Analysis of short chain organic acids in tobacco and in some flavored e-liquids

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Background

- Short chain organic acids from acetic to caprylic play an important role in tobacco flavor, and may be present in the flavors added to the liquids (e-liquids) used in electronic nicotine delivery systems (ENDS).
- Until recently, the direct GC/MS quantitation of these acids, without derivatization, has been problematic due to the reactivity of the analytes within the column phase that lead to the lack of reproducibility of results.
- The use of a new GC column, DB-624UI from Agilent leads to much better reproducibility.
- The GC/MS direct determination of the levels of acids in tobacco after extraction (for example with CH_3CN) shows significant interferences from the tobacco matrix.
- A solid phase extraction (SPE) step is necessary to clean up the extract.
- The analysis of e-liquids can be done directly following the dilution of the sample in CH_3OH and injection in the GC/MS instrument.

Analytical method summary

- Tobacco was extracted with CH_3CN.
- The tobacco extract was cleaned on a Strata SAX 500 mg/3mL SPE cartridge.
- The analytes were eluted with CH_3OH containing 5,000 µg/mL HCOOH.
- The analysis was performed on two different GC/MS systems with different sensitivity with the goal of extending the range of concentrations of the analyzed acids from a lower sensitivity of the first instrument to a higher one.
- One system was a 6890N/5973 GC/MS system from Agilent, and the second system was a 7890B/5977B GC/MS with High Efficiency Source (HES) which is about ten times more sensitive.
- Both systems were equipped with a DB-624UI 30 m x 0.25 mm, 1.4 µm film thickness from Agilent [1].

1. J. Oostdijk, K. Lynam, A. Vickers, Trace analysis of volatile organic acids with the Agilent J7W DB-624UI GC column, Agilent Application Note 5991-0845EN.

Sample processing summary

500 mg tobacco are extracted with 2.5 mL CH_3CN at 73 °C for 30 min

↓

To 1 mL extract is added 20 µL I.S. with 11,060 µg/mL CD_3COOD

↓

1 mL extract + I.S. is added to a conditioned Strata SAX 500 mg/3mL SPE cartridge

↓

The cartridge is washed with 5 portions of 1 mL CH_3CN and dried

↓

The sample is eluted with 1 mL CH_3OH that contains 5000 µg/mL HCOOH and 200 µg/mL decanol

