Analysis of the ‘butter-type‘ flavor compounds diacetyl, acetoin, and 2,3-pentanedione in e-cigarette liquids

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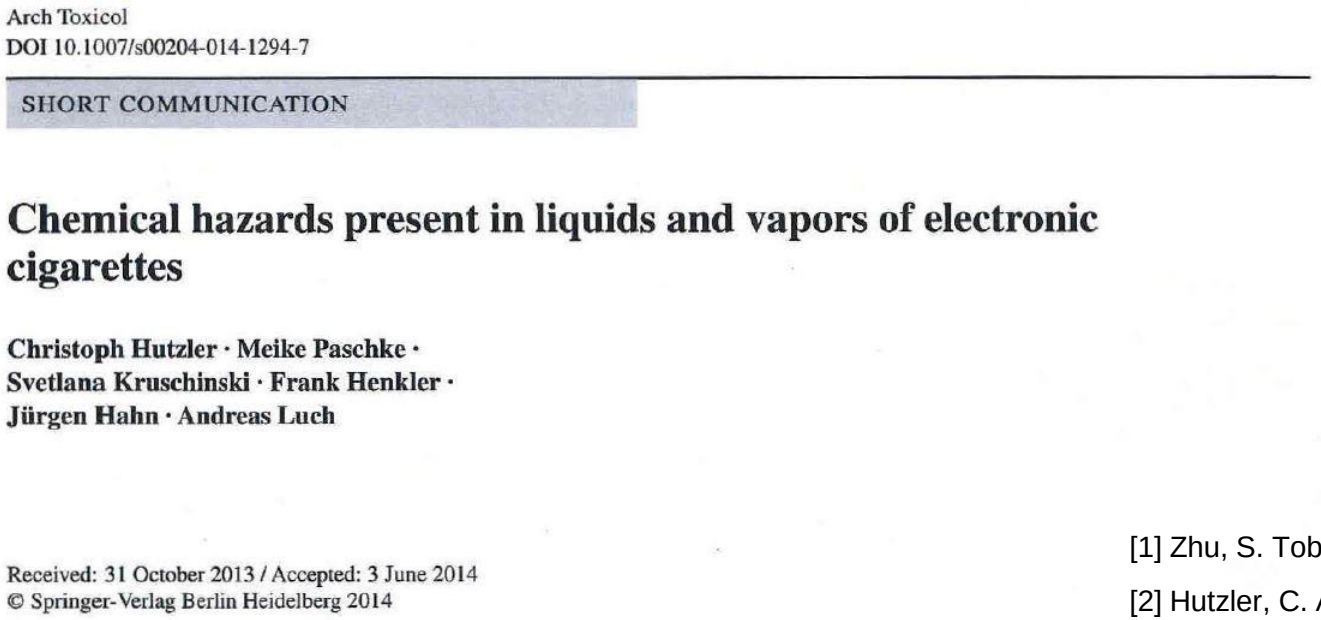
Flavors in e-liquids

- Use of flavors and flavor mixtures depends on the desired taste of the e-cigarette liquid (e-liquid)
- Large variety of flavors currently used in e-liquids: > 7000 e-cig flavors[1]
- German Federal Institute of Risk Assessment (BfR) screened e-cig flavors[2]



Arch Toxicol
DOI 10.1007/s00204-014-1294-7

SHORT COMMUNICATION

Chemical hazards present in liquids and vapors of electronic cigarettes

Christoph Hutzler · Meike Paschke · Svetlana Kruschinski · Frank Henkler · Jürgen Hahn · Andreas Luch

Received: 31 October 2013 / Accepted: 3 June 2014

[1] Zhu, S. Tobacco Control **2014**

[2] Hutzler, C. Archives of Toxicology **2014**

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No.	Compound name	Frequency in 28 e-cigarette liquids	MW (g/mol)	BP (°C)	Case no.
1	Vanillin	22	152	285–286	121-33-5
2	Ethyl maltol	16	140	161	4940-11-8
14	Citral	5	152	229	5392-40-5
15	Corylon	5	112	245	80-71-7
16	Anisaldehyde propylene glycol acetal	4	194	287–289	6414-32-0
17	Benzaldehyde	4	106	178	100-52-7
18	Benzyl benzoate	4	212	323	120-51-4
19	Coumarin	4	146	302	91-64-5
20	γ-Octalactone	4	142	239	104-50-7

