The Creation Of The Calibration Methods And The Analysis Of Alcoholic Drinks Using 3-Pentanol Or Ethanol As Internal Standards (ISTD)

in Agilent ChemStation B.04.03

Contents

Determination of the quantitative content of volatile compounds in alcoholic drinks when testing using the “Traditional ISTD” and “Ethanol ISTD” methods includes the following stages.

1. Registration of chromatograms of standard samples. The measurement of each standard samples is repeated 3 times.

2. Calculation of calibration characteristics such as relative response factors using the one of the standard solution (SS_{calibr}). The creation of calibration methods “Traditional ISTD” and “Ethanol ISTD”.

3. Uncertainty analysis and linearity control using the set of the standard solution (SS_j, $j \geq 3$) excluding the calibration standard solution (SS_{calibr}).

4. Registration of chromatograms of the alcoholic drink samples (2 repeated measurements).

5. The application of calibration methods “Traditional ISTD” and “Ethanol ISTD” to chromatograms of the alcoholic drink samples. Calculation of average values and formation of final reports.

Abbreviation and Definitions of Symbols

AA – absolute alcohol;

$A_{i,j,k}$ – the value of detector response on volatile compound *i* obtained in result of the measurement *k* of SS_j;

$A_{\text{ethanol},j,k}$ – the value of detector response on ethanol obtained in result of the measurement *k* of SS_j;

$A_{\text{3-pentanol},j,k}$ – the value of detector response on 3-pentanol obtained in result of the measurement *k* of SS_j;

$bias_{i,j},\%$ – accuracy calculated for the compound *i* measured concentration in SS_j;

$\tilde{C}_{i,j}^{certified}$ – the certified value of compound *i* concentration in SS_j (µg/g);

$C_{i,j}^{certified}$ – the certified value of compound *i* concentration in SS_j (mg/L AA);

$\tilde{C}_{\text{3-pentanol},j}^{certified}$ – the certified value of 3-pentanol concentration in SS_j (µg/g);

$\tilde{C}_{i,j,k}^{measured}$ – the value of compound *i* concentration calculated on the base of the measurement *k* of SS_j (µg/g);

$C_{i,j,k}^{measured}$ – the value of compound *i* concentration calculated on the base of the measurement *k* of SS_j (mg/L AA) ;

$\left\langle \tilde{C} \right\rangle_{i,\text{calibr}}^{measured}$ – the average value of measured concentration (µg/g) in the case of 3-pentanol as ISTD;

$\left\langle C \right\rangle_{i,\text{calibr}}^{measured}$ – the average value of measured concentration (mg/L AA) when ethanol used as ISTD;

index *i* – the number of volatile compound (*i* = 1, …, 13);

index *j* – the number of concentration level of SS_j that used for linearity control;

index *k* – the number of repeat measurement of SS_j;

index "calibr" – marked the concentration level of calibration SS_{calibr};

ISTD – internal standard;

M – the total number of repeat measurements of SS_j;

N – the total number of the concentration levels;

R_i^2 – coefficient of determination for compound *i*;

$RRF_i^{\text{3-pentanol}}$ – relative response factor when 3-pentanol using as ISTD (X - axis Rel.area, Y - axis Rel.amount);

RRF_i^{ethanol} – relative response factor when ethanol using as ISTD (X - axis Rel.area, Y - axis Rel.amount);

$RSD_i,\%$ – relative standard deviation calculated for the compound *i*;

SD_i – standard deviation calculated for the compound *i*;

SS_j – standard solution at the concentration level *j*;

$rRF_i^{\text{3-pentanol}}$ – relative response factor when 3-pentanol using as ISTD (X - axis Rel.amount, Y - axis Rel.area);

rRF_i^{ethanol} – relative response factor when ethanol using as ISTD (X - axis Rel.amount, Y - axis Rel.area);

X (axis Rel.amount) – $\tilde{C}_{i,j}^{certified} / \tilde{C}_{\text{3-pentanol},j}^{certified}$ (if 3-pentanol is ISTD) or $C_{i,j}^{certified} / \rho_{\text{ethanol}}$ (if ethanol is ISTD);

Y (axis Rel.area) – $A_{i,j,k} / A_{\text{3-pentanol},j,k}$ (if 3-pentanol is ISTD) or $A_{i,j,k} / A_{\text{ethanol},j,k}$ (if ethanol is ISTD);

ρ_{ethanol} – the density of ethanol at temperature 20 °C (789270 mg/L).

1. The creation of calibration methods

1.1. The synchronic creation of calibration methods

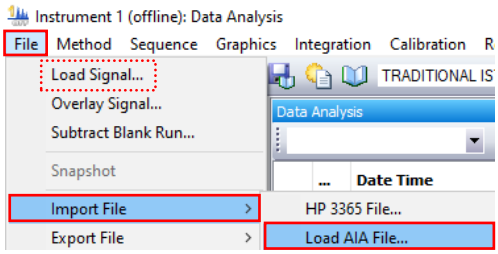
1.1.1. Acquire the chromatograms of standard solution for calibration (SS_{calibr}).

1.1.2. Click “Method” in the main menu and press “Load Method…” in the list.

1.1.3. Select any method and then save the method with new name: “Traditional ISTD” in the case of the using 3-pentanol as internal standard and “Ethanol ISTD” in the case of the using ethanol as internal standard.

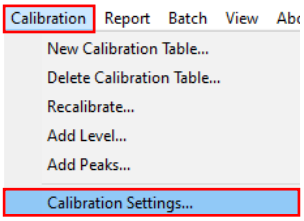
1.1.4. Add “Comment for audit trail”, for example, “3-pentanol ISTD” or “Ethanol ISTD”.

1.1.5. Click “File” in the main menu and press “Load Signal…” in the list for native data of ChemStation. But if the files of measured chromatograms have format *.cdf, then press “Import File” and “AIA File…”. Open the file of the first chromatogram of SS_{calibr} (SS 1-1).

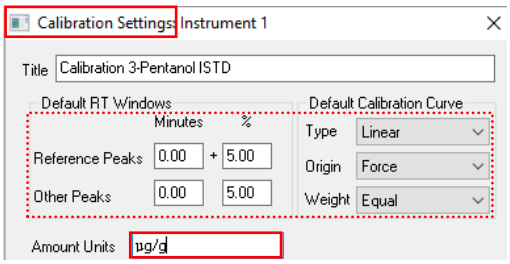


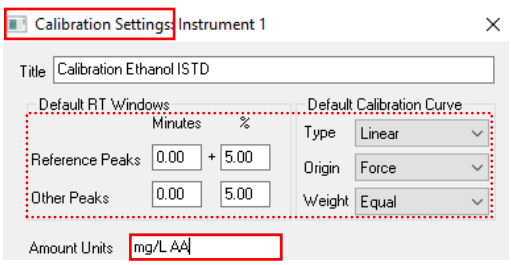
1.1.6. Set and integrate the peaks of the volatiles compounds in the chromatogram.

1.1.7. Click “Calibration” in the main menu and press “Calibration Setting…” in the list.



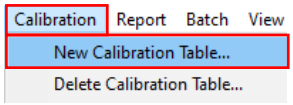
1.1.8. Fill the input lines in dialog window “Calibration Setting”. Set “Type” (Lineal), “Origin” (Force), “Weight” (Equal) and other parameters as shown in the screenshot. Fill in the cell “Amount Units”: “µg/g” in the case of “Traditional ISTD” project and “mg/L AA” in the case of “Ethanol ISTD” project. Press “OK” button.

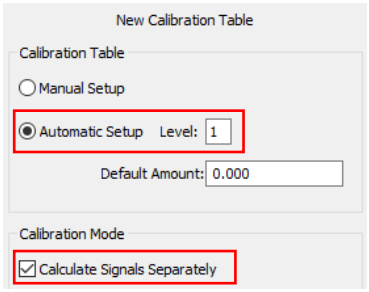




The units of concentration: µg/g in “Traditional ISTD” project and mg/L AA in “Ethanol ISTD” project

1.1.9. Press “New calibration table” in “Calibration”. Set “Level”: 1, “Calibration Mode” – “Calculate Signals Separately” as shown in the screenshot. Press “OK” button.





