

GUIDE

Calculation of Compound Concentrations in Standard Solutions and Calibration Factors

*during preparation the solutions in the accordance
of the new OIV method OENO-SCMA 24-756 Et5_2026,
using contained ethanol as a reference standard (EIS method)*



Version Calc_SS-EIS-1500

2026

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Preparation of Standard Solutions

Equipment and Laboratory Glassware

- Analytical balance, capable of measuring to four decimal places
- Pycnometer or alcoholometer
- Thermometer
- 1-liter volumetric flask with ground glass stopper
- 100-mililiter volumetric flasks with ground glass stopper
- 500-militer graduated cylinder
- Pipette dispensers with variable volume from 100 to 1000 mL

Reagents, Materials and Conditions

Use only reagents of a purity greater than 97.0 %, with certificate of purity, free from other volatile compounds at test dilution and only water of at least grade 3 as defined in ISO 3696. Acetal and acetaldehyde must be stored in the dark at $\leq 5.0\text{ }^{\circ}\text{C}$, all other reagents should be stored according to the supplier's instructions.

- Ethanol absolute (CAS 64-17-5)
- Methanol (CAS 67-56-1)
- Propan-1-ol (CAS 71-23-8)
- 2-Methylpropan-1-ol (CAS 78-33-1)
- 2-Methylbutan-1-ol (CAS 137-32-6)
- 3-Methylbutan-1-ol (CAS 123-51-3)
- Ethyl acetate (CAS 141-78-6)
- Butan-1-ol (CAS 71-36-3)
- Butan-2-ol (CAS 78-92-2)
- Acetaldehyde (CAS 75-07-0)
- Acetal (CAS 105-57-7)

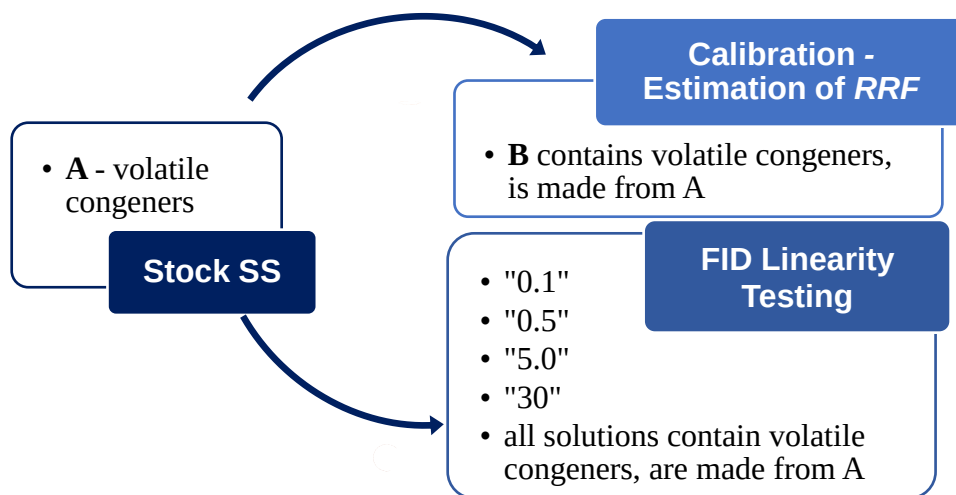
Preparation of 40 % v/v Ethanol Solution in Water (WES)

To prepare a 400 mL/L ethanol solution in water (WES), measure 400 mL of absolute ethanol using a graduated cylinder or measuring flask of the appropriate volume and pour it into a 1-liter volumetric flask. Bring the solution volume up to the 1-liter mark by adding distilled water and mix thoroughly. After preparing the WES, accurately determine the volumetric ethanol content of the prepared solution (ABV, %) and the density of the WES (ρ_{WES} , g/L) using standard methods.