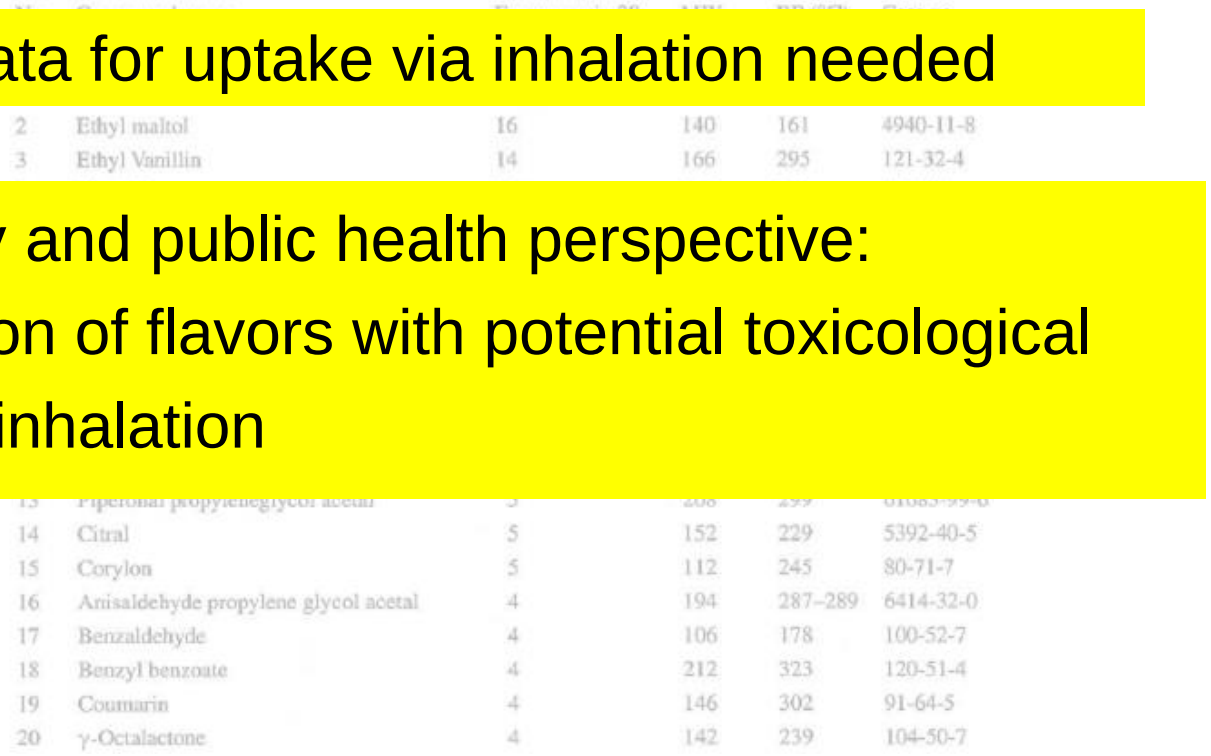
141 additives detected in 28 e-liquids

[1] Zhu, S. Tobacco Control **2014**

[2] Hutzler, C. Archives of Toxicology **2014**

Flavors in e-liquids

- Applied flavors ‘generally regarded as safe’ (GRAS) for oral uptake
- Metabolic fate after inhalation unknown
- Concerns raised on potential health risks



2	Ethyl maltol	16	140	161	4940-11-8
3	Ethyl Vanillin	14	166	295	121-32-4
14	Citral	5	152	229	5392-40-5
15	Corylon	5	112	245	80-71-7
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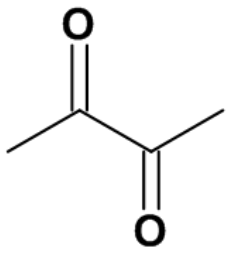
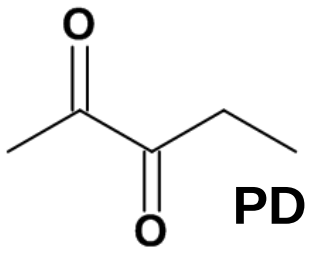
Reliable data for uptake via inhalation needed