1.1.10. Input the names of the compounds in “Compound” column and the certified values of compound concentration in “Amt[µg/g]” or “Amt[mg/L AA]” column. It will be named these concentration values of compound i (i = 1, …, 13) in SS_{calibr} as $\tilde{C}_{i,calibr}^{certified}$ (µg/g) in the case of 3-pentanol as ISTD and $C_{i,calibr}^{certified}$ (mg/L AA) in the case of ethanol as ISTD. Concentration of ethanol in mg/L AA is equal to ethanol density ($\rho_{ethanol}$ = 789270 mg/L).

Calibration Table

Enter | Delete | Insert... | Print | OK

#	RT	Compound	ISTD	Lvl	Amt[µg/g]
1	6.2	acetaldehyde	No	1	180.68
2	8.6	methyl acetate	No	1	287.96
3	10.5	ethyl acetate	No	1	260.11
4	10.7	acetal	No	1	233.31
5	11.6	methanol	No	1	203.11
6	13.9	ethanol	No	1	333000.00
7	18.4	2-butanol	No	1	210.07
8	19.3	n-propanol	No	1	204.23
9	23.1	2-methyl-1-propanol	No	1	200.14
10	24.2	3-pentanol	Ye / No / Yes	1	226.16
11	26.9	n-butanol	No	1	209.60
12	31.2	2-methyl-1-butanol	No	1	216.87
13	31.3	3-methyl-1-butanol	No	1	207.88

Calibration Table

Enter | Delete | Insert... | Print | OK | Help

#	RT	Signal	Compound	ISTD	Lvl	Amt[mg/L AA]
1	6.181	FID1 A	acetaldehyde	No	1	428.250
2	8.554	FID1 A	methyl acetate	No	1	682.510
3	10.471	FID1 A	ethyl acetate	No	1	616.510
4	10.693	FID1 A	acetal	No	1	552.980
5	11.557	FID1 A	methanol	No	1	481.410
6	13.912	FID1 A	ethanol	Ye / No / Yes	1	789270.000
7	18.434	FID1 A	2-butanol	No	1	497.890
8	19.318	FID1 A	n-propanol	No	1	484.060
9	23.118	FID1 A	2-methyl-1-propan	No	1	474.370
10	24.168	FID1 A	3-pentanol	No	1	536.030
11	26.872	FID1 A	n-butanol	No	1	496.770
12	31.155	FID1 A	2-methyl-1-butano	No	1	514.010
13	31.348	FID1 A	3-methyl-1-butano	No	1	492.710

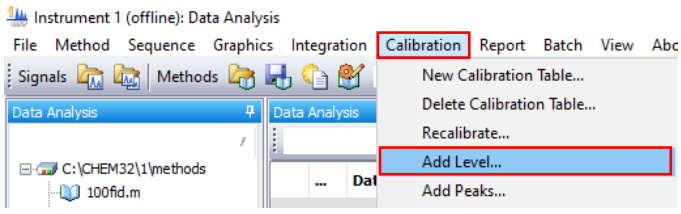
The units of concentration: µg/g in “Traditional ISTD” project and mg/L AA in “Ethanol ISTD” project

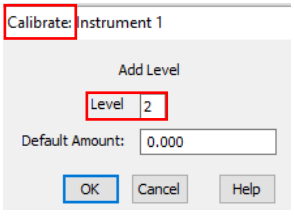
1.1.11. Mark 3-pentanol in “Traditional ISTD” project and ethanol in “Ethanol ISTD” project as internal standard in “ISTD” column (set “Yes” in the cell).

1.1.12. To determine calibration coefficients, need to add 2 another implementations of SS_{calibr}. Rename column “Lvl” into “Implem.#” for the insertion of the implementation number.

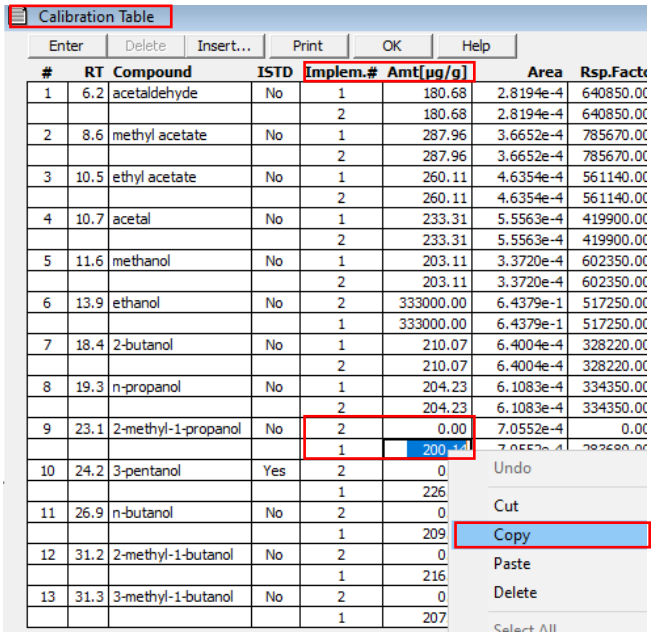
1.1.13. Click “File” in the main menu and press “Load Signal…” or “Import File” and “AIA File…” to open the file of the second chromatogram of SS_{calibr} (SS 1-2).

1.1.14. Click “Add Level…” in “Calibration”. Input the value of SS_{calibr} implementation number (“2”) in the cell “Level” and press “OK” button.





1.1.15. Copy the values of compound concentration from the cells of implementation 1 into the cells of implementation 2 in the calibration table for all components.



#	RT	Compound	ISTD	Implem.#	Amt[µg/g]	Area	Rsp.Facto
1	6.2	acetaldehyde	No	1	180.68	2.8194e-4	640850.00
				2	180.68	2.8194e-4	640850.00
2	8.6	methyl acetate	No	1	287.96	3.6652e-4	785670.00
				2	287.96	3.6652e-4	785670.00
3	10.5	ethyl acetate	No	1	260.11	4.6354e-4	561140.00
				2	260.11	4.6354e-4	561140.00
4	10.7	acetal	No	1	233.31	5.5563e-4	419900.00
				2	233.31	5.5563e-4	419900.00
5	11.6	methanol	No	1	203.11	3.3720e-4	602350.00
				2	203.11	3.3720e-4	602350.00
6	13.9	ethanol	No	2	333000.00	6.4379e-1	517250.00
				1	333000.00	6.4379e-1	517250.00
7	18.4	2-butanol	No	1	210.07	6.4004e-4	328220.00
				2	210.07	6.4004e-4	328220.00
8	19.3	n-propanol	No	1	204.23	6.1083e-4	334350.00
				2	204.23	6.1083e-4	334350.00
9	23.1	2-methyl-1-propanol	No	2	0.00	7.0552e-4	0.00
				1	200		
10	24.2	3-pentanol	Yes	2	0		
				1	226		
11	26.9	n-butanol	No	2	0		
				1	209		
12	31.2	2-methyl-1-butanol	No	2	0		
				1	216		
13	31.3	3-methyl-1-butanol	No	2	0		
				1	207		

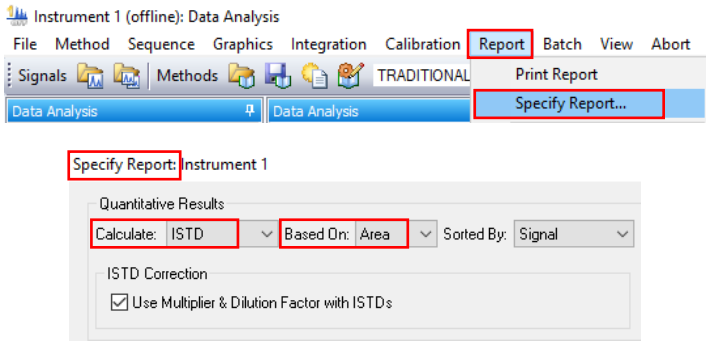
1.1.16. Repeat the actions as in 1.1.13-1.1.15 for the third implementation of SS_{calibr}. Thus, the calibrations are ready.