↓

The eluate is analyzed by GC/MS

100 mg of e-liquid are weighed in a 2 mL GC vial

↓

To the vial are added 1000 µL CH_3OH

↓

To the same vial is added 20 µL I.S. with 11,060 µg/mL CD_3COOH

↓

The solution is analyzed by GC/MS

GC operating parameters

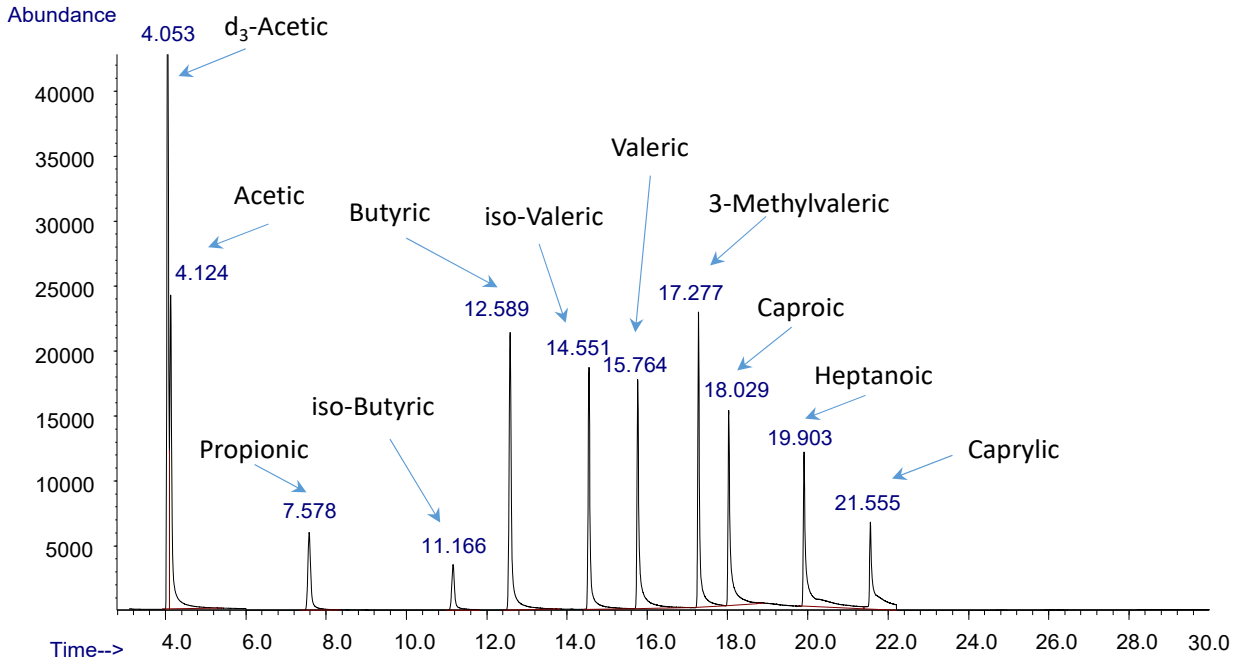
Parameter	*Description*	*Parameter*	*Description*
Initial oven temp.	70 °C	Carrier gas	Helium
Initial time	10 min	Split ratio	25:1
Oven ramp rate	10 °C/min	Split flow	20 mL/min
Oven final temp.	230 °C	Injection volume	1.0 µL
Oven final time	4 min	Flow mode	Constant flow
Total run time	30 min	Flow rate	0.8 mL/min
Inlet temp.	250 °C	Nominal initial pressure	6.72 psi
Inlet type	Split/Splitless	Outlet pressure	Vacuum
Inlet mode	Split	Transfer line heater	280 °C

MS operating parameters

Parameter for 5973 MS	*Description*	*Parameter for 5977B (HES)*	*Description*
Ion source temp.	230 °C	Ion source temp.	230 °C
Quadrupole temp.	150 °C	Quadrupole temp.	150 °C
Resulting EM Voltage	1752 V	Resulting EM Voltage	1523 V
MSD solvent delay	3.0 min	MSD solvent delay	4.0 min
MSD acquisition mode	SIM	MSD acquisition mode	SIM
SIM time for ions 63*, 60	3.0 min	SIM time for ions 63*, 60	4.0 min
SIM time for ions 74.1	6.0 min	SIM time for ions 74.1	8.0 min
SIM time for ions 73.1	10.0 min	SIM time for ions 73.1	11.0 min
SIM time for ions 60.1	11.8 min	SIM time for ions 60.1	13.2 min
SIM time for ions 112	22.2 min	SIM time for ions 112	22.9 min

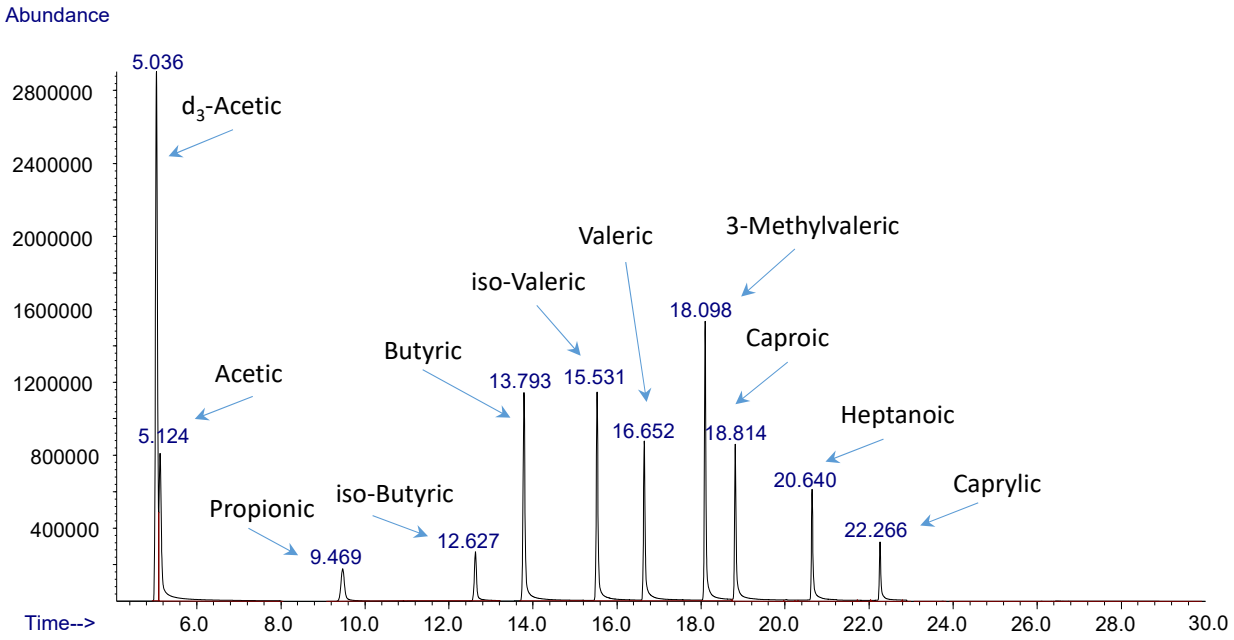
*Note: Due to the isotopic exchange in the presence of protons, the CD_3COOD is changed into CD_3COOH.