Regulatory and public health perspective:
Identification of flavors with potential toxicological risks after inhalation

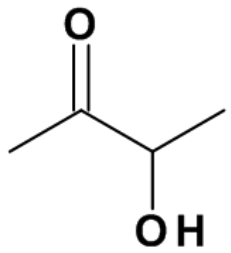
Diacetyl, pentanedione and acetoin



DA



PD



AC

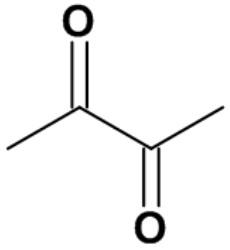
2,3-Butanedione = **Diacetyl** **2,3-Pentanedione** 3-Hydroxy-2-butanone = **Acetoin**

- Use as ‘butter-type’ flavor e.g. in microwave popcorn production
- Diacetyl, pentanedione, acetoin approved for ingestion but associated with chronic bronchitis and other respiratory diseases
- Strong association between disease state and inhalation in workers exposed to DA, PD, AC
- Disease state (bronchiolitis obliterans) in those workers referred to as “popcorn lung”[3]

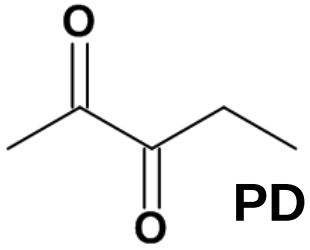
➢ NIOSH inhalation exposure limits for DA, PD published in 2011

[3] Allen, Environmental Health Perspectives **2016**

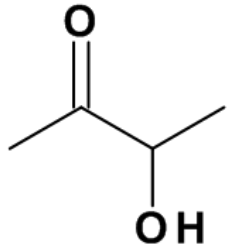
Diacetyl, pentanedione and acetoin



DA



PD



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3-Hydroxy-2-butanone = **Acetoin**

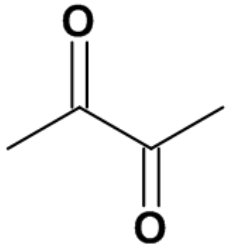
Flavoring Chemicals in E-Cigarettes: Diacetyl, 2,3-Pentanedione, and Acetoin in a Sample of 51 Products, Including Fruit-, Candy-, and Cocktail-Flavored E-Cigarettes

Joseph G. Allen, Skye S. Flanigan, Mallory LeBlanc, Jose Vallarino, Piers MacNaughton, James H. Stewart, and David C. Christiani

Harvard T.H. Chan School of Public Health. Boston. Massachusetts. USA

Environmental Health Perspectives • VOLUME 124 | NUMBER 6 | June 2016

Diacetyl, pentanedione and acetoin



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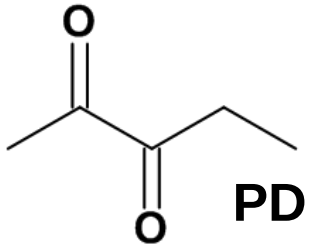
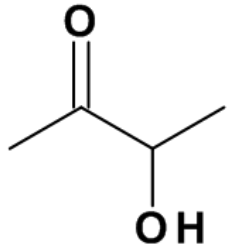