#	RT	Compound	ISTD	Implem.#	Amt[µg/g]	Area	Rsp.Factor
1	6.2	acetaldehyde	No	1	180.68	2.82e-4	640847
				2	180.68	2.86e-4	630730
				3	180.68	2.84e-4	637261
2	8.6	methyl acetate	No	1	287.96	3.67e-4	785668
				2	287.96	3.69e-4	779607
				3	287.96	3.62e-4	795822
3	10.5	ethyl acetate	No	1	260.11	4.64e-4	561140
				2	260.11	4.64e-4	560645
				3	260.11	4.57e-4	568818
4	10.7	acetal	No	1	233.31	5.56e-4	419903
				2	233.31	5.60e-4	416793
				3	233.31	5.49e-4	425099
5	11.6	methanol	No	1	203.11	3.37e-4	602347
				2	203.11	3.39e-4	599041
				3	203.11	3.35e-4	606212
6	13.9	ethanol	No	2	333000.00	0.649	513446
				3	333000.00	0.639	520885
				1	333000.00	0.644	517252
7	18.4	2-butanol	No	1	210.07	6.40e-4	328216
				2	210.07	6.48e-4	324187
				3	210.07	6.38e-4	329344
8	19.3	n-propanol	No	1	204.23	6.11e-4	334346
				2	204.23	6.14e-4	332515
				3	204.23	6.03e-4	338490
9	23.1	2-methyl-1-propanol	No	1	200.14	7.06e-4	283679
				2	200.14	7.16e-4	279593
				3	200.14	7.02e-4	285178
10	24.2	3-pentanol	Yes	1	226.16	7.60e-4	297519
				2	226.16	7.68e-4	294411
				3	226.16	7.54e-4	299922
11	26.9	n-butanol	No	1	209.60	6.87e-4	304936
				2	209.60	6.93e-4	302559
				3	209.60	6.83e-4	306882
12	31.2	2-methyl-1-butanol	No	1	216.87	7.71e-4	281380
				2	216.87	7.75e-4	279748
				3	216.87	7.67e-4	282590
13	31.3	3-methyl-1-butanol	No	1	207.88	7.30e-4	284585
				2	207.88	7.32e-4	283981
				3	207.88	7.25e-4	286813

#	RT	Compound	ISTD	Implem.#	Amt[mg/L AA]	Area	Rsp.Factor
1	6.2	acetaldehyde	No	1	428.25	2.82e-4	1518944
				2	428.25	2.86e-4	1494965
				3	428.25	2.84e-4	1510443
2	8.5	methyl acetate	No	1	682.51	3.67e-4	1862156
				2	682.51	3.69e-4	1847790
				3	682.51	3.62e-4	1886223
3	10.5	ethyl acetate	No	1	616.51	4.64e-4	1329996
				2	616.51	4.64e-4	1328834
				3	616.51	4.57e-4	1348206
4	10.7	acetal	No	1	552.98	5.56e-4	995234
				2	552.98	5.60e-4	987862
				3	552.98	5.49e-4	1007548
5	11.6	methanol	No	1	481.41	3.37e-4	1427679
				2	481.41	3.39e-4	1419844
				3	481.41	3.35e-4	1436840
6	13.9	ethanol	Yes	1	789270.00	0.644	1225966
				2	789270.00	0.649	1216959
				3	789270.00	0.639	1234591
7	18.4	2-butanol	No	1	497.89	6.40e-4	777910
				2	497.89	6.48e-4	768361
				3	497.89	6.38e-4	780582
8	19.3	n-propanol	No	1	484.06	6.11e-4	792457
				2	484.06	6.14e-4	788117
				3	484.06	6.03e-4	802278
9	23.1	2-methyl-1-propanol	No	1	474.37	7.06e-4	672372
				2	474.37	7.16e-4	662689
				3	474.37	7.02e-4	675926
10	24.2	3-pentanol	No	1	536.03	7.60e-4	705161
				2	536.03	7.68e-4	697794
				3	536.03	7.54e-4	710856
11	26.9	n-butanol	No	1	496.77	6.87e-4	722724
				2	496.77	6.93e-4	717091
				3	496.77	6.83e-4	727336
12	31.2	2-methyl-1-butanol	No	1	514.01	7.71e-4	666907
				2	514.01	7.75e-4	663039
				3	514.01	7.67e-4	669775
13	31.3	3-methyl-1-butanol	No	1	492.71	7.30e-4	674513
				2	492.71	7.32e-4	673081
				3	492.71	7.25e-4	679794

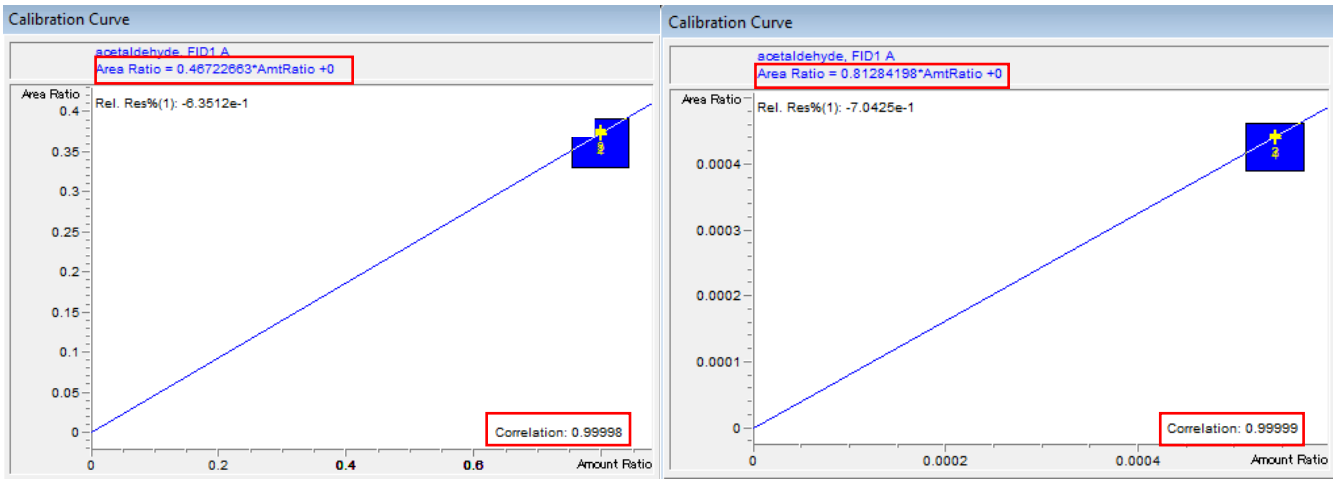
The calibration tables in the methods: “Traditional ISTD” (3-pentanol as ISTD) and “Ethanol ISTD”

1.1.17. Click “Report” in the main menu and press “Specify Report…”. Set in “Quantitative Results”: “Calculate” – “ISTD” and “Based on” – “Area”.



1.2. The results of calibration

1.2.1. The lineal dependences of Y (the area ratio, FID responses) on X (the amount ratio) of values obtained for the volatile compound relative to the values obtained for the ISTD (3-pentanol or ethanol) are shown in “Calibration curve” for compound *i* (*i* = 1, …, 13).



The calibration curves in the methods: “Traditional ISTD” (3-pentanol as ISTD) and “Ethanol ISTD”

1.2.2. There is the window shown the calibration curve for the compound *i* marked in the visible table. It is a function $Y = rRF_i \cdot X$, when the value of coefficient rRF_i (rRF – relative response factor) calculated on the base of all calibration dependences. The coefficients rRF_i ($rRF_i^{\text{3-pentanol}}$ if 3-pentanol is ISTD; rRF_i^{ethanol} if ethanol is ISTD) for *i* = 1, …, 13 are calculated by the method of root mean squares according to the following formulae:

$$rRF_i^{\text{3-pentanol}} = \frac{\sum_{k=1}^{M}\left(A_{i,\text{calibr},k} / A_{\text{3-pentanol,calibr},k}\right)}{M \cdot \left(\tilde{C}_{i,\text{calibr}}^{certified} / \tilde{C}_{\text{3-pentanol,calibr}}^{certified}\right)} \quad (1),$$

$$rRF_i^{\text{ethanol}} = \frac{\sum_{k=1}^{M}\left(A_{i,\text{calibr},k} / A_{\text{ethanol},\text{calibr},k}\right)}{M \cdot \left(C_{i,\text{calibr}}^{certified} / \rho_{\text{ethanol}}\right)} \quad (2),$$

