Analytical methods for the determination of carbonyls in aerosol from e-vapor products and cigarette smoke and their urinary biomarkers of exposure

Landmesser A. (1, 2); Scherer M. (1); Scherer G. (1); Pluym N. (1)

(1) Analytisch-Biologisches Forschungslabor (ABF) GmbH, Semmelweisstraße 5, Planegg, Germany

(2) Chair for Analytical Chemistry, Technische Universität München, Marchioninstraße 17, 81377 Munich, Germany

Introduction

Potential risks associated with the formation of aldehydes, such as acrolein, formaldehyde or acetaldehyde, during the consumption of e-vapor products (EVPs) are of current interest in the scientific and public health community. The presence of aldehydes in EVP aerosol was mostly attributed to their formation from the e-liquid constituents propylene glycol (PG) and glycerol (G).

An e-liquid, containing stable isotope-labelled PG and G, and conventional non-filter cigarettes spiked with the same labelled compounds, serving as a positive control, were used to assess the formation of aldehydes in EVP aerosol as well as in mainstream smoke of spiked cigarettes. Methods were also developed for related metabolites in urine (mercapturic acids, glutathione adducts) for a clinical study using these isotype-labeled products.

Mass selective detection allows the unequivocal discrimination between labelled and unlabeled compounds. Hence, analysis of the labelled aldehydes by means of LC-MS/MS revealed the amounts exclusively formed from PG and G during vaping/smoking and their metabolism in the human body. Aerosol analysis comprised the simultaneous detection of 7 aldehyde derivatives after trapping with 2,4-dinitrophenylhydrazine (DNPH) solution. Urinary bioanalysis included the mercapturic acids formed from acrolein and crotonaldehyde using previously developed assays, while metabolites of formaldehyde (thiazolidine-4-carboxylic acid (TCA)) and acetaldehyde (methyl-thiazolidine-4-carboxylic acid (MTCA)) were investigated by means of a newly developed and validated LC-MS/MS method.

Determination of carbonyls in aerosol using LC-MS/MS

Carbonylic compounds were analyzed both in their labeled and unlabeled form in the aerosol/smoke by means of a validated LC-MS/MS method. The method covers 7 carbonyls, namely formaldehyde, acetaldehyde, acrolein, acetone, propanal, crotonaldehyde and methacrolein.

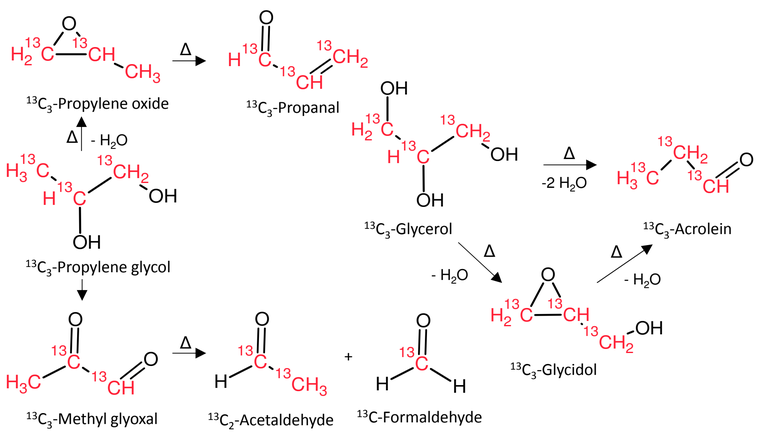

Figure 1: Predicted Formation of the carbonyls from propylene glycol and glycerol. (Adapted from [1])

Sample preparation

- Trapping of the carbonyls in 2 impingers containing 20 ml DNPH-solution (2.5 mM) with perchloric acid (2.7 mM)
- Trapping: 1 cigarette or 20 puffs of an e-cigarette (puff duration: 4 sec, puff interval: 30 sec)
- 10 µL internal standard (FA-DNPH-d_3, AA-DNPH-d_3, ACR-DNPH-d_3, Acetone-DNPH-d_3, Propanal-DNPH-d_3, CRO-DNPH-d_3, MACR-DNPH-d_3) are spiked to 100 µL undiluted and diluted (1:10, 1:000) DNPH-Solution

Analytical method

- LC-MS/MS: Sciex 6500$^+$Qtrap (MS); Shimadzu Nexera X2 (HPLC)
- Chromatographic separation (Figure 2):

  Phenomenex Kinetex EVO C18 (150 x 2.1 mm, 5 µm)

  Injection volume: 5 µL

  Eluents: water (A) – ACN (B); Flow rate: 0.6 mL/min

  Gradient: 0-5.0 min: 35-55% B; 5.0-7.0 min: 70% B; 7.0-7.1 min: 70-35% B; 7.1 – 10. min: 35% B