Example of a chromatogram for a standard on the 6890N/5973 GC/MS



The standard containing 113.25 µg/mL acetic acid and around 50 µg/mL of the other acids (no decanol).

Example of a chromatogram for a standard on a 7890B/5977B GC/MS HES



The standard containing 56.63 µg/mL acetic acid and around 25 µg/mL of the other acids (no decanol).

Standards used for the quantitation in µg/mL

Acid	Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6	Std. 7	Std. 8
Acetic	226.50	113.25	56.63	28.31	14.16	7.08	3.54	1.77
Propionic	152.00	76.00	38.00	19.00	9.50	4.75	2.38	1.19
Iso-Butyric	103.25	51.63	25.81	12.91	6.45	3.23	1.61	0.81
Butyric	164.75	82.38	41.19	20.59	10.30	5.15	2.57	1.29
Iso-Valeric	121.50	60.75	30.38	15.19	7.59	3.80	1.90	0.95
Valeric	116.25	58.13	29.06	14.53	7.27	3.63	1.82	0.91
3-Methylvaleric	160.75	80.38	40.19	20.09	10.05	5.02	2.51	1.26
Caproic	134.00	67.00	33.50	16.75	8.38	4.19	2.09	1.05
Heptanoic	142.75	71.38	35.69	17.84	8.92	4.46	2.23	1.12
Caprylic	119.75	59.88	29.94	14.97	7.48	3.74	1.87	0.94

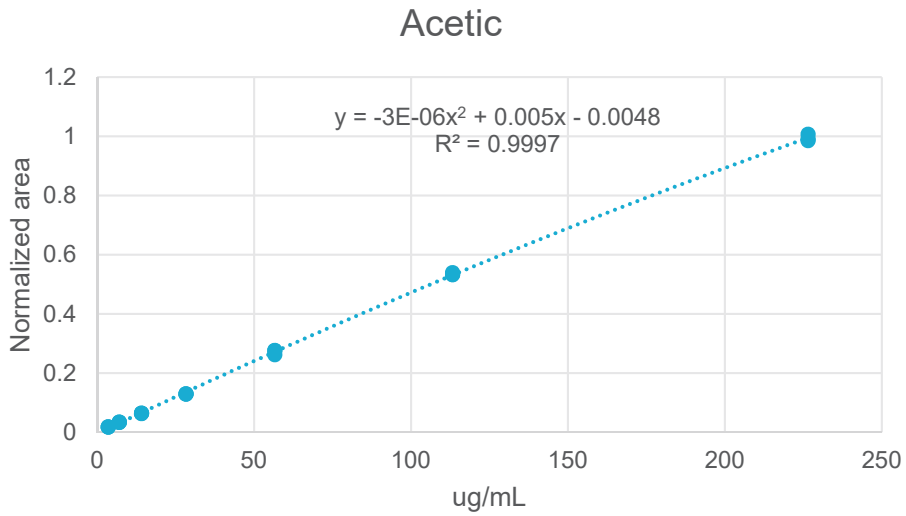
Quantitation using quadratic equations of the form $Y = a X^2 + b X + c$ where Y is the analyte concentration and X is the normalized peak area by the area of the internal standard for the 6890N/5973 GC/MS system