X – $\tilde{C}_{i,\text{calibr}}^{certified} / \tilde{C}_{\text{3-pentanol},\text{calibr}}^{certified}$ (if 3-pentanol is ISTD) or $C_{i,\text{calibr}}^{certified} / \rho_{\text{ethanol}}$ (if ethanol is ISTD);

Y – $A_{i,\text{calibr},k} / A_{\text{3-pentanol},\text{calibr},k}$ (if 3-pentanol is ISTD) or $A_{i,\text{calibr},k} / A_{\text{ethanol},\text{calibr},k}$ (if ethanol is ISTD);

$A_{i,\text{calibr},k}$ – the value of detector response on volatile compound *i* (excluding ISTD) obtained in result of the measurement *k* of SS_{calibr};

$A_{\text{3-pentanol},\text{calibr},k}$ – the value of detector response on 3-pentanol obtained in result of the measurement *k*;

$A_{\text{ethanol},\text{calibr},k}$ – the value of detector response on ethanol obtained in result of the measurement *k* of SS_{calibr};

$\tilde{C}_{i,\text{calibr}}^{certified}$ – the certified value of compound *i* concentration in SS_{calibr} (μg/g);

$\tilde{C}_{\text{3-pentanol},\text{calibr}}^{certified}$ – the certified value of 3-pentanol concentration in SS_{calibr} (μg/g);

$C_{i,\text{calibr}}^{certified}$ – the certified value of compound *i* concentration in SS_{calibr} (mg/L AA);

ρ_{ethanol} – the certified value of ethanol concentration in SS_{calibr} (mg/L AA), it is equal to ethanol density 789270 mg/L;

M – the total number of repeat measurements of SS_{calibr}.

i	Compound name	$rRF_i^{\text{3-pentanol}}$	$1/rRF_i^{\text{3-pentanol}}$	rRF_i^{ethanol}	$1/rRF_i^{\text{ethanol}}$
1	acetaldehyde	0.4672	2.140	0.8128	1.230
2	methyl acetate	0.3777	2.648	0.6572	1.522
3	ethyl acetate	0.5275	1.896	0.9178	1.090
4	acetal	0.7068	1.415	1.2297	0.813
5	methanol	0.4934	2.027	0.8584	1.165
6	ethanol	0.5448	1.836	1.0000	1.000
7	2-butanol	0.9084	1.101	1.5805	0.633
8	n-propanol	0.8871	1.127	1.5433	0.648
9	2-methyl-1-propanol	1.0512	0.951	1.8288	0.547
10	**3-pentanol**	1.0000	1.000	1.7398	0.575
11	n-butanol	0.9754	1.025	1.6969	0.589
12	2-methyl-1-butanol	1.0570	0.946	1.8390	0.544
13	3-methyl-1-butanol	1.0426	0.959	1.8139	0.551
		Correlation > 0.99998		Correlation > 0.99999	

According to EC2870: $RRF_{i,k}^{\text{ISTD}} = RF_{i,k} / RF_{\text{ISTD},k}$; average: $1/M \cdot \sum_{k=1}^{M}\left(RF_{i,k} / RF_{\text{ISTD},k}\right) \approx 1/rRF_i^{\text{ISTD}}$.

Thus, $RRF_i^{\text{3-pentanol}} = 1/M \cdot \sum_{k=1}^{M}\left(RF_{i,k} / RF_{\text{3-pentanol},k}\right) \approx 1/rRF_i^{\text{3-pentanol}}$;

$$RRF_i^{\text{ethanol}} = 1/M \cdot \sum_{k=1}^{M}\left(RF_{i,k} / RF_{\text{ethanol},k}\right) \approx 1/rRF_i^{\text{ethanol}}$$

2. The control of detector response linearity

2.1. The processing of the chromatograms which are used for the control of linearity

2.1.1. The control of detector response linearity is carried out using standard solutions (SS_j, j =1, ..., N) which don't use for the estimation of calibration coefficients during creation the "Traditional ISTD" and "Ethanol ISTD" methods. Acquire the chromatograms of standard solutions for linearity control (SS_j – standard solution at the concentration level j).

2.1.2. Linearity is controlled by the same algorithms in the both projects. Therefore, duplicate all actions in the both projects.

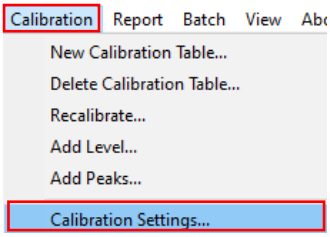
2.1.3. Click "Method" in the main menu and press "Load Method…" in the list.

2.1.4. Select any method and then save the method with new name: "LINEARITY_3_PENTANOL_ISTD" in the case of the using 3-pentanol as internal standard and "LINEARITY_ETHANOL_ISTD" in the case of the using ethanol as internal standard. Add "Comment for audit trail".

2.1.5. Click "File" in the main menu and press "Load Signal…" in the list for native data of ChemStation. But if the files of measured chromatograms have format *.cdf, then press "Import File" and "AIA File…". Open the file of the first chromatogram of SS_j (j = 1, "SS 0_1-1").

2.1.6. Set and integrate the peaks of the volatiles compounds in the chromatogram.

2.1.7. Click "Calibration" in the main menu and press "Calibration Setting…" in the list.



2.1.8. Fill the input lines in dialog window "Calibration Setting". Set "Type" (Lineal), "Origin" (Force), "Weight" (Equal) and other parameters as shown in the screenshot. Fill in the cell "Amount Units": "µg/g" in the case of "Traditional ISTD" project and "mg/L AA" in the case of "Ethanol ISTD" project. Press "OK" button.

2.1.9. Press "New calibration table" in "Calibration". Set "Level": 1, "Calibration Mode" – "Calculate Signals Separately" as shown in the screenshot. Press "OK" button.

2.1.10. Input the names of the compounds in "Compound" column and the certified values of compound concentration in "Amt[µg/g]" or "Amt[mg/L AA]" column. It will be named these concentration values of compound i (i = 1, …, 13) in SS_j as $\tilde{C}_{i,j}^{certified}$ (µg/g) in the case of 3-pentanol as ISTD or $C_{i,j}^{certified}$ (mg/L AA) in the case of ethanol as ISTD. Concentration of ethanol in mg/L AA is equal to ethanol density ($\rho_{ethanol}$ = 789270 mg/L).

2.1.11. Mark 3-pentanol in "Traditional ISTD" project and ethanol in "Ethanol ISTD" project as internal standard in "ISTD" column (set "Yes" in the cell).

2.1.12. Click "File" in the main menu and press "Load Signal…" or "Import File" and "AIA File…" to open the file of the second chromatogram of SS_j (j = 1, "SS 0_1-2").

2.1.13. Click "Add Level…" in "Calibration". Input the value of SS_j implementation number ("2") in the cell "Level" and press "OK" button.

2.1.14. Copy the values of compound concentration from the cells of implementation 1 into the cells of implementation 2 in the calibration table for all components.

2.1.15. Repeat the actions as in 2.1.12-2.1.14 for the third implementation of SS_j (j = 1, "SS 0_1-3").

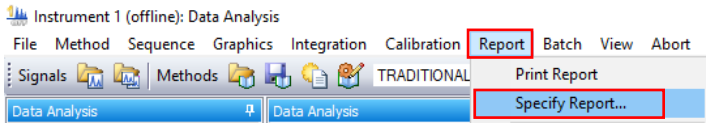
2.1.16. Repeat the actions as in 2.1.9-2.1.15 for the each other SS_j (j = 2, …, N) and their implementations k = 1, …, M. Number the concentration levels subsequently up to value $N \times M$: levels 1-3 of SS_1, levels 4-6 of SS_2, levels 7-9 of SS_3, levels 10-12 of SS_4.