PD



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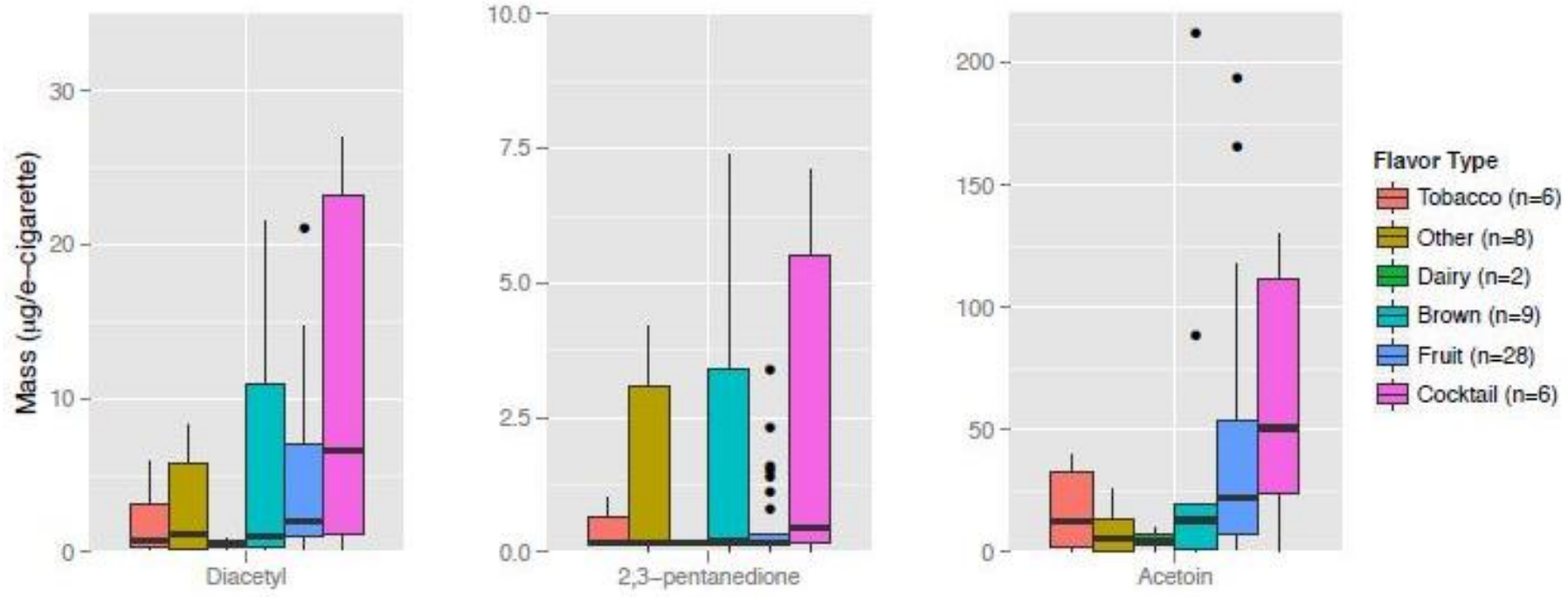
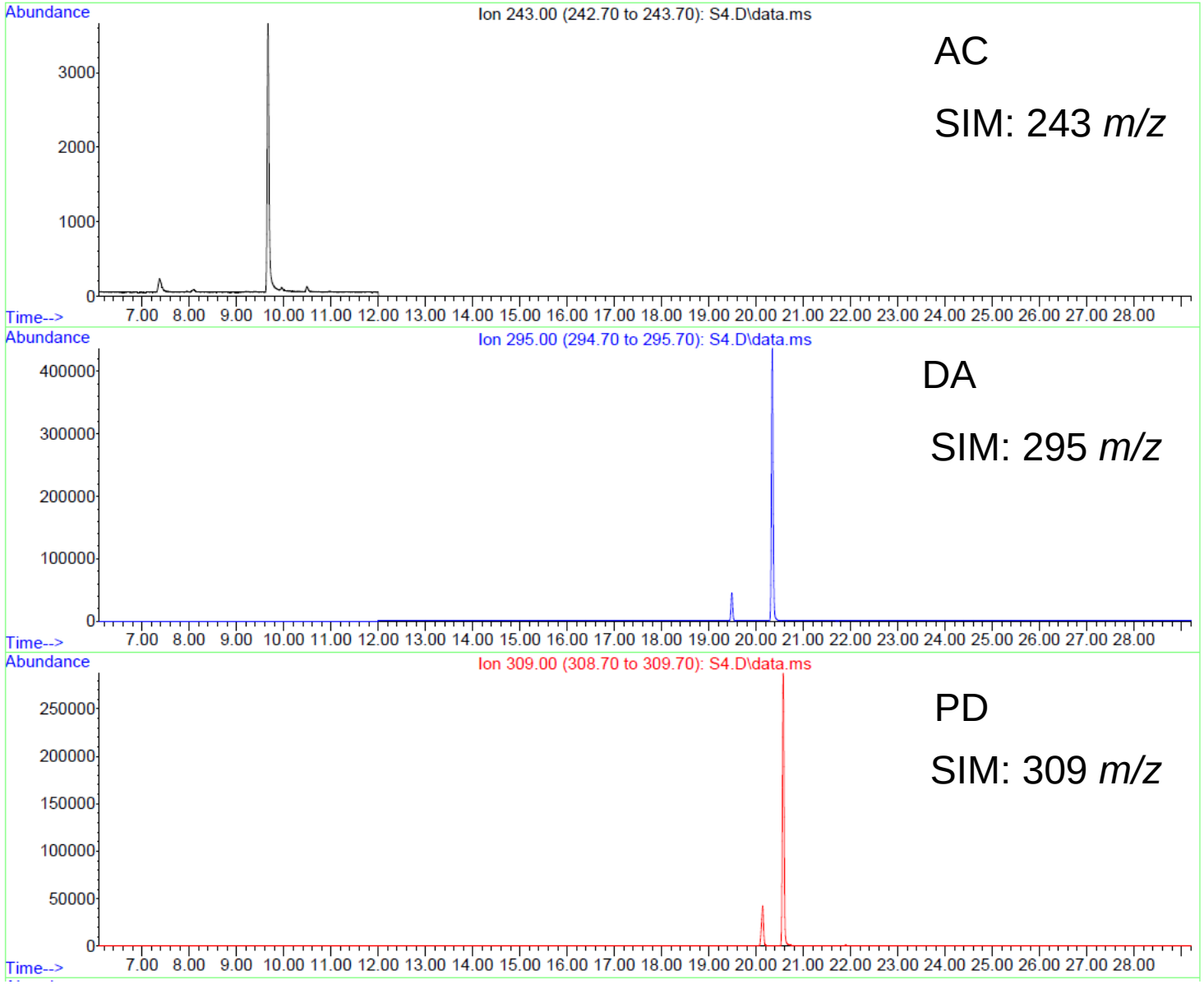