Acid	*a*	*b*	*c*
Acetic	32.1561	194.4075	1.1569
Propionic	-124.8711	387.3005	1.4084
iso-Butyric	-445.7606	505.7048	1.0635
Butyric	-34.2539	166.1949	2.9002
iso-Valeric	-59.8341	179.1659	1.2961
Valeric	-83.9905	195.5604	2.1173
3-Methylvaleric	-64.2050	210.5282	2.2880
Caproic (C6)	-127.5748	257.7616	3.3604
Heptanoic	-214.2129	346.0608	2.7550
Caprylic (C8)	-834.5090	627.3591	2.1256

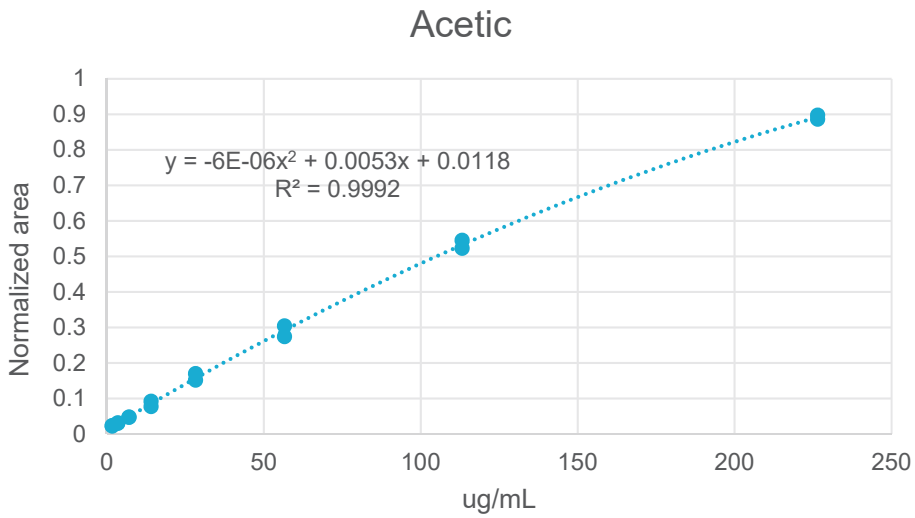
Quantitation using quadratic equations of the form $Y = a X^2 + b X + c$ where Y is the analyte concentration and X is the normalized peak area by the area of the internal standard for the 7890B/5977B (HES) GC/MS system

Acid	*a*	*b*	*c*
Acetic	98.9104	166.8879	-1.7722
Propionic	-306.4462	382.3322	0.1329
iso-Butyric	-230.3076	252.7045	0.4175
Butyric	-27.1954	111.7221	0.2729
iso-Valeric	-34.3868	97.5451	0.5296
Valeric	-64.4255	126.5227	-0.0216
3-Methylvaleric	-33.2636	111.4624	0.7443
Caproic (C6)	-102.3509	168.8759	-0.4925
Heptanoic	-256.7466	274.6734	-1.3843
Caprylic (C8)	-740.2224	435.8324	-2.2187

Calibration curve for acetic acid on the 6890N/5973 GC/MS system.



Calibration curve for acetic acid on the 7890B/5977B (HES) GC/MS system.



Method validation

- ***Specificity/selectivity***. Assured by the GC/MS separation, SIM mode analysis and SPE cleanup for tobacco extracts.
- ***Precision*** using four repeated injections for the standard Std. 6. showed relative standard deviations below 7%.
- ***Accuracy*** was verified using back-calculation of standards for both GC/MS systems. The results were within 5-8% of the level of initial level taken in the standard for each acid.
- ***LOQ and LOD*** are typically based on signal to noise (S/N) ratios between the peak of interest and a specific noise region from baseline. The values for the S/N obtained for Std. 7 as obtained using the 6890N/5973 GC/MS system, and for Std. 8 as obtained using the 7890B/5977B (HES) GC/MS system are given in the table from the next slide. However, measurements outside the calibration range are not recommended because of the non-linearity of the calibrations.
- ***Extraction efficiency*** for the acids from the tobacco was measured only on one sample of Oriental tobacco, by performing the extraction for 30 min, 60 min and 90 min. The results were within 10% RSD for all the analyzed acids.
- ***The SPE recovery*** has been verified for standards.