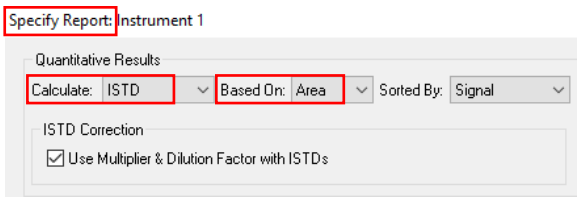
RT	Compound	ISTD	Implem.#	Amt[µg/g]	Area	Rsp.Factor
23.1	2-methyl-1-propanol	No	1	21.32	7.50e-5	284125
			2	21.32	7.39e-5	288591
			3	21.32	7.39e-5	288415
			4	106.15	3.72e-4	285710
			5	106.15	3.74e-4	283554
			6	106.15	3.73e-4	284682
			7	307.72	1.10e-3	280641
			8	307.72	1.11e-3	278169
			9	307.72	1.11e-3	278242
			10	411.77	1.48e-3	278422
			11	411.77	1.48e-3	278057
			12	411.77	1.44e-3	286932
24.2	3-pentanol	Yes	10	225.77	7.74e-4	291542
			11	225.77	7.77e-4	290662
			12	225.77	7.53e-4	299983
			4	227.65	7.65e-4	297597
			5	227.65	7.74e-4	294279
			6	227.65	7.75e-4	293892
			7	227.81	7.71e-4	295584
			8	227.81	7.76e-4	293723
			9	227.81	7.75e-4	293908
			1	228.73	7.74e-4	295536
			2	228.73	7.74e-4	295401
			3	228.73	7.73e-4	295720

RT	Compound	ISTD	Implem.#	Amt[mg/L AA]	Area	Rsp.Factor
13.9	ethanol	Yes	1	789270.00	0.648	1217535
			2	789270.00	0.647	1219415
			3	789270.00	0.647	1220484
			4	789270.00	0.642	1229754
			5	789270.00	0.646	1221628
			6	789270.00	0.647	1219933
			7	789270.00	0.647	1219313
			8	789270.00	0.653	1208364
			9	789270.00	0.651	1212062
			10	789270.00	0.652	1210348
			11	789270.00	0.654	1207665
			12	789270.00	0.634	1244302
18.4	2-butanol	No	1	53.03	6.84e-5	775543
			2	53.03	6.69e-5	792221
			3	53.03	6.83e-5	776098
			4	263.61	3.35e-4	785954
			5	263.61	3.41e-4	772311
			6	263.61	3.42e-4	771715
			7	765.51	9.96e-4	768749
			8	765.51	1.01e-3	759946
			9	765.51	1.00e-3	764447
			10	1024.40	1.34e-3	764076
			11	1024.40	1.35e-3	760832
			12	1024.40	1.31e-3	784794

The parts of calibration tables in the methods: "LINEARITY_3_PENTANOL_ISTD" and "LINEARITY_ETHANOL_ISTD"

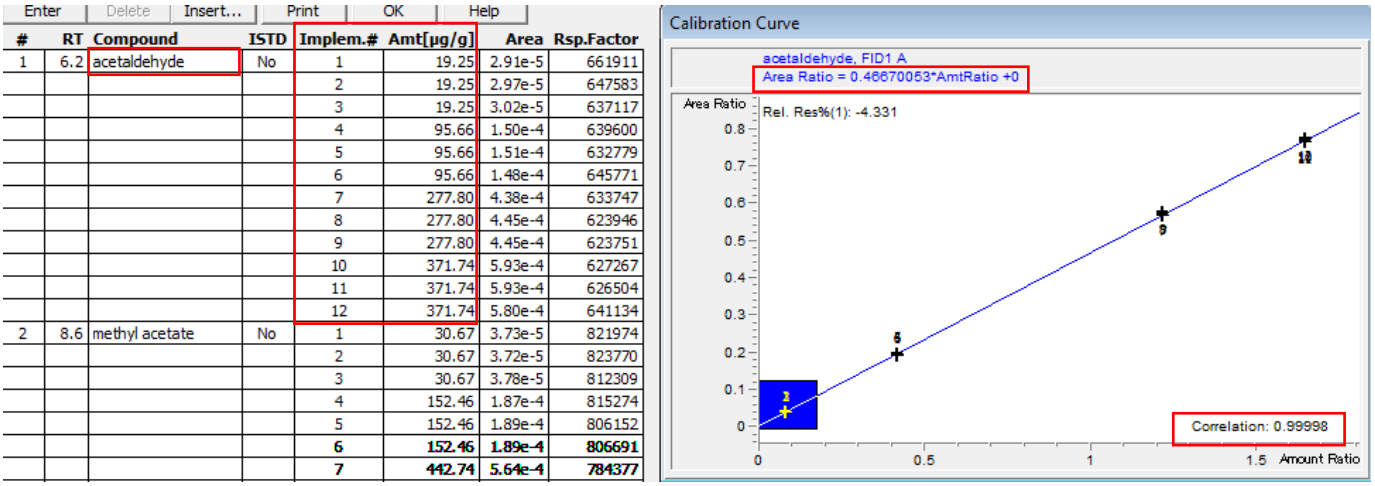
2.1.1. Click "Report" in the main menu and press "Specify Report…". Set in "Quantitative Results": "Calculate" – "ISTD" and "Based on" – "Area".





2.2. The results of the control of detector response linearity

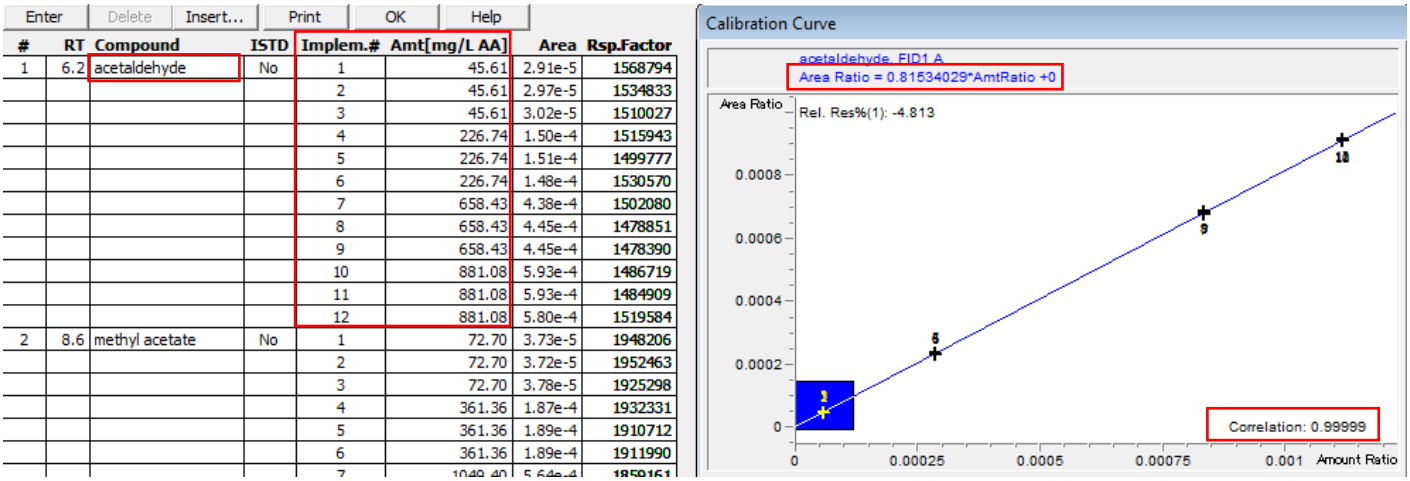
2.2.1. The lineal dependences of Y (the area ratio, FID responses) on X (the amount ratio) - the values obtained for the volatile compound relative to the values obtained for the ISTD (3-pentanol or ethanol) are shown in "Calibration curve" for compound *i* (*i* = 1, …, 13).



