.8
II	11.28	11.52	102.1	13.84	12.8	92.5	13.5	13.7	101.5
III	51.28	49.92	97.3	53.85	53.76	99.8	53.5	52.9	98.9

Repeatability and reproducibility. Repeatability was checked with 10 repetitions in one day, while reproducibility was checked with 3 repetitions in 3 consecutive days, both performed on real samples (white wine, red wine and brandy) (Table 6).

Values for the standard deviations were very low for all methods, ranging from 0.15 to 0.70 for repeatability and 0.13 to 0.75 for reproducibility, confirming that methods are accurate and can be applied for determination of SO_2 (free and total) and reducing sugars in white wines, red wines and alcoholic beverages.

Table 6. Results for repeatability and reproducibility of SO_2 (free and total) and reducing sugars in white wines, red wines and alcoholic beverages

Samples	Content of free SO_2/mg/L	Content of total SO_2/mg/L	Content of reducing sugars/g/L
White wine	***Repeatability*** *(10 replicates in one day)*		
<*x*>	33.8	74.9	1.15
SD	0.66	0.70	0.15
RSD (%)	1.95	0.94	13.7
Red wine			
<*x*>	15.8	41.8	2.81
SD	0.66	0.62	0.15
RSD (%)	4.17	1.47	5.16
Brandy			
<*x*>	2.16	3.68	1.3
SD	0.31	0.49	0.15
RSD (%)	14.2	13.6	11.1
White wine	***Reproducibility*** *(3 replicates x 5 days)*		
<*x*>	33.5	75.1	1.24
SD	0.57	0.75	0.13
RSD (%)	1.7	0.99	10.8
Red wine			
<*x*>	15.6	41.9	2.84
SD	0.57	0.57	0.13
RSD (%)	3.67	1.36	4.72
Brandy			
<*x*>	2.06	3.52	1.3
SD	0.44	0.70	0.13
RSD (%)	21.1	20.0	10.3

<*x*> - average, SD – standard deviation, *RSD* – relative standard deviation

3.2. Application of methods for analysis of wines and brandies

Validated volumetric methods for determination of free and total SO_2 and reducing sugars were applied on real samples, including white wines (Smederevka, Chardonnay, Riesling, Muscat Ottonel and Sauvignon blanc), red wines (Vranec, Merlot, Cabernet Sauvignon, Pinot Noir and Stanušina) and alcoholic beverages (yellow brandy, white brandy and mastika). All wines were protected from oxidation and microbial contamination, containing sufficient levels of free and total SO_2 (free SO_2: 20.48 to 47.37 mg/L for white wines and 10.24 to 26.88 for red wines; total SO_2: 88.32 to 112.6 for white wines and 44.8 to 65.28 for red wines). All wines were considered as dry wines, presenting low values of reducing sugars, ranging from 1 to 2.9 g/L). Brandies also contained low levels of sugars (0.3 to 3.8 g/L). Results were in accordance to previous published data for Macedonian wines (Ivanova-Petropulos et al. 2015).

Table 7. Content of SO_2 (free and total) and reducing sugars in wines and alcoholic beverages

Wines	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Reducing sugars (g/L)
White wines			
Smederevka	42.24	112.6	2.9
Chardonnay	30.72	98.56	1.6
Riesling	35.85	88.32	1.3
Muscat Ottonel	20.48	102.4	1.9
Sauvignon blanc	47.37	90.88	2.6
Red wines			
Vranec	15.36	44.80	2.9
Merlot	21.76	60.16	2.9
Cabernet Sauvignon	10.24	65.28	1.3
Pinot Noir	11.52	49.92	1.0
Stanušina	26.88	60.16	1.3
Alcoholic beverages			
White brandy	1.28	3.84	0.3
Yellow brandy	1.28	3.84	2.2
Mastika	2.26	5.12	3.8

4. Conclusion

Volumetric methods for determination of SO_2 (free and total) and reducing sugars in wines and alcoholic beverage were checked. Validation parameters confirmed its accuracy and precision. These methods are fast and very easily available in every laboratory. These methods are widely applicable in wineries for control of the content of SO_2 and sugars during the wine production. The content of SO_2 was higher in the white wines compared to the red wines since white wines are easily oxidizable and therefore higher dose of SO_2 is needed for protection of oxidation. All wines were dry, containing low value of reducing sugars (< 5 g/L).

5. References

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