Signal to noise (S/N) values for the peaks in the chromatogram of Std. 7 as obtained using the 6890N/5973 GC/MS system, and of Std. 8 as obtained using the 7890B/5977B (HES) GC/MS system.

Acid	*S/N Std. 7 6890N/5973*	*S/N Std. 8 7890B/5977B*
Acetic	49.7	234.6
Propionic	24.4	63.4
iso-Butyric	10.2	20.4
Butyric	15.5	120.6
iso-Valeric	15.6	84.4
Valeric	18.8	111.6
3-Methylvaleric	14.3	105.0
Caproic (C6)	15.7	137.7
Heptanoic	14.6	147.7
Caprylic (C8)	13.5	117.2

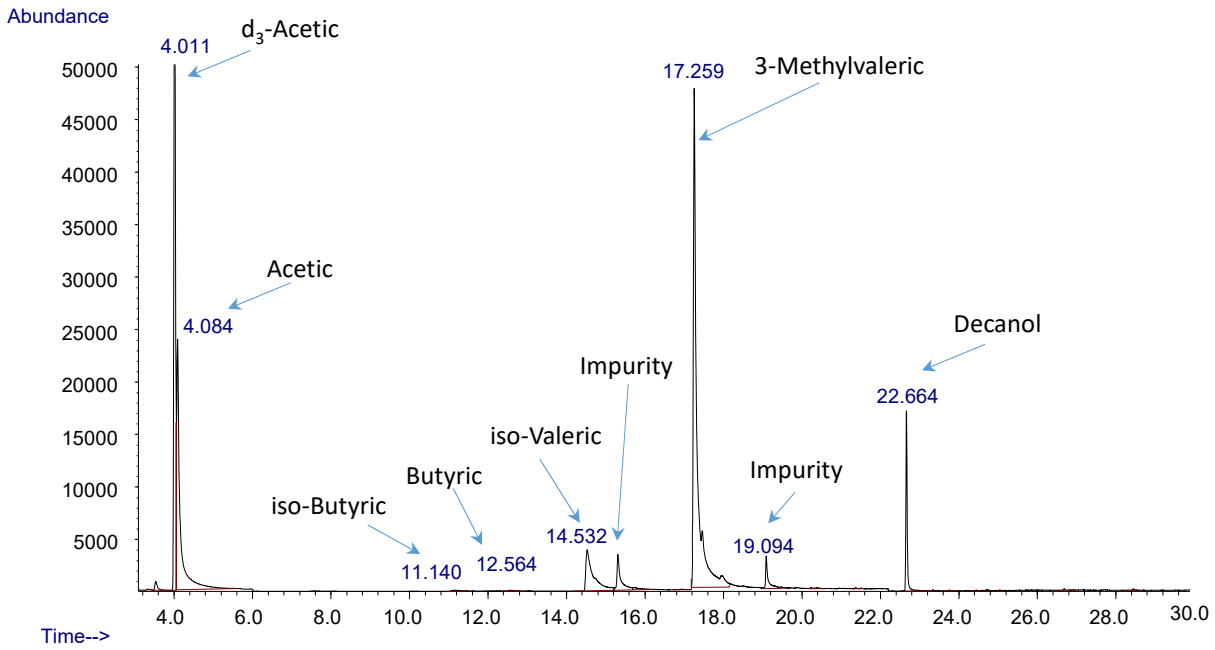
The list of tobaccos analyzed in this study and the moisture level