Enter | Delete | Insert... | Print | OK | Help

#	RT	Compound	ISTD	Implem.#	Amt[µg/g]	Area	Rsp.Factor
1	6.2	acetaldehyde	No	1	19.25	2.91e-5	661911
				2	19.25	2.97e-5	647583
				3	19.25	3.02e-5	637117
				4	95.66	1.50e-4	639600
				5	95.66	1.51e-4	632779
				6	95.66	1.48e-4	645771
				7	277.80	4.38e-4	633747
				8	277.80	4.45e-4	623946
				9	277.80	4.45e-4	623751
				10	371.74	5.93e-4	627267
				11	371.74	5.93e-4	626504
				12	371.74	5.80e-4	641134
2	8.6	methyl acetate	No	1	30.67	3.73e-5	821974
				2	30.67	3.72e-5	823770
				3	30.67	3.78e-5	812309
				4	152.46	1.87e-4	815274
				5	152.46	1.89e-4	806152
				6	**152.46**	**1.89e-4**	**806691**
				7	**442.74**	**5.64e-4**	**784377**

The one of the lineal dependences in the method: "LINEARITY_3_PENTANOL_ISTD"



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#	RT	Compound	ISTD	Implem.#	Amt[mg/L AA]	Area	Rsp.Factor
1	6.2	acetaldehyde	No	1	45.61	2.91e-5	1568794
				2	45.61	2.97e-5	1534833
				3	45.61	3.02e-5	1510027
				4	226.74	1.50e-4	1515943
				5	226.74	1.51e-4	1499777
				6	226.74	1.48e-4	1530570
				7	658.43	4.38e-4	1502080
				8	658.43	4.45e-4	1478851
				9	658.43	4.45e-4	1478390
				10	881.08	5.93e-4	1486719
				11	881.08	5.93e-4	1484909
				12	881.08	5.80e-4	1519584
2	8.6	methyl acetate	No	1	72.70	3.73e-5	1948206
				2	72.70	3.72e-5	1952463
				3	72.70	3.78e-5	1925298
				4	361.36	1.87e-4	1932331
				5	361.36	1.89e-4	1910712
				6	361.36	1.89e-4	1911990

The one of the lineal dependences in the method: "LINEARITY_ETHANOL_ISTD"

2.2.2. There is the window shown the calibration curve for the compound *i* marked in the visible table. It is a function $Y = rRF_i \cdot X$, when the value of coefficient rRF_i (rRF – relative response factor) calculated on the base of all calibration dependences. The coefficients rRF_i ($rRF_i^{\text{3-pentanol}}$ if 3-pentanol is ISTD; rRF_i^{ethanol} if ethanol is ISTD) are calculated by the method of root mean squares according to the following formulae:

$$rRF_i^{\text{3-pentanol}} = \frac{\sum_{j=1}^{N}\sum_{k=1}^{M}\left(\left(\tilde{C}_{i,j}^{certified} / \tilde{C}_{\text{3-pentanol},j}^{certified}\right)\cdot\left(A_{i,j,k} / A_{\text{3-pentanol},j,k}\right)\right)}{M \cdot \sum_{j=1}^{N}\left(\tilde{C}_{i,j}^{certified} / \tilde{C}_{\text{3-pentanol},j}^{certified}\right)^2} \quad (3),$$

and when ethanol is ISTD:

$$rRF_i^{\text{ethanol}} = \frac{\sum_{j=1}^{N}\sum_{k=1}^{M}\left(\left(C_{i,j}^{certified} / \rho_{\text{ethanol}}\right)\cdot\left(A_{i,j,k} / A_{\text{ethanol},j,k}\right)\right)}{M\cdot\sum_{j=1}^{N}\left(C_{i,j}^{certified} / \rho_{\text{ethanol}}\right)^2} \quad (4),$$

X – $\tilde{C}_{i,j}^{certified} / \tilde{C}_{\text{3-pentanol},j}^{certified}$ (if 3-pentanol is ISTD) or $C_{i,j}^{certified} / \rho_{\text{ethanol}}$ (if ethanol is ISTD);

Y – $A_{i,j,k} / A_{\text{3-pentanol},j,k}$ (if 3-pentanol is ISTD) or $A_{i,j,k} / A_{\text{ethanol},j,k}$ (if ethanol is ISTD);

$A_{i,j,k}$ – the value of detector response on compound *i* obtained in result of the measurement *k* of SS_j;

$A_{\text{3-pentanol},j,k}$ – the value of detector response on 3-pentanol obtained in result of the measurement *k* of SS_j;

$A_{\text{ethanol},j,k}$ – the value of detector response on ethanol obtained in result of the measurement *k* of SS_j;

$\tilde{C}_{i,j}^{certified}$ – the certified value of compound *i* concentration in SS_j (µg/g);

$\tilde{C}_{\text{3-pentanol},j}^{certified}$ – the certified value of 3-pentanol concentration in SS_j (µg/g);

$C_{i,j}^{certified}$ – the certified value of compound *i* concentration SS_j (mg/L AA);

C_p (correlation) – coefficient of lineal correlation;

ρ_{ethanol} – the certified value of ethanol concentration in SS_j (mg/L AA), is equal to density 789270 mg/L;

M – the total number of repeat measurements of SS_j; *N* – the total number of concentration levels.

i	Compound name	$rRF_i^{\text{3-pentanol}}$	$1/rRF_i^{\text{3-pentanol}}$	rRF_i^{ethanol}	$1/rRF_i^{\text{ethanol}}$
1	acetaldehyde	0.467	2.143	0.815	1.227
2	methyl acetate	0.375	2.669	0.655	1.528
3	ethyl acetate	0.523	1.914	0.913	1.095
4	acetal	0.700	1.428	1.223	0.817
5	methanol	0.490	2.039	0.857	1.167
6	ethanol	0.573	1.745	1.000	1.000
7	2-butanol	0.908	1.102	1.586	0.631
8	n-propanol	0.885	1.130	1.546	0.647
9	2-methyl-1-propanol	1.049	0.954	1.832	0.546
10	**3-pentanol**	1.000	1.000	1.745	0.573
11	n-butanol	0.978	1.023	1.708	0.585
12	2-methyl-1-butanol	1.061	0.942	1.854	0.539
13	3-methyl-1-butanol	1.050	0.952	1.834	0.545

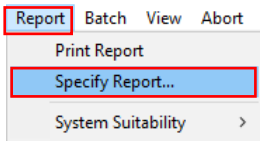
Compounds	C_p (Traditional ISTD)	C_p (Ethanol ISTD)
acetaldehyde	0.99998	0.99999
methyl acetate	0.99998	0.99998
ethyl acetate	0.99998	0.99999
acetal	0.99998	0.99999
methanol	0.99998	0.99999
2-butanol	0.99999	0.999999
n-propanol	0.99999	0.999999
2-methyl-1-propanol	0.99999	0.999999
n-butanol	0.99999	0.999999
2-methyl-1-butanol	0.99998	0.99999
3-methyl-1-butanol	0.99998	0.99999

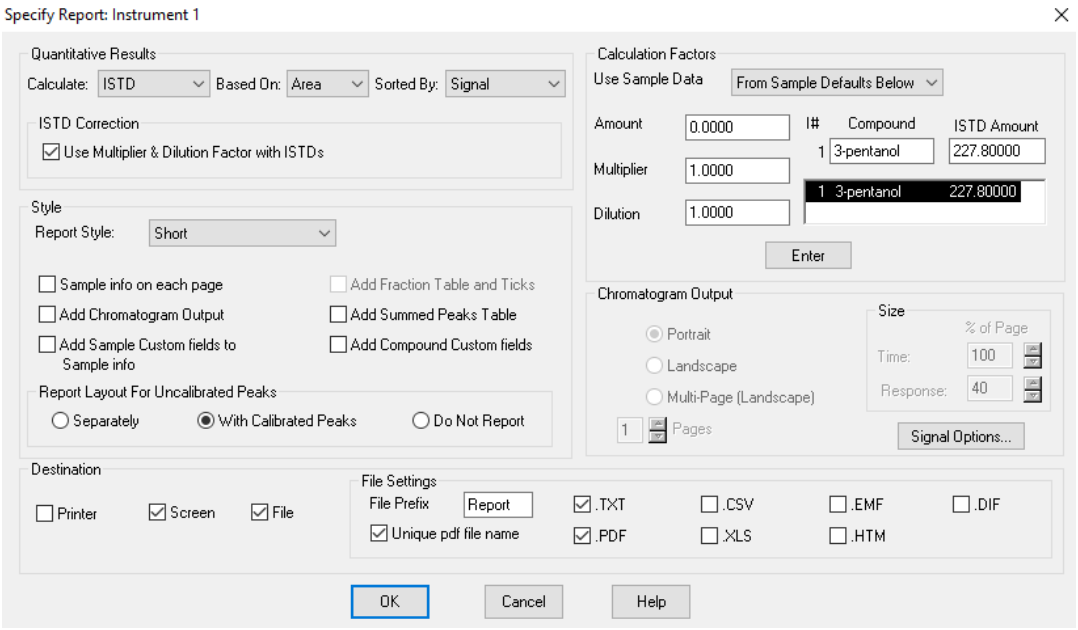
2.3. The estimation of statistic parameters for "Traditional ISTD" and "Ethanol ISTD" methods

2.3.1. Click "Method" in the main menu and press "Load Method…" in the list. Select method "Traditional ISTD" or "Ethanol ISTD".

