

VALIDATION OF A GAS CHROMATOGRAPHIC METHOD FOR METHANOL DETERMINATION

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Abstract

In this study we developed and validated a gas chromatographic method for methanol determination from alcoholic distillates. In order to determine methanol concentrations in alcoholic distillates, an aliquot of samples is analyzed by a gas chromatographic - mass spectrometric (GC-MS) method with an Agilent Technologies 7890 A Chromatograph equipped with a Zebron – Phenomenex, ZB-WAXplus column (60 m x 0.25 mm, 0.25 μ m) and Agilent Technologies 5975C inert MSD detector. The temperature program begin from 50°C (constant for 20 minutes), after that, the temperature increase with 10°C/min to 250°C, with He as carrier gas (1 mL/min) and MS detection. The temperature of the source was 230°C and of the quadrupole was 150°C. In these conditions, the method is linear in the 5 – 20000 μ g/mL range, the detection limit is 146.4 μ g/mL, the quantification limits is 443.5 μ g/mL. For precision were obtained the following results: for system precision RSD = 1.0621 %, for method precision RSD = 2.6601 %, for intermediate precision RSD = 2.3336 %. The accuracy was 95.9% in 92.4 – 97.9% range. Under these conditions, methanol was determined from alcoholic distillates with good results.

Rezumat

Lucrarea prezintă o nouă metodă de determinare a metanolului din distilate alcoolice. Probele au fost analizate prin cromatografie de gaze cuplată cu spectrometria de masă pe un cromatograf de gaze tip Agilent Technologies 7890 A echipat cu o coloană Zebron–Phenomenex, ZB-WAXplus (60 m x 0,25 mm, 0,25 μ m) și un detector Agilent Technologies 5975C inert MSD. Programul de temperatură a început la 50°C (constant 20 minute), apoi temperatura a fost crescută cu 10°C/min până la 250°C. S-a utilizat un debit de 1mL/min pentru faza mobilă (He). Detecția s-a realizat prin spectrometrie de masă. Temperatura sursei a fost de 230°C iar cea a quadrupolului de 150°C. În aceste condiții, metoda s-a dovedit liniară în domeniul 5 – 20000 μ g/mL, limita de detecție a fost de 146,4 μ g/mL, iar cea de cuantificare de 443,5 μ g/mL. S-a determinat precizia sistemului (RSD = 1,0621%), precizia metodei (RSD = 2,6601%), precizia intermediară (RSD = 2.3336%) și exactitatea (95,9% în domeniul 92,4–97,9%). În aceste condiții, metanolul a fost determinat din distilate alcoolice cu rezultate bune.

Keywords: methanol, GC-MS, validation.

Introduction

The methanolic intoxication continues to be a real problem in the entire world regarding its action on morbidity and mortality. Methanol can induce professional intoxications (in chemical, pharmaceutical, varnish and paints or detergents industry etc.) or non-professional intoxications (consuming of artisanal distillate preparations or fruit juices enzymatically cleared). The severity and complexity of methanolic simpatomatologies impose the development of new toxicological methods of analysis.

In some studies on methanol determination by gas chromatography (GC), the studied compound was analyzed directly on a medium polar or a polar column. To make the analysis as simple as possible, a polar column was used. The method is relatively rapid, efficient and has a sensibility and selectivity comparable with other methods.

Material and methods

Materials

An Agilent Technologies 7890 A Gas Chromatograph was used, equipped with an Agilent Technologies 5975C inert MSD detector, a DB 5 MS column (30 m x 0.25 mm, 0.25 μ m) and a Zebron–Phenomenex, ZB-WAXplus column (60 m x 0.25 mm, 0.25 μ m). High purity methanol, ethanol and water (HPLC grade - Merck) were used.

Chromatographic conditions

The mixtures were analysed by gas chromatography (GC), operated with temperature programming from 50°C (held for 20 minutes) to 250°C (held for 5 min) at 10°C/min, with He as carrier gas (1 mL/min). For injection we used the SPLIT mode with the split ratio of 1/50; the volume of injection was 0.1 μ L. For mass spectrometric (MS) detection the source temperature was 230°C and the quadrupole temperature was fixed at 150°C; the analysis was performed in SCAN mode, acquiring the mass spectra in the range of 15 – 400 units. The standard of methanol is prepared by dissolving 0.5 mL methanol in 9.5 mL mixture of ethanol/water (1/1) and the samples for method validation were prepared by dilution of the standard in a mixture of ethanol/water (1/1); the concentrations of methanol were in the 5–20000 μ g/mL range. The compound identification is performed by comparing the retention times and the mass spectra of the principal peak from the chromatograms with the mass spectra from Willey library.