No	Acronym	Description	Moisture %
1	FC L (1)	Eastern NC belt, lower stalk (lug) flue-cured	8.21
2	FC U (1)	Eastern NC belt, upper stalk (leaf & some tips) flue-cured	9.63
3	FC L (2)	South Carolina belt, lower stalk (lug) flue-cured	10.18
4	FC U (2)	South Carolina belt, upper stalk (leaf & some tips) flue-cured	12.25
5	FC off L	Brazil, lower stalk (lugs & primings) flue-cured	10.45
6	FC off U	Brazil, upper stalk (leaf & tips) flue-cured	9.85
7	Bu L (1)	Kentucky & Tennessee, lower stalk (flyings & cutters) burley	7.89
8	Bu U (1)	Kentucky & Tennessee, upper stalk (leaf) burley	7.63
9	Bu L (2)	North Carolina & Virginia, lower stalk (flyings & cutters) burley	9.31
10	Bu U (2)	North Carolina & Virginia, upper stalk (leaf) burley	8.21
11	Bu off L	Malawi, lower stalk (flyings & cutters) burley	10.8
12	Bu off U	Malawi, upper stalk (leaf) burley	10.63
13	O SA U	Turkey, good quality middle to upper stalk, Samsun Oriental	7.74
14	O Iz U	Turkey, good quality middle to upper stalk, Izmir Oriental	10.2
15	Commercial cig.	Tobacco blend	9.21
16	O Pri	Prilip Oriental	7.52
17	O Kat	Katherini Oriental	8.24
18	O Sa (2)	Old Turkey Samsun (2004)	8.22
19	O Iz (2)	Old Turkey Izmir (2004)	9.12
20	3R4F cig.	Tobacco blend	8.46

The list of e-liquids analyzed in this study

No.	*e-liquid*
1	e-Liq (1)
2	e-Liq (2)
3	e-Liq (3)
4	e-Liq (4)
5	e-Liq (5)
6	E-Liq (6): 65% glycerin, 30% PG, 5% water, 122.03 mg/mL iso-Valeric acid

Chromatogram for an Oriental tobacco sample (Spl. 14) as obtained on the 6890N/5973 GC/MS system



Results in µg/g for the analyzed acids in tobacco samples 1 to 7

Tobacco /	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Compound	*FC L (1)*	*FC U (1)*	*FC L (2)*	*FC U (2)*	*FC off L*	*FC off U*	*Bu L (1)*
Acetic	320.99	367.72	552.49	560.20	255.37	253.19	174.13
Propionic	N.D.*	N.D.	<LOQ	<LOQ	19.14	17.40	17.24
iso-Butyric	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Butyric	<LOQ**	<LOQ	<LOQ	<LOQ	21.09	19.93	18.26
iso-Valeric	<LOQ	11.13	N.D.	12.08	<LOQ	<LOQ	10.12
Valeric	N.D.	N.D.	N.D.	N.D.	<LOQ	<LOQ	<LOQ
3-Methylvaleric	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Caproic (C6)	<LOQ	<LOQ	<LOQ	24.86	<LOQ	<LOQ	<LOQ
Heptanoic	N.D.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Caprylic (C8)	N.D.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

* Note: N.D. indicates Not Detected or, below LOD.
** Note: <LOQ indicates that a trace is present but the measured level is below LOQ.

Results in µg/g for the analyzed acids in tobacco samples 8 to 14

Tobacco /	*8*	*9*	*10*	*11*	*12*	*13*	*14*
Compound	*Bu U (1)*	*Bu L (2)*	*Bu U (2)*	*Bu off L*	*Bu off U*	*O SA U*	*O Iz U*
Acetic	231.51	257.81	369.32	274.39	217.03	252.69	461.10
Propionic	23.27	68.70	<LOQ	22.32	31.11	N.D.	<LOQ
iso-Butyric	<LOQ	11.64	N.D.	<LOQ	N.D.	15.06	17.15
Butyric	23.08	48.92	<LOQ	20.76	35.26	<LOQ	<LOQ
iso-Valeric	15.24	35.95	11.30	18.19	24.22	90.83	110.85
Valeric	<LOQ	22.20	<LOQ	<LOQ	24.56	<LOQ	<LOQ
3-Methylvaleric	<LOQ	13.76	<LOQ	32.46	32.34	812.51	700.80
Caproic (C6)	<LOQ	28.61	<LOQ	22.34	28.07	<LOQ	<LOQ
Heptanoic	<LOQ	<LOQ	N.D.	<LOQ	16.69	<LOQ	<LOQ
Caprylic (C8)	<LOQ	19.31	N.D.	<LOQ	27.82	N.D.	<LOQ