2.3.2. Click "File" in the main menu and press "Load Signal…" in the list for native data of ChemStation. But if the files of measured chromatograms have format *.cdf, then press "Import File" and "AIA File…". Open the first chromatogram file of control sample.

2.3.3. The calculation of concentration values of volatile compounds in analyzed sample is made during creation of the report. Press "Report" and "Specify Report". Set the parameters as shown in the screenshots and enter the concentration of 3-pentanol in the control sample.





2.3.4. Press "Report" and "Print Report". Duplicate these actions using the both methods.

```
Sample ISTD Information:
ISTD  ISTD Amount   Name
  #   [mg/L AA]
----|------------|--------------------
  1   7.89270e5    ethanol
```

Signal 1: FID1 A, Front Signal

RetTime [min]	Type	ISTD	Area used	[V*s]	Amt/Area ratio	Amount [mg/L AA]	Grp	Name
6.175	BB		1	4.45370e-4	1.23025	664.10906		acetaldehyde
8.552	BB		1	5.66553e-4	1.52169	1044.94145		methyl acetate
10.471	BB		1	7.13272e-4	1.08959	941.98646		ethyl acetate
10.690	BB		1	8.54955e-4	8.13209e-1	842.69422		acetal
11.553	BB		1	5.23888e-4	1.16502	739.77027		methanol
13.912	BB	I	1	6.51180e-1	1.00000	7.89270e5		ethanol
18.433	BB		1	1.00138e-3	6.32719e-1	767.95626		2-butanol
19.320	BB		1	9.51137e-4	6.47943e-1	746.97288		n-propanol
23.120	BB		1	1.10594e-3	5.46821e-1	732.99524		2-methyl-1-propanol
24.174	BB		1	7.75096e-4	5.74786e-1	539.99079		3-pentanol
26.880	BB		1	1.07927e-3	5.89298e-1	770.88278		n-butanol
31.162	BB		1	1.21142e-3	5.43773e-1	798.42876		2-methyl-1-butanol
31.349	BB		1	1.14876e-3	5.51296e-1	767.60644		3-methyl-1-butanol

2.3.5. The table contains the results with the average values of measured concentration $\langle \tilde{C} \rangle_i^{measured}$ (μg/g) in the case of 3-pentanol as ISTD or $\langle C \rangle_i^{measured}$ (mg/L AA) in the case of ethanol as ISTD in control sample, relative standard deviation ($RSD_i, \%$) and accuracy ($bias_{i,j}, \%$) calculated on the base of M implementations according to the following formulae when 3-pentanol is ISTD:

$$\tilde{C}_{i,k}^{measured} = 1/rRF_i^{\text{3-pentanol}} \cdot \tilde{C}_{\text{3-pentanol}}^{certified} \cdot A_{i,k} / A_{\text{3-pentanol},k} \quad (5);$$

$$\langle \tilde{C} \rangle_i^{measured} = \left(\sum_{k=1}^{M} \tilde{C}_{i,k}^{measured} \right) / M \quad (6),$$

$$S\tilde{D}_i = \sqrt{\sum_{k=1}^{M} \frac{\left(\tilde{C}_{i,k}^{measured} - \langle \tilde{C} \rangle_i^{measured} \right)^2}{M-1}} \quad (7);$$

$$RSD_i, \% = \frac{S\tilde{D}_i}{\langle \tilde{C} \rangle_i^{measured}} \cdot 100\% \quad (8);$$

$$bias_i, \% = \frac{\langle \tilde{C} \rangle_i^{measured} - \tilde{C}_i^{certified}}{\tilde{C}_i^{certified}} \cdot 100\% \quad (9);$$

and when ethanol is ISTD:

$$C_{i,k}^{measured} = 1/rRF_i^{\text{ethanol}} \cdot \rho_{\text{ethanol}} \cdot A_{i,k} / A_{\text{ethanol},k} \quad (10);$$

$$\langle C \rangle_i^{measured} = \left(\sum_{k=1}^{M} C_{i,k}^{measured} \right) / M \quad (11);$$

$$SD_i = \sqrt{\sum_{k=1}^{M} \frac{\left(C_{i,k}^{measured} - \langle C \rangle_i^{measured} \right)^2}{M-1}} \quad (12);$$

$$RSD_i, \% = \frac{SD_i}{\langle C \rangle_i^{measured}} \cdot 100\% \quad (13);$$

$$bias_i, \% = \frac{\langle C \rangle_i^{measured} - C_i^{certified}}{C_i^{certified}} \cdot 100\% \quad (14);$$

$A_{i,k}$ – the value of detector response on volatile compound i (excluding ISTD) obtained in result of the measurement k of analyzed sample;

$A_{\text{3-pentanol},k}$ – the value of detector response on 3-pentanol obtained in result of the measurement k of analyzed sample;

$A_{\text{ethanol},k}$ – the value of detector response on ethanol obtained in result of the measurement k of the sample;

$\tilde{C}_{\text{3-pentanol},k}^{certified}$ – the certified value of 3-pentanol concentration in analyzed sample (μg/g);

$\tilde{C}_{i,j}^{certified}$ – the certified value of compound i concentration in analyzed sample (μg/g);

$C_{i,j}^{certified}$ – the certified value of compound *i* concentration in analyzed sample (mg/L AA);

ρ_{ethanol} – the certified value of ethanol concentration in the sample (mg/L AA), is equal to the ethanol density 789270 mg/L;

$\tilde{C}_{i,k}^{measured}$ – the value of compound *i* concentration in analyzed sample (μg/g) calculated on the base of measurement *k*;

$\left\langle \tilde{C} \right\rangle_i^{measured}$ – the average value of compound *i* concentration in analyzed sample (μg/g);

$C_{i,k}^{measured}$ – the value of compound *i* concentration in analyzed sample (mg/L AA) as the result of measurement *k*;

$\left\langle C \right\rangle_i^{measured}$ – the average value of compound *i* concentration in analyzed sample (mg/L AA);

M – the total number of repeat measurements of analyzed sample.

3-pentanol ISTD							
i	Compound	$C^{certified}$, μg/g	$C^{certified}$, mg/L AA	$\left\langle \tilde{C} \right\rangle_i^{measured}$, μg/g	$\left\langle C \right\rangle_i^{measured}$, mg/L AA	*RSD, %*	*bias, %*
1	acetaldehyde	277.8	658.4	279.1	661.6	0.6	0.5
2	methyl acetate	442.7	1049.4	441.5	1046.3	0.1	-0.3
3	ethyl acetate	399.9	947.9	398.5	944.5	0.2	-0.4
4	acetal	358.7	850.2	357.8	848.0	0.8	-0.3
5	methanol	312.3	740.2	312.3	740.1	0.2	0.0
6	*ethanol*	333004.2	789270.0	333207.7	789752.2	0.1	0.1
7	2-butanol	323.0	765.5	324.6	769.3	0.3	0.5
8	n-propanol	314.0	744.2	314.7	745.9	0.1	0.2
9	2-methyl-1-propanol	307.7	729.3	308.9	732.1	0.2	0.4
10	*3-pentanol*	227.8	539.9	227.8	539.9	-	-
11	n-butanol	322.3	763.8	325.0	770.3	0.3	0.9
12	2-methyl-1-butanol	333.4	790.3	337.3	799.5	0.2	1.2
13	3-methyl-1-butanol	319.6	757.5	323.8	767.3	0.1	1.3

Ethanol ISTD					
i	Compound	$C^{certified}$, mg/L AA	$\left\langle C \right\rangle_i^{measured}$, mg/L AA	*RSD, %*	*bias, %*
1	acetaldehyde	658.4	661.2	0.5	0.4
2	methyl acetate	1049.4	1045.7	0.1	-0.3
3	ethyl acetate	947.9	943.9	0.2	-0.4
4	acetal	850.2	847.4	0.6	-0.3
5	methanol	740.2	739.7	0.1	-0.1
6	*ethanol*	789270.0	789270.0	-	-
7	2-butanol	765.5	768.8	0.2	0.4
8	n-propanol	744.2	745.5	0.2	0.2
9	2-methyl-1-propanol	729.3	731.7	0.2	0.3
10	*3-pentanol*	539.9	539.6	0.1	-0.1
11	n-butanol	763.8	769.8	0.2	0.8
12	2-methyl-1-butanol	790.3	799.0	0.3	1.1
13	3-methyl-1-butanol	757.5	766.9	0.1	1.2

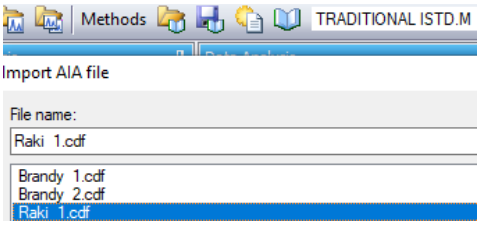
3. Analysis of alcoholic drinks

3.1. The application of the methods “Traditional ISTD” and “Ethanol ISTD” for analysis of samples of alcoholic drinks

3.1.1. Acquire the chromatograms of samples of alcoholic drinks.

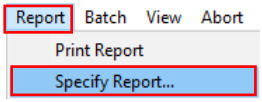
3.1.2. Click “Method” in the main menu and press “Load Method…” in the list. Select method “Traditional ISTD” or “Ethanol ISTD”.