For the quantitative determination, the method was validated, establishing the linearity, detection and quantification limits, precision and accuracy.

Results and discussion

Method development

The analysis was performed, first, on a DB 5 MS column (30 m x 0.25 mm, 0.25 μ m) but the resolution between the peak of methanol and ethanol was poor. According to the literature, we separated the methanol from ethanol on a more polar column, such as Zebron – Phenomenex, ZB-WAXplus column (60 m x 0.25 mm, 0.25 μ m). In the mentioned conditions the resolution was calculated. Therefore, the retention times (RT) and the band width (W) were recorded from the chromatogram, after analysis. The RT was 6.650 min for methanol and 7.712 min for ethanol, and, the band width is 0.049 for methanol and 0.117 for ethanol, so, the resolution is 12.795.

In order to determine the quantity of analyzed sample, in the same conditions were performed injections at different split ratios (1/10, 1/50, 1/100 and 1 / 1000). The optimum split ratio was 1/50, because at this value there were obtained the best results from peak area of ethanol and methanol.

Method validation

Linearity

Because our interest is to determine traces of methanol, from the concentration range (5 – 20000 μ g/mL), the 4.9 – 2768.5 μ g/mL range was selected. For the linearity [1-7], three sets of standard solution of methanol were prepared in the 5 – 20000 μ g/mL concentration range. From these solutions, volumes of 0.1 μ L were injected. The results are showed in table I. These results were statistical evaluated.

Table I
Experimental results for the linearity determination

No.	Methanol μ g / mL	Peak area (mAU • sec.)			
		I	II	III	Average
1	4.94375	36389	35141	35525	35685
2	9.8875	85520	85313	85337	85390
3	19.775	148405	145501	143339	145748.3
4	39.55	260743	253396	260079	258072.7
5	158.2	1071516	1073457	1077307	1074093
6	237.3	1544154	1554464	1541829	1546816
7	395.5	2676912	2676668	2681345	2678308
8	791.0	5309579	5307579	5305277	5307478

Table I (continued)

No.	Methanol $\mu\text{g} / \text{mL}$	Peak area (mAU • sec.)			
		I	II	III	Average
9	1186.5	7367555	7368638	7367709	7367967
10	1582.0	9635432	9647369	9639818	9640873
11	1977.5	13353055	13181091	13155824	13229990
12	2373.0	15202307	14819460	15453882	15158550
13	2768.5	18469310	18397688	18204241	18357080
14	3164.0	19481271	19353438	19616579	19483763
15	3559.5	20842477	20783003	20137936	20587805
16	3955.0	21347315	21786692	21788479	21640829
17	5932.5	29777327	29593805	29299185	29556772
18	7910.0	39816755	40830519	39741944	40129739
19	9887.5	48459719	49251958	49784256	49165311
20	11865.0	58540381	58547273	58416812	58501489
21	19775.0	94684620	98666792	99029409	97460274

For two different concentration ranges (4.9–2768.5 $\mu\text{g}/\text{mL}$ and 2768.5–19775 $\mu\text{g}/\text{mL}$ respectively) the calibration curves are showed in figure 1.

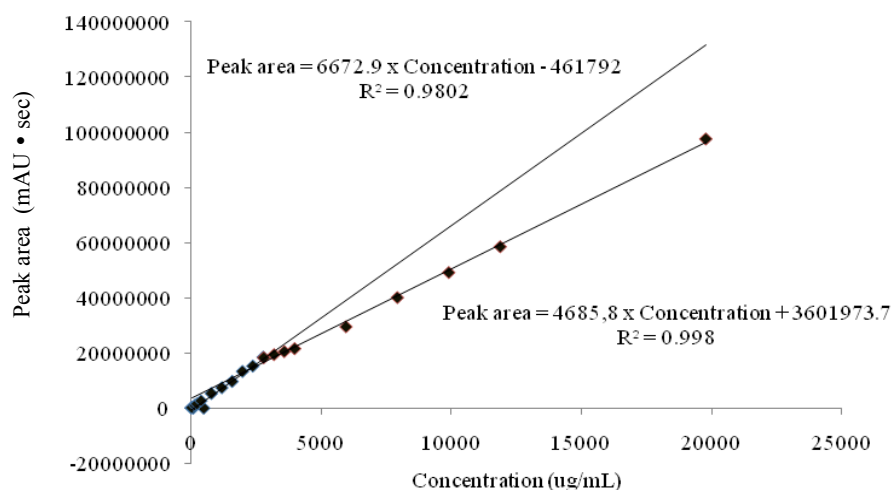


Figure 1
Calibration curves

The equations of calibration curves are: peak area = 6500.4 x amount ($\mu\text{g}/\text{mL}$) - 11679.5 ($r = 0.9991$; standard error (SE) = 288313.9) for 4.9–2768.5 $\mu\text{g}/\text{mL}$ and peak area = 4685.8 x amount ($\mu\text{g}/\text{mL}$) + 3601973.7 ($r = 0.9990$, standard error = 1243540.6) for 2768.5 – 19775 $\mu\text{g}/\text{mL}$.

From the statistical evaluation the detection limit (LD) and quantification limit (LQ) were calculated using the following formulas [1-7]:

$$LD = \frac{3.3 \cdot SE}{slope} = \frac{3.3 \cdot 288313.9}{6500.4} = 146.4 \mu\text{g} / \text{mL}$$

$$LQ = \frac{10 \cdot SE}{slope} = \frac{10 \cdot 288313.9}{6500.4} = 443.5 \mu\text{g} / \text{mL}$$

where SE is the standard error of regression.

Precision

For precision determination it was analyzed the system precision for a number of 5 injections at the same concentration of 5932 $\mu\text{g}/\text{mL}$. The experimental results are showed in table II.

Table II
System precision

No.	Peak area (mAU • sec)
1	29181942
2	29366891
3	29827788
4	29884137
5	29351896
Average	29522531
SD	313560.9
RSD	1.0621 %

SD – standard deviation, RSD – relative standard deviation

The precision of the method [1-7] was determined at 3 concentration levels. The results are showed in table III.

Table III
Method precision for methanol determination by GC-MS

Method precision for melatonin determination by GC-MS				
No.	Theoretical concentration ($\mu\text{g} / \text{mL}$)	Peak area (mAU • sec)	Calculated concentration ($\mu\text{g} / \text{mL}$)	%
1	3559.5	19987590	3496.9	98.2
2		19941825	3487.1	98.0
3		20100442	3521.0	98.9
4	5932.5	29254146	5474.4	92.3
5		29754916	5581.3	94.1
6		29672735	5563.8	93.8
7	9887.5	49055098	9700.2	98.0
8		49372967	9768.0	98.7
9		49365475	9766.4	98.7
Statistical data			Average	96.7 %
			SD	2.5735
			RSD	2.6601 %

The intermediate precision [6-7] was determined at 3 concentration levels. The results are showed in table IV.

Table IV
Intermediate precision for methanol determination by GC-MS

No.	Theoretical concentration ($\mu\text{g} / \text{mL}$)	Peak area ($\text{mAU} \cdot \text{sec}$)	Calculated concentration ($\mu\text{g} / \text{mL}$)	%
1	3559.5	19970889	3493.3	98.1
2		20112781	3523.6	99.0
3		20071228	3514.7	98.7
4	5932.5	29390148	5503.5	92.8
5		29763692	5583.2	94.1
6		29757259	5581.8	94.1
7	9887.5	48931933	9673.9	97.7
8		48513789	9584.7	96.8
9		48538743	9590.0	96.9
Statistical data			Average	96.5 %
			SD	2.2511
			RSD	2.3336 %

Over the entire range, the SD is **2.2511** and RSD is **2.3336 %**.

Accuracy

The accuracy of the method [1-7] was determined at 3 concentration levels. The results are showed in table V.

Table V
Accuracy for methanol determination by GC-MS

No.	Theoretical concentration (µg / mL)	Peak area (mAU • sec)	Calculated concentration (µg / mL)	Recovery (%)
1	3559.5	19922628	3483.0	97.9
2		19934729	3485.6	97.9
3		19925152	3483.5	97.9
4	5932.5	29292047	5482.5	92.4
5		29298045	5483.8	92.4
6		29489809	5524.7	93.1
7	9887.5	48660751	9616.0	97.2
8		48587881	9600.5	97.0
9		48858334	9658.2	97.6
Statistical data			Average	95.9 %
			Min	92.4 %
			Max	97.9 %

Over the entire concentration range, the obtained values are: mean recovery = 95.9% (Min = 92.4 %, Max = 97.9%).

Conclusions

The linearity of the assay method for methanol in the range 5–20000 µg/mL is excellent since the correlation coefficients, r , were 0.9991 and 0.9990. The detection limit (LD) is 146.4 µg/mL and the quantification limit (LQ) is 443.5 µg/mL.

The GC – MS method for the determination of methanol is precise.

The system precision has an RSD value of 1.0621%, the method precision has a RSD value of 2.6601% and the intermediate precision has a RSD value of 2.3336%. The RSD value limit is: $RSD \leq 10.0\%$.

The GC – MS method for the determination of methanol is accurate. The calculated values for errors (%) are within limits. The mean recovery found for methanol is 95.9% with a recovery range of 92.4 – 97.9%.

The method was applied with good results for methanol determination from alcoholic distillates.

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