NCI-GC-MS anaylsis

- 100 – 120 mg of liquid, accurately weighed, diluted with 300 µl water
- + 20 µl internal std solution
- Derivatization with PFBHA
- LLE with hexane; acidification with $H_2SO_{4(conc.)}$
- Injection of 1µl of org. phase into GC-MS system (Agilent)
- GC column: Rxi-17, 30m x 0.25mm, 0.25µ (Resteck)
- NCI (CH_4); Selected Ion Monitoring (SIM)

Analyte	Retention time / min	Quantifier / m/z	Qualifier / m/z
Acetoin	9.7	243	-
Diacetyl	20.3	295	276
Diacetyl-d_6	20.2	301	282
2,3-Pentanedione	20.6	309	290
2,3-Pentanedione-d_5	20.5	314	295

Chromatography



AC

SIM: 243 *m/z*

DA

SIM: 295 *m/z*

PD

SIM: 309 *m/z*

Calibration matrix: PG/VG 50/50

Spike: 1.6 µg/g liquid

Method validation

- Assessment of method performance parameters according to FDA guidelines for bioanalytical method validation:
  - ✓ Linearity and calibration range: in PG/VG 50/50 (dev. from target: ±15%)
  - ✓ Sensitivity: LLOQ determined for 3 different e-liq spiked at LLOQ
  - ✓ Accuracy / Precision: 5 / 6 independent sample preparations (Acc./CV: ±15%)
  - ✓ Reproducibility after reinjection and carryover

- Stability:
  - ✓ short-term stability: 7 days at r.t.
  - ✓ post-preparative stability: 5 days at 10°C in autosampler