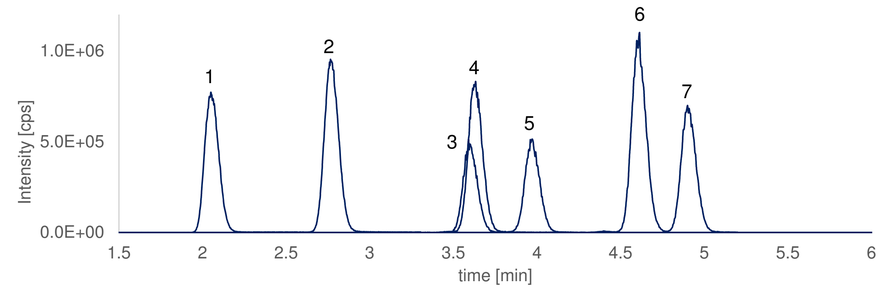

Figure 2: Chromatographic separation of DNPH-Carbonyls. The numbers are explained in Table 1.

Table 1: MS parameters (IS: internal standard, RT: retention time) for the Carbonyl-DNPH-derivatives.

Analyte	MRM [*m/z*]	IS (MRM) [*m/z*]	RT [min]	LLOQ [ng/ml]
^{13}C-Formaldehyde (1)	210 → 164	Formaldehyde-DNPH-d_3 (212 → 166)	2.1	0.10
$^{13}C_2$-Acetaldehyde (2)	225 → 151	Acetaldehyde-DNPH-d_3 (226 → 166)	2.8	0.10
$^{13}C_3$-Acrolein (3)	238 → 164	Acrolein-DNPH-d_3 (238 → 166)	3.6	0.10
$^{13}C_3$-Acetone (4)	240 → 151	Aceton-DNPH-d_3 (240 → 154)	3.7	0.10
$^{13}C_3$-Propanal (5)	240 → 164	Propanal-DNPH-d_3 (240 → 166)	4.0	0.10
$^{13}C_{2/4}$-Crotonaldehyde (6)	251/253 → 173	Crotonaldehyde-DNPH-d_3 (252 → 175)	4.6	0.10
$^{13}C_4$-Methacrolein (7)	253 → 164	Crotonaldehyde-DNPH-d_3 (252 → 175)	4.9	0.10

Newly developed and validated LC-MS/MS method for the biomarkers of formaldehyde (TCA) and acetaldehyde (MTCA)

As yet, no biomarkers for the smoking-related exposure to formaldehyde and acetaldehyde are available. The reaction products of formaldehyde and acetaldehyde with cysteine, namely TCA and MTCA, respectively, are excreted into the urine.

We successfully developed a method for the urinary analysis of TCA and MTCA by means of LC-MS/MS. The method was validated according to FDA guidelines and showed excellent results in terms of accuracy, precision lower limit of quantification and robustness. However, a question still remains about the long-term stability of MTCA in urine which is currently under evaluation.

The method was applied to the urine study samples in their labeled and unlabeled form.

Sample preparation

- Urine samples spiked with internal standard ($^{13}C_3$-^{15}N-TCA, $^{13}C_3$-^{15}N-MTCA)
- Solid-phase extraction using Oasis MCX (Waters)
- Evaporation of the organic phase and resolve the residue in waters
- Derivatisation with propyl chloroformate and propanol
- Liquid-liquid-extraction with isooctane
- Evaporation of the organic phase
- Reconstitution of the residue in methanol

Table 2: MS parameters (IS: internal standard, RT: retention time) for ^{13}C-TCA and $^{13}C_2$MTCA

Analyte)	MRM [*m/z*]	IS (MRM) [*m/z*]	RT [min]	LLOQ [ng/ml]
TCA	262 → 174	$^{13}C_3$-^{15}N-TCA (266 → 177)	4.1	0.5
MTCA	276 → 188	$^{13}C_3$-^{15}N-MTCA (280 → 191)	5.1	0.5
^{13}C-TCA	263 → 175	$^{13}C_3$-^{15}N-TCA (266 → 177)	4.1	0.5
$^{13}C_2$-MTCA	278 → 190	$^{13}C_3$-^{15}N-MTCA (280 → 191)	5.1	0.5

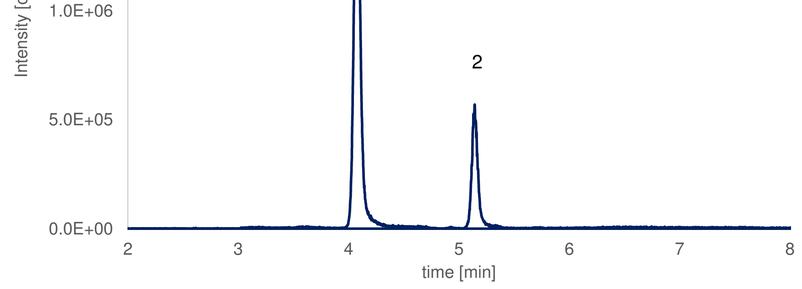

Figure 4: Chromatographic separation of ^{13}C-TCA (1) and $^{13}C_2$-MTCA (2).