Results in µg/g for the analyzed acids in tobacco samples 15 to 20

Tobacco /	*15*	*16*	*17*	*18*	*19*	*20*
Compound	*Commercial.*	*O Pri*	*O Kat*	*O Sa (2)*	*O Iz (2)*	*3R4F*
Acetic	279.12	294.03	198.17	162.20	161.35	571.34
Propionic	11.59	12.13	20.25	21.20	29.31	18.47
iso-Butyric	N.D.	<LOQ	12.74	19.82	14.62	<LOQ
Butyric	<LOQ	<LOQ	22.59	25.41	26.77	19.89
iso-Valeric	11.78	35.00	121.56	148.19	91.47	12.23
Valeric	<LOQ	<LOQ	<LOQ	15.17	15.57	<LOQ
3-Methylvaleric	38.44	316.02	698.45	736.21	603.35	45.56
Caproic (C6)	<LOQ	32.08	28.48	28.11	34.82	<LOQ
Heptanoic	<LOQ	N.D.	<LOQ	<LOQ	<LOQ	N.D.
Caprylic (C8)	<LOQ	<LOQ	19.52	17.26	22.96	<LOQ

Results in µg/g for the analyzed acids in e-liquid samples obtained using the 6890N/5973 GC/MS system

e-Liquid / compound	*e-Liq (1)*	*e-Liq (2)*	*e-Liq (3)*	*e-Liq (4)*	*e-Liq (5)*	*e-Liq (6)*
Acetic	7.24	395.69	14.48	12.83	25.38	<LOQ
Propionic	<LOQ	<LOQ	51.21	<LOQ	126.43	N.D.
iso-Butyric	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Butyric	56.60	<LOQ	63.11	8.81	157.09	N.D.
iso-Valeric	N.D.	N.D.	N.D.	N.D.	<LOQ	133.50
Valeric	N.D.	<LOQ	<LOQ	N.D.	<LOQ	<LOQ
3-Methylvaleric	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Caproic (C6)	<LOQ	<LOQ	32.46	<LOQ	77.35	N.D.
Heptanoic	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Caprylic (C8)	N.D.	N.D.	<LOQ	N.D.	18.68	N.D.

Results in µg/g for the analyzed acids in e-liquid samples obtained using the 7890B/5977B (HES) GC/MS system

e-Liquid / compound	*e-Liq (1)*	*e-Liq (2)*	*e-Liq (3)*	*e-Liq (4)*	*e-Liq (5)*	*e-Liq (6)*
Acetic	7.87	405.27	15.15	13.65	27.90	N.D.
Propionic	<LOQ	<LOQ	55.25	N.D.	133.38	N.D.
iso-Butyric	<LOQ	N.D.	<LOQ	N.D.	<LOQ	<LOQ
Butyric	56.91	<LOQ	68.07	9.10	163.80	<LOQ
iso-Valeric	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	135.96
Valeric	<LOQ	N.D.	<LOQ	N.D.	<LOQ	<LOQ
3-Methylvaleric	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Caproic (C6)	<LOQ	7.32	33.38	<LOQ	78.21	<LOQ
Heptanoic	N.D.	<LOQ	N.D.	<LOQ	<LOQ	N.D.
Caprylic (C8)	<LOQ	N.D.	<LOQ	<LOQ	19.96	N.D.

Conclusions

- A method for the analysis of several short chain organic acids including acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, 3-methylvaleric, caproic, heptanoic and caprylic in tobacco and e-liquids has been developed and validated.
- The analysis was performed on two different GC/MS systems, a 6890N/5973 GC/MS system, and a 7890B/5977B GC/MS with High Efficiency Source (HES) with the sensitivity about ten times higher on the second system.
- 20 tobaccos and six different e-liquids were successfully analyzed for short chain organic acids by this procedure.
- The analysis of tobaccos involved a cleanup procedure using a Strata SAX 500 mg/3mL SPE cartridge.
- The results were very similar between the analysis on 6890N/5973 GC/MS and the 7890B/5977B GC/MS (HES) systems regarding the levels of acids in either the 20 tobaccos or the six different e-liquid samples with less than 10% differences between the results on the two GC/MS systems.