3.1.3. Click “File” in the main menu and press “Load Signal…” in the list for native data of ChemStation. But if the files of measured chromatograms have format *.cdf, then press “Import File” and “AIA File…”. Open the first chromatogram file of alcoholic drink sample.



3.1.4. Set and integrate the peaks of the volatiles compounds in the chromatogram.

3.1.5. The calculation of concentration values of volatile compounds in analyzed sample is made during creation of the report. Press “Report” and “Specify Report”. Set the parameters as shown in the screenshots and enter the concentration of 3-pentanol in the analyzed sample.



3.1.6. Press “Report” and “Print Report”. Duplicate these actions using the both methods.

```
Sorted By             :      Signal
Calib. Data Modified  :      Thursday, December 26, 2024 7:15:13 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
Sample ISTD Information:
ISTD  ISTD Amount   Name
  #     [µg/g]
----|-----------|-------------
  1   227.80000   3-pentanol

Signal 1: FID1 A, Front Signal

RetTime  Type  ISTD    Area      Amt/Area    Amount    Grp   Name
 [min]         used   [V*s]       ratio     [µg/g]
-------|------|----|----------|----------|----------|--|-----------------
  6.185 BB         1 9.02712e-5    2.14029   58.17736    acetaldehyde
  8.534 BB         1 1.48048e-5    2.64738   11.80194    methyl acetate
 10.446 BB         1 8.49700e-4    1.89561   485.00482   ethyl acetate
 10.668 BB         1 1.39654e-4    1.41479   59.49469    acetal
 11.523 BB         1 4.37130e-3    2.02682 2667.82824    methanol
 13.930 BB         1 7.71661e-1    1.73975 4.04246e5     ethanol
 18.434            1      -          -         -        2-butanol
 19.271 BB         1 4.53447e-4    1.12726   153.91588   n-propanol
 23.052 BB         1 3.04911e-4 9.51327e-1   87.34440    2-methyl-1-propanol
 24.102 BB    I    1 7.56521e-4    1.00000   227.80000   3-pentanol
 26.802 BB         1 9.14371e-5    1.02527   28.22876    n-butanol
 31.091 BB         1 1.38481e-4 9.46042e-1   39.44860    2-methyl-1-butanol
 31.284 BB         1 6.80836e-4 9.59114e-1   196.62790   3-methyl-1-butanol
```

```
Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, December 24, 2024 7:10:13 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
Sample ISTD Information:
ISTD  ISTD Amount   Name
  #     [mg/L AA]
----|-----------|-------------
  1   7.89270e5   ethanol

Signal 1: FID1 A, Front Signal

RetTime  Type  ISTD    Area      Amt/Area    Amount    Grp   Name
 [min]         used   [V*s]       ratio     [mg/L AA]
-------|------|----|----------|----------|----------|--|-----------------
  6.185 BB         1 9.02712e-5    1.23025   113.59049   acetaldehyde
  8.534 BB         1 1.48048e-5    1.52169    23.04247   methyl acetate
 10.446 BB         1 8.49700e-4    1.08959   946.95446   ethyl acetate
 10.668 BB         1 1.39654e-4 8.13209e-1   116.15915   acetal
 11.523 BB         1 4.37130e-3    1.16502  5208.86353   methanol
 13.930 BB    I    1 7.71661e-1    1.00000  7.89270e5    ethanol
 18.431            1      -          -         -        2-butanol
 19.271 BB         1 4.53447e-4 6.47943e-1   300.51277   n-propanol
 23.052 BB         1 3.04911e-4 5.46821e-1   170.53630   2-methyl-1-propanol
 24.102 BB         1 7.56521e-4 5.74786e-1   444.76045   3-pentanol
 26.802 BB         1 9.14371e-5 5.89298e-1    55.11329   n-butanol
 31.091 BB         1 1.38481e-4 5.43773e-1    77.02034   2-methyl-1-butanol
 31.284 BB         1 6.80836e-4 5.51296e-1   383.90693   3-methyl-1-butanol
```

The examples of the reports which be obtained for the first chromatogram of the sample “Raki” using the methods “Traditional ISTD” (3-pentanol as ISTD) and “Ethanol ISTD”

3.1.7. The table contains the results with the average values of measured concentration $\langle \tilde{C} \rangle_i^{measured}$ (μg/g) in the case of 3-pentanol as ISTD or $\langle C \rangle_i^{measured}$ (mg/L AA) if ethanol is ISTD and relative standard deviation ($RSD_i, \%$) calculated on the base of M implementations according to the formulae (5)-(8) when 3-pentanol is ISTD and (10)-(13) when ethanol is ISTD.

3-pentanol ISTD		Raki		Brandy		Whiskey	
i	Compound	Average, μg/g	*RSD*, %	Average, μg/g	*RSD*, %	Average, μg/g	*RSD*, %
1	acetaldehyde	58.6	1.1	36.5	1.1	24.1	0.3
2	methyl acetate	11.5	3.7	82.1	1.6	-	-
3	ethyl acetate	477.2	2.3	53.5	0.5	75.0	1.3
4	acetal	58.3	2.9	17.4	1.1	10.9	4.3
5	methanol	2625.2	2.3	32.0	2.9	30.6	1.1
6	*ethanol*	*398333.5*	*2.1*	*285264.0*	*0.2*	*340146.5*	*1.2*
7	2-butanol	-	-	-	-	-	-
8	n-propanol	151.4	2.3	145.5	0.5	152.7	1.8
9	2-methyl-1-propanol	85.8	2.5	154.0	0.4	176.8	1.4
10	*3-pentanol*	*227.8*	*0.0*	*188.7*	*0.0*	*224.1*	*0.0*
11	n-butanol	28.4	0.8	-	-	-	-
12	2-methyl-1-butanol	38.2	4.6	46.5	0.6	62.4	1.5
13	3-methyl-1-butanol	192.7	2.9	128.2	2.3	157.1	1.2

Ethanol ISTD		Raki		Brandy		Whiskey	
i	Compound	Average, mg/L AA	*RSD*, %	Average, mg/L AA	*RSD, %*	Average, mg/L AA	*RSD, %*
1	acetaldehyde	116.2	3.2	101.1	0.9	56.0	1.5
2	methyl acetate	22.8	1.6	227.8	1.6	-	-
3	ethyl acetate	945.6	0.2	148.1	0.8	173.9	0.1
4	acetal	115.5	0.8	48.1	0.9	25.4	3.1
5	methanol	5201.6	0.2	88.5	2.7	71.0	0.0
6	*ethanol*	*789270.0*	-	*789270.0*	-	*789270.0*	-
7	2-butanol	-	-	-	-	-	-
8	n-propanol	300.0	0.2	402.6	0.2	354.4	0.6
9	2-methyl-1-propanol	170.1	0.4	426.1	0.6	410.2	0.2
10	*3-pentanol*	*451.5*	*2.1*	*522.1*	*0.2*	*520.0*	*1.2*
11	n-butanol	56.3	2.9	-	-	-	-
12	2-methyl-1-butanol	75.7	2.5	128.6	0.8	144.8	0.3
13	3-methyl-1-butanol	381.8	0.8	354.8	2.6	364.5	0.1