Method validation

Method validation:

- Assessment of method performance parameters according to FDA guidelines for bioanalytical method validation:

Validation initially performed using 1 ISTD: DA-d_6

  - Reproducibility after reinjection and carryover

- Stability:
  - ✓ short-term stability: 7 days at r.t.
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Precision

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Analyte	Level	Precision (CV) intraday	Precision (CV) interday	Accuracy
Diacetyl	Low	1.3 %	4.1 %	
	Med	4.4 %	4.5 %	
	High	6.9 %	8.0 %	
Pentanedione	Low	1.0 %	4.9 %	
	Med	3.6 %	3.9 %	
	High	7.4 %	8.8 %	
Acetoin	Low	3.4 %	6.7 %	
	Med	2.4 %	7.1 %	
	High	6.1 %	8.7 %	

⇨acceptance criteria were met for intraday and interday precision

Accuracy

6 sample preps spiked with the analytes at low, med, high

independent sample preparations for 3 different levels (low, med, high)
⇨different e-liquids from different suppliers were used for each level to determine the influence of the matrix (different flavors, different PG/VG composition) on accuracy of the method: six different liquids were used per level

Pentanedione	Low	1.0 %	4.9 %	95.7 %
	Med	3.6 %	3.9 %	85.5 %
	High	7.4 %	8.8 %	80.3 %
Acetoin	Low	3.4 %	6.7 %	74.6 %
	Med	2.4 %	7.1 %	87.7 %
	High	6.1 %	8.7 %	89.9 %

⇨Accuracy of DA: acceptance criteria met for all levels

⇨Accuracy of PD: acceptance criteria met for low and med

⇨Accuracy of AC: acceptance criteria met for med and high

Accuracy

6 sample preps spiked with the analytes at low, med, high

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		%	8.8 %	80.3 %
		3.4 %	6.7 %	74.6 %
	Med	2.4 %	7.1 %	87.7 %
	High	6.1 %	8.7 %	89.9 %

⇨A cy of DA: acceptance criteria met for all levels

⇨Accuracy of PD: acceptance criteria met for low and med

⇨Accuracy of AC: acceptance criteria met for med and high

Accuracy – Influence of the ISTD

Matrix-dependent variability in accuracy exemplified for medium level:
6 different e-liquid formulations were spiked with the analytes at medium level

Liquid	Accuracy		
	Diacetyl	Pentanedione	Acetoin
L4	107.4%	82.4%	79.3%
L13	109.6%	98.6%	108.6%
L21	103.4%	93.7%	100.3%
L23	107.0%	89.1%	85.2%
L27	105.1%	88.3%	76.0%
L33	104.9%	61.0%	76.8%

- Quantification most accurate for DA ⇨ correction of matrix-effects by ISTD DA-d_6
- 4/6 liq. with acceptable accuracy for PD, even when quantified with DA-d_6
- Sufficient accuracy only for 3/6 liq. in case of AC
- DA and PD similar reactivity (derivatization of both carbonyl moieties) ⇨ similar polarity
- Most differences for AC due to differences in chemical structure (only one carbonyl group)
- ISTD DA-d_6 more suitable for DA/PD