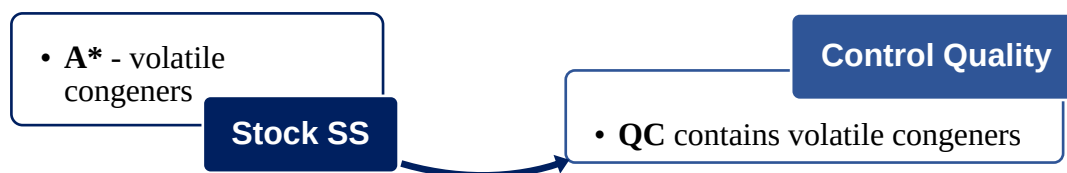
Standard Solutions

To work according to the methods, the following solutions of volatile compounds and internal standard are prepared:

- stock standard solutions (A, A*) – concentrated solutions not subjected to chromatography,
- 1 standard solution B for chromatograph calibration and establishing the *RRF*,
- standard solutions ("0.1", "0.5", "5.0", "30") for the control of the linearity of the flame ionisation detector (FID) response,
- 1 standard solution QC for checking the quality of the calibration.



In order to maintain analytical continuity and an effective quality control, prepare a quality control standard QC using the previously prepared standard A or, preferably, prepare a control standard as indicated for standard A*, but using different batches or suppliers of reagents.



Preparation and storage of standard solutions: the calibration ranges should be adapted to the nature of the different types of products analysed by each laboratory. All standard solutions must be stored at ≤ 5.0 °C and be prepared freshly on a monthly basis. Masses of components and solutions should be recorded to the nearest 0.1 mg.

Preparation of Standard Solution A

Pipette the following reagents (Table 1) into a 100 mL volumetric flask, containing approximately 60.0 mL of WES to minimise component evaporation, make up to volume with WES and mix thoroughly. Record the weight of the flask, each component added and the total final weight of contents in the Table 1. Approximate mass concentration of reagents in standard solution A is about 5,000 g/100 L AA.

Table 1

Reagent i	Reagent purity P_i^i , %	Volume, mL	Approximate mass, mg	The exact value of the mass m_A^i and m_A^{WES} , mg
Methanol		2.0	2000.0	
Propan-1-ol		2.0	2000.0	
2-methylpropan-1-ol		2.0	2000.0	
2-methylbutan-1-ol		2.0	2000.0	
3-methylbutan-1-ol		2.0	2000.0	
Ethyl acetate		2.0	2000.0	
Butan-1-ol		2.0	2000.0	
Butan-2-ol		2.0	2000.0	
Acetaldehyde		2.0	2000.0	
Acetal		2.0	2000.0	
WES		60.0 20.0	60,000 20,000	
Mass of the flask, mg				
Total final weight of contents, mg				

Preparation of Standard Solution B

Pipette 5 mL standard solution A into a 100 mL volumetric flask containing approximately 60.0 mL of WES, make up to volume with WES and mix thoroughly. Record the weight of the flask, each component added and the total final weight of contents in the Table 2. Approximate concentration of reagents in solution B is about 250.0 g/100 L AA.

Table 2

Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	5.0	5000.0		m_B^A
WES	60.0 35.0	60,000 35,000		m_B^{WES}
Mass of the flask, mg				
Total final weight of contents, mg				

Preparation of Standard Solutions “0.1”, “0.5”, “5.0”, “30” used to check the Linearity of FID Response

Into separate 100 mL volumetric flasks, containing approximately 60.0 mL of WES, pipette 0.1, 0.5, 5.0, 30.0 mL standard solution A into and make up to volume with ethanol solution (WES) and mix thoroughly (Table 3). Record the weight of the flask, each component added and the total final weight of contents in the Table 3. Approximate mass concentration values of reagents in these solutions are about 5.0, 25.0, 250, 1500 g/100 L AA.

Table 3

Standard Solution “0.1” - approximate concentration of volatile components 5 g/100 L AA				
Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	0.1	100.0		$m_{0.1}^A$
WES	60.0 39.9	60,000 39,900		$m_{0.1}^{WES}$
Mass of the flask, mg				
Total final weight of contents, mg				

Standard Solution “0.5” - approximate concentration of volatile components 25 g/100 L AA				
Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	0.5	500.0		$m_{0.5}^A$
WES	60.0 39.5	60,000 39,500		$m_{0.5}^{WES}$

Mass of the flask, mg	
Total final weight of contents, mg	

Standard Solution “5” - approximate concentration of volatile components 250 g/100 L AA				
Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	5.0	5000.0		m_5^A
WES	60.0 35.0	60,000 35,000		m_5^{WES}

Mass of the flask, mg	
Total final weight of contents, mg	

Standard Solution “30” - approximate concentration of volatile components 1500 g/100 L AA				
Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	30.0	30,000		m_{30}^A
WES	60.0 10.0	60,000 10,000		m_{30}^{WES}

Mass of the flask, mg	
Total final weight of contents, mg	

Preparation of Standard Solution QC

In order to maintain analytical continuity and an effective quality control, prepare a quality control standard using the previously prepared standard A or, preferably, prepare a control standard as indicated for standard A*, but using different batches or suppliers of reagents.

Pipette 4.5 mL solution A or A* into a 100 mL volumetric flask containing approximately 60.0 mL of WES, make up to volume with WES and mix thoroughly. Record the weight of the flask, each component added and the total final weight of contents in the Table 4. Approximate concentration of reagents in solution QC is about 225.0 g/100 L AA.

Table 4

Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	4.5	4500.0		m_{QC}^A
WES	60.0 35.5	60,000 35,500		m_{QC}^{WES}
Mass of the flask, mg				
Total final weight of contents, mg				

Approximate Concentrations

Calculation of Approximate Values of Volatile Compound Concentration in Standard Solutions

To calculate the "certified" values of component concentrations C^i in standard solutions, it is necessary to accurately determine the masses of the substances using an analytical balance and record them in the tables 1-4. *You can use the free web-application "Calculator of volatile compound concentrations in standard solutions prepared in the accordance of OIV method" for automatic calculation the mass concentration values of volatile compounds in standard solutions and RRF values.*

The new method of internal standard with using ethanol as reference substance (method EIS) differ on the methods of traditional internal standard (methods TIS). In the EIS method, the concentrations of substances in standard solutions are calculated in units of g/100 L of anhydrous alcohol (g/100 L AA).

Zero approximation (0_ap). In the zero approximation (0_ap), the calculations assume that ethanol reagent and WES consist only of water and ethanol, and that other compounds such as volatile congeners are absent, as are their fractions.

The mass part W_i^i of the main volatile compound i in the initial reagent in units of mg/mg is calculated using the formula:

$$W_i^i = \frac{P_i^i}{100 \%}, \quad (1)$$

where P_i^i is the purity of the main substance i in the initial reagent of the volatile compound i being determined, specified in the manufacturer's data sheet for the corresponding volatile compound i , in % by mass.

In the EIS method, the mass part W_{WES}^{Eth} of ethyl alcohol in WES in mg/mg of the solution is calculated using the formula:

$$W_{WES}^{Eth} = \frac{\rho_{Eth}}{\rho_{WES}} \cdot \frac{ABV}{100}, \quad (2)$$

where ABV is the volume content of ethyl alcohol in the WES, %; ρ_{WES} is the density of WES,

in g/L under normal conditions; ρ_{Eth} is the density of anhydrous alcohol at temperature of 20 °C, $\rho_{Eth} = 789.270$ g/L.

The mass part W_A^{Eth} of ethyl alcohol in solution A in mg/mg of the solution is calculated using the formula:

$$W_A^{Eth} = \frac{m_A^{WES}}{m_A^{WES} + \sum_i m_A^i} \cdot W_{WES}^{Eth}, \quad (3)$$

where m_A^i – the mass of initial substance of the volatile compound i being determined, introduced into solution A during its preparation, mg; m_A^{WES} – the mass of WES added into solution A during its preparation, mg.

The mass part of the volatile component i in solution A in mg/mg of the solution, to a n -order approximation, is calculated using the formula:

$$W_A^i(n_ap) = \frac{W_i^i \cdot m_A^i + W_{WES}^i(n_ap) \cdot m_A^{WES}}{m_A^{WES} + \sum_i m_A^i}, \quad (4)$$

where n is the number of approximation order; $W_{WES}^i(n_ap)$ – the mass part of volatile compound i in WES in n -order approximation, mg/mg of the mixture.

In zero approximation, ethanol reagent and WES doesn't contain volatile congeners $W_{WES}^i(0_ap) = 0$ mg/mg and therefore:

$$W_A^i(0_ap) = \frac{W_i^i \cdot m_A^i}{m_A^{WES} + \sum_i m_A^i}. \quad (5)$$

The mass concentration $C_B^i(n_ap)$ of compound i in standard solution B in units of g/100 L AA in n -order approximation is calculated using the formula:

$$C_B^i(n_ap) = \frac{W_A^i(n_ap) \cdot m_B^A + W_{WES}^i(n_ap) \cdot m_B^{WES}}{W_A^{Eth} \cdot m_B^A + W_{WES}^{Eth} \cdot m_B^{WES}} \cdot \rho_{Eth}, \quad (6)$$

where m_B^A is the mass of solution A added into solution B during its preparation, mg;

m_B^{WES} – the mass of WES added into solution B during its preparation, mg; ρ_{Eth} is the density of anhydrous alcohol at temperature of 20 °C, $\rho_{Eth} = 78927$ g/100 L.

In zero approximation $W_{WES}^i(0_ap) = 0$ mg/mg and therefore:

$$C_B^i(0_ap) = \frac{W_i^i \cdot m_A^i \cdot m_B^A}{\left(m_A^{WES} + \sum_i m_A^i\right) \cdot (W_A^{Eth} \cdot m_B^A + W_{WES}^{Eth} \cdot m_B^{WES})} \cdot \rho_{Eth}, \quad (7)$$

where $C_B^i(0_ap)$ is the mass concentration of compound i in standard solution B in zero approximation, g/100 L AA; $\rho_{Eth} = 78927$ g/100 L.

As zero approximation, the compound concentration values in solution A $C_A^i(0_ap)$ and other standard solutions (SS) $C_{SS}^i(0_ap)$ in g/100 L AA can be calculated using the formulas:

$$C_A^i(0_ap) = \frac{W_i^i \cdot m_A^i}{W_{WES}^{Eth} \cdot m_A^{WES}} \cdot \rho_{Eth}, \quad (8)$$

$$C_{SS}^i(0_ap) = \frac{W_i^i \cdot m_A^i \cdot m_{SS}^A}{\left(m_A^{WES} + \sum_i m_A^i\right) \cdot (W_A^{Eth} \cdot m_{SS}^A + W_{WES}^{Eth} \cdot m_{SS}^{WES})} \cdot \rho_{Eth}, \quad (9)$$

where m_{SS}^A is the mass of solution A added into solution SS during its preparation, mg; m_{SS}^{WES} is the mass of WES added into solution SS during its preparation, mg; ρ_{Eth} is the density of anhydrous alcohol at temperature of 20 °C, $\rho_{Eth} = 78927$ g/100 L; SS – standard solutions such as QC, “0.1”, “0.5”, “5.0”, “30”.

The first and second approximations are calculations taking into account the impurity content in the WES. The first and second approximations allow to obtain the most accurate values for the concentration of volatile compounds and values of *RRF*.

Initially, the compound concentrations in solution B are calculated to a zero approximation. Then, the WES solution and standard solution B are chromatographed, the peaks of volatile compounds and ethanol are integrated and obtained values of peak area are recorded. After that, calculations are performed to the first and second approximations. This allows for more accurate values for component concentrations in standard solutions and values of *RRF*.

Exact concentrations and RRF

Equipment for Gas Chromatography (GC) and GC Conditions

- A temperature programmed gas chromatograph equipped with a flame ionisation detector (FID), S/SL injector and integrator or other data handling system capable of measuring peak areas
- Gas chromatographic column(s), capable of separating the analytes such that the minimum resolution between the individual components (other than 2-methylbutan-1-ol and 3-methylbutan-1-ol) is, as a guide, at least 1.3, if a simple visual examination of the chromatogram is not sufficient
- Vial crimper, chromatographic vials, septa and caps for them

NOTE – The following columns and GC conditions are given as suitable examples (Table 5):

Table 5

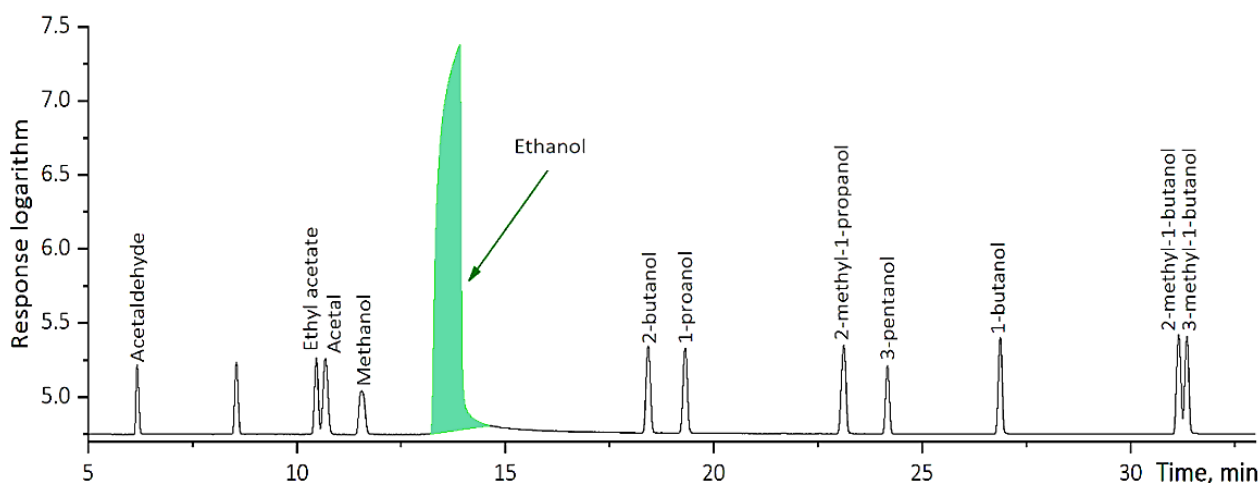
1	A retention gap 1 m x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 0.32 mm i.d. 0.2 µm film thickness (stabilised polyethylene glycol) followed by a Carbowax 400 column 50 m x 0.32 mm i.d. 0.2 µm film thickness. (Columns are connected using press-fit connectors.)
	Carrier gas and pressure: Helium (135 kPa)
	Column temperature: 35 °C for 17 min., 35 °C to 70 °C at 12 °C/min., hold at 70 °C for 25 min.
	Injector temperature: 150 °C
	Detector temperature: 250 °C
	Injection volume: 1 µL, split 20 to 100:1
2	A retention gap 1 m x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 0.32 mm i.d. 0.2 µm film thickness (stabilised polyethylene glycol). (Retention gap is connected using a press-fit connector.)
	Carrier gas and pressure: Helium (65 kPa)
	Column temperature: 35 °C for 10 min., 35 °C to 110 °C at 5 °C/min., 110 °C to 190 °C at 30 °C/min., hold at 190 °C for 2 min.
	Injector temperature: 260 °C
	Detector temperature: 300 °C
	Injection volume: 1 µL, split 55:1

Blank test, Chromatography of WES

Acquire 2-3 chromatograms of 40% v/v ethanol solution (WES). Measure peak areas for volatile compounds and ethanol and record the peak area values. When integrating the ethanol peak, it is recommended to use a valley-to-valley baseline.

Preliminary test, Chromatography of Standard Solution B

Inject standard solution B into chromatograph to ensure that all of the analytes are separated with a minimum resolution of 1.3 (except 2-methyl-1-butanol and 3-methyl-1-butanol). Measure peak areas for volatile compounds and ethanol and record the peak area values. When integrating the ethanol peak, it is recommended to use a valley-to-valley baseline. The calibration solution B is chromatographed at least twice.



Calculation of Calibration Coefficients RRF and Certified Values of Volatile Compound Concentration in Standard Solutions

Calibration characteristics are named relative response factors (*RRF*) expressing the dependence of the peak area ratio of volatile compound *i* and internal standard (ethyl alcohol or traditional IS) on the concentration ratios of the volatile compound *i* and internal standard (ethyl alcohol or traditional IS) are established using standard solution B.

The calibration coefficients are calculated as zero approximation using the formula:

$$RRF_i^{Eth}(0_ap) = \frac{C_B^i(0_ap)}{\rho_{Eth}} \cdot \frac{\sum_{j=1}^{N_B} (A_{B,j}^i / A_{B,j}^{Eth})}{\sum_{j=1}^{N_B} (A_{B,j}^i / A_{B,j}^{Eth})^2}, \quad (10)$$

where $RRF_i^{Eth}(0_ap)$ is the relative response factor of the compound i to ethyl alcohol in zero approximation; $C_B^i(0_ap)$ is the value of concentration of compound i in standard solution B in zero approximation (formula 7), g/100 L AA; $A_{B,j}^i$ is the detector response value to the compound i , obtained as a result of the measurement j of solution B, in peak area units; $A_{B,j}^{Eth}$ is the detector response to ethyl alcohol obtained as a result of the measurement j of solution B, units of peak area; N_B is the number of measurements of solution B ($N_B \geq 2$).

The first and second approximations are calculations taking into account the impurity content in the WES (if it is necessary) and allow to obtain the most accurate values for the concentration of volatile compounds and values of RRF .

In the first approximation, the mass concentration $C_{WES}^i(1_ap)$ of the compound i in the WES in g/100 L AA and the mass fraction $W_{WES}^i(1_ap)$ of the compound i in the WES in mg/mg of solution are calculated using the formulas:

$$C_{WES}^i(1_ap) = RRF_i^{Eth}(0_ap) \cdot \rho_{Eth} \cdot \frac{1}{N_{WES}} \sum_{j=1}^{N_{WES}} \frac{A_{WES,j}^i}{A_{WES,j}^{Eth}}, \quad (11)$$

$$W_{WES}^i(1_ap) = \frac{C_{WES}^i(1_ap) \cdot ABV}{\rho_{WES} \cdot 100}, \quad (12)$$

where $A_{WES,j}^i$ is the detector response value to the compound i , obtained as a result of the measurement j of WES, in peak area units; $A_{WES,j}^{Eth}$ is the detector response to ethyl alcohol obtained as a result of the measurement j of WES, units of peak area; N_{WES} is the number of measurements of WES ($N_{WES} \geq 2$); $\rho_{Eth} = 78927$ g/100 L; ABV is the volume content of ethyl alcohol in the WES, %; ρ_{WES} is the density of WES in units of g/100 L, $\rho_{WES} [g/100 L] = \rho_{WES} [g/L] \cdot 100$.

In the first approximation, the mass part $W_A^i(1_ap)$ of the volatile component i in solution A

in mg/mg of the solution is calculated using the formula:

$$W_A^i(1_ap) = \frac{W_i^i \cdot m_A^i + W_{WES}^i(1_ap) \cdot m_A^{WES}}{m_A^{WES} + \sum_i m_A^i}. \quad (4'')$$

In the first approximation, the mass concentration $C_B^i(1_ap)$ of compound i in standard solution B in units of g/100 L AA is calculated using the formula:

$$C_B^i(1_ap) = \frac{W_A^i(1_ap) \cdot m_B^A + W_{WES}^i(1_ap) \cdot m_B^{WES}}{W_A^{Eth} \cdot m_B^A + W_{WES}^{Eth} \cdot m_B^{WES}} \cdot \rho_{Eth}. \quad (6'')$$

The calibration coefficients $RRF_i^{Eth}(1_ap)$ are calculated as first approximation using the formula:

$$RRF_i^{Eth}(1_ap) = \frac{C_B^i(1_ap)}{\rho_{Eth}} \cdot \frac{\sum_{j=1}^{N_B} (A_{B,j}^i / A_{B,j}^{Eth})}{\sum_{j=1}^{N_B} (A_{B,j}^i / A_{B,j}^{Eth})^2}. \quad (10'')$$

In the second approximation, the mass concentration $C_{WES}^i(2_ap)$ of the compound i in the WES in g/100 L AA and the mass fraction $W_{WES}^i(2_ap)$ of the compound i in the WES in mg/mg of solution are calculated using the formulas:

$$C_{WES}^i(2_ap) = RRF_i^{Eth}(1_ap) \cdot \rho_{Eth} \cdot \frac{1}{N_{WES}} \sum_{j=1}^{N_{WES}} \frac{A_{WES,j}^i}{A_{WES,j}^{Eth}}, \quad (11'')$$

$$W_{WES}^i(2_ap) = \frac{C_{WES}^i(2_ap) \cdot ABV}{\rho_{WES} \cdot 100}. \quad (12'')$$

In the second approximation, the mass part $W_A^i(2_ap)$ of the volatile component i in solution A in mg/mg of the solution is calculated using the formula:

$$W_A^i(2_ap) = \frac{W_i^i \cdot m_A^i + W_{WES}^i(2_ap) \cdot m_A^{WES}}{m_A^{WES} + \sum_i m_A^i}. \quad (4''')$$

In the second approximation, the mass concentration $C_B^i(2_ap)$ of compound i in standard solution B in units of g/100 L AA is calculated using the formula:

$$C_B^i(2_ap) = \frac{W_A^i(2_ap) \cdot m_B^A + W_{WES}^i(2_ap) \cdot m_B^{WES}}{W_A^{Eth} \cdot m_B^A + W_{WES}^{Eth} \cdot m_B^{WES}} \cdot \rho_{Eth}. \quad (6'')$$

The most exact concentration values of volatile compounds in solution A $C_A^i(2_ap)$ and other standard solutions SS in units of g/100 L AA $C_{SS}^i(2_ap)$ are calculated as the second approximation using the formulas:

$$C_A^i(2_ap) = \frac{W_i^i \cdot m_A^i + W_{WES}^i(2_ap) \cdot m_A^{WES}}{W_{WES}^{Eth} \cdot m_A^{WES}} \cdot \rho_{Eth}, \quad (13)$$

$$C_{SS}^i(2_ap) = \frac{W_A^i(2_ap) \cdot m_{SS}^A + W_{WES}^i(2_ap) \cdot m_{SS}^{WES}}{W_A^{Eth} \cdot m_{SS}^A + W_{WES}^{Eth} \cdot m_{SS}^{WES}} \cdot \rho_{Eth}. \quad (14)$$

The calibration coefficients $RRF_i^{Eth}(2_ap)$ are calculated as second approximation using the formula:

$$RRF_i^{Eth}(2_ap) = \frac{C_B^i(2_ap)}{\rho_{Eth}} \cdot \frac{\sum_{j=1}^{N_B} (A_{B,j}^i / A_{B,j}^{Eth})}{\sum_{j=1}^{N_B} (A_{B,j}^i / A_{B,j}^{Eth})^2}. \quad (10'')$$

The Testing of FID Response Linearity

The calibration should be checked using the following procedure. Inject three times standard solutions “0.1”, “0.5”, “5.0”, “30” and WES for the testing of FID response linearity into chromatograph. Measure peak areas for volatile compounds and internal standard (ethanol or traditional IS) and record the peak area values. When integrating the ethanol peak, it is recommended to use a valley-to-valley baseline.

Plot the following relationships using obtained data for SS “0.1”, “0.5”, “5.0”, “30” and WES:

- x-axis: certified concentration (g/100 L AA) of the volatile compound i in linearity standard solution / density of anhydrous ethanol (78927 g/100 L) C_{SS}^i / ρ_{Eth} ;
- y-axis: the detector response to the volatile compound i (peak area), obtained during

measurement of linearity standard solution / detector response to the ethanol (peak area), obtained during measurement of linearity standard solution $A_{SS,j}^i / A_{SS,j}^{Eth}$.

Concentration of ethanol in solutions in units of g/100 L AA is equal to density of anhydrous ethanol 78927 g/100 L, therefore $C_{SS}^{Eth} / \rho_{Eth} = 1$.

A linear plot should be obtained, with a correlation coefficient of at least 0.99.

The linearity of the FID response is verified by measuring all standard linearity solutions at least every six months, as well as during column replacement and after chromatograph repair. When verifying the linearity of the detector response, it is recommended to use linear regression without an intercept. The Pearson linear correlation coefficient or the R^2 determination coefficient is established using the gas chromatograph software.