Accuracy – Influence of the ISTD

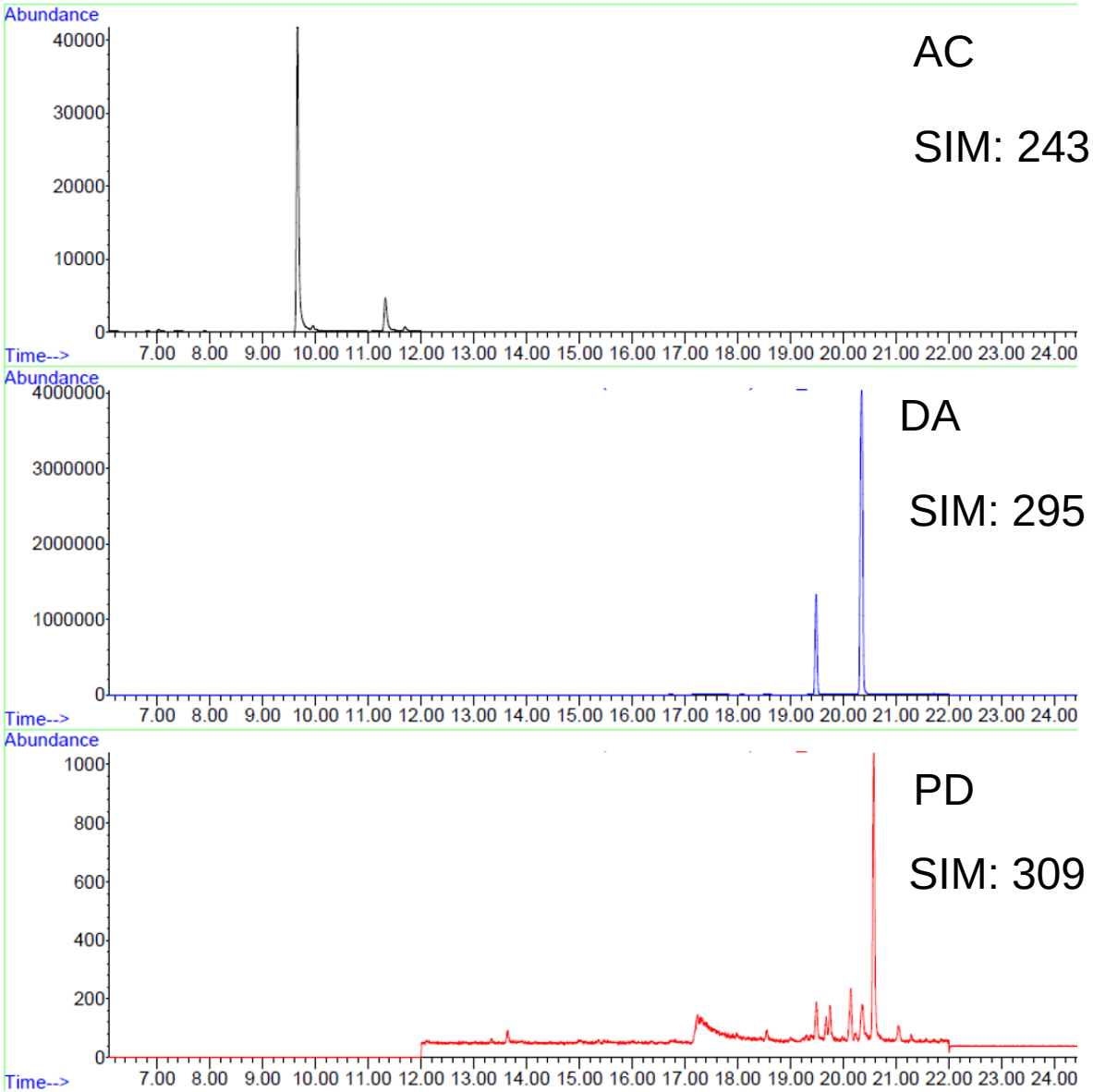
Investigation of accuracy repeated with ISTD for PD (PD-d_5)

Liquid	Accuracy		
	Diacetyl	Pentanedione	Acetoin
L4	107.4%	91.2%	79.3%
L13	109.6%	94.3%	108.6%
L21	103.4%	95.0%	100.3%
L23	107.0%	99.1%	85.2%
L27	105.1%	100.8%	76.0%
L33	104.9%	90.3%	76.8%

- Improved accuracy for PD with the authentic internal standard
- Purchase of authentic ISTD Acetoin-$^{13}C_4$ for AC ongoing
- Validation experiments to be repeated with authentic ISTDs also for acetoin

Analysis of 38 e-liquids using the new analytical method

Analysis of 38 e-liquids: Results (I)



AC

SIM: 243 *m/z*

DA

SIM: 295 *m/z*

PD

SIM: 309 *m/z*

Chromatogram of e-liquid “L32”:

Flavor: “waffle”; manufactured in Poland

Acetoin:	66.3 µg/g
Diacetyl:	177.5 µg/g
Pentanedione:	0.05 µg/g

NIOSH limit DA: 65 µg
NIOSH limit PD: 137 µg
LLOQ: 0.013 µg/g

Analysis of 38 e-liquids: Results (II)

Liquid	AC (µg/g)	DA (µg/g)	PD (µg/g)	Liquid	AC (µg/g)	DA (µg/g)	PD (µg/g)
3	0.0196	0.053	0.078	27	<LLOQ	0.034	<LLOQ
4	0.0184	0.041	<LLOQ	28	0.0174	0.037	<LLOQ
5	0.1427	0.159	0.607	29	<LLOQ	0.032	0.024
6	0.8518	0.073	<LLOQ	30	0.0410	0.057	<LLOQ
7	17.4598	58.456	<LLOQ	31	0.0264	0.518	<LLOQ
8	0.0419	0.827	<LLOQ	32	66.3392	177.522	0.051
9	0.0301	0.027	0.066	33	0.0066	0.028	<LLOQ
10	0.8434	0.101	<LLOQ	38	n.a.	11.550	0.035
13	<LLOQ	0.035	0.014	39	n.a.	0.114	0.026
14	1.1060	0.274	<LLOQ	41	n.a.	0.040	<LLOQ
15	2.1406	0.210	<LLOQ	42	n.a.	<LLOQ	<LLOQ
18	0.0304	0.523	<LLOQ	44	n.a.	0.023	<LLOQ
19	0.0365	0.077	0.168	46	n.a.	6.949	<LLOQ
21	<LLOQ	<LLOQ	<LLOQ	48	n.a.	0.335	<LLOQ
22	<LLOQ	0.031	<LLOQ	49	n.a.	0.561	<LLOQ
23	0.0149	0.017	<LLOQ	51	n.a.	7.078	0.029
24	<LLOQ	0.033	<LLOQ	57	n.a.	43.649	0.025
25	0.0900	0.051	0.035	62	n.a.	<LLOQ	0.032
26	0.0390	0.193	0.054	63	n.a.	<LLOQ	<LLOQ

Analysis of 38 e-liquids: Results (III)

Statistics (N = 38)	AC (µg/g)	DA (µg/g)	PD (µg/g)
Mean	3.44	8.15	0.04
SD	13.3	30.6	0.10
Median	0.03	0.07	0.01
90 percentile	6.74	14.76	0.07
Maximum	66.34	177.52	0.61
N > LLOQ	19 (73 %)	34 (89 %)	14 (37 %)
> NIOSH	n.a.	3 (8 %)	0

LLOQ: 0.013 µg/g liquid
NIOSH DA: 65 µg (8 hr exposure in workers)

Conclusion

- Development of an NCI-GC-MS method for the simultaneous quantification of diacetyl, 2,3-pentanedione and acetoin

- Initial validation with only one available ISTD showed very promising results
  - ✓ large linear range
  - ✓ sufficient sensitivity for risk assessment after inhalation
  - ✓ highly precise (robust derivatization)
  - ✓ highly accurate in case of DA for quantification with authentic internal standards

- Improved accuracy for PD after quantification with authentic ISTD PD-d_5

Conclusion

- Application to 38 e-liquids revealed numerous “hits” for the flavors DA, PD, AC

- DA / AC were quantifiable in majority of e-liquids
  - ➢ Sufficient sensitivity of the method proven

- NIOSH limit applied for risk evaluation assuming a consumption of 1.5 - 2g e-liq/d
  - ➢ 3 e-liquids exceeded NIOSH limit for DA
  - ➢ Flavor characteristics: “waffle”, “vanilla”, “caramel” → DA/AC present in sweet flavors

- ➢ High levels of DA may contribute to health risk after inhalation

- ➢ Toxicological assessment for respiratory risks of GRAS additives needed

- ➢ Quantitative analysis for Quality Control of e-liquids needed for risk evaluation

Acknowledgment

Kirsten Riedel

Dr. Max Scherer

Prof. Gerhard Scherer

Thank you for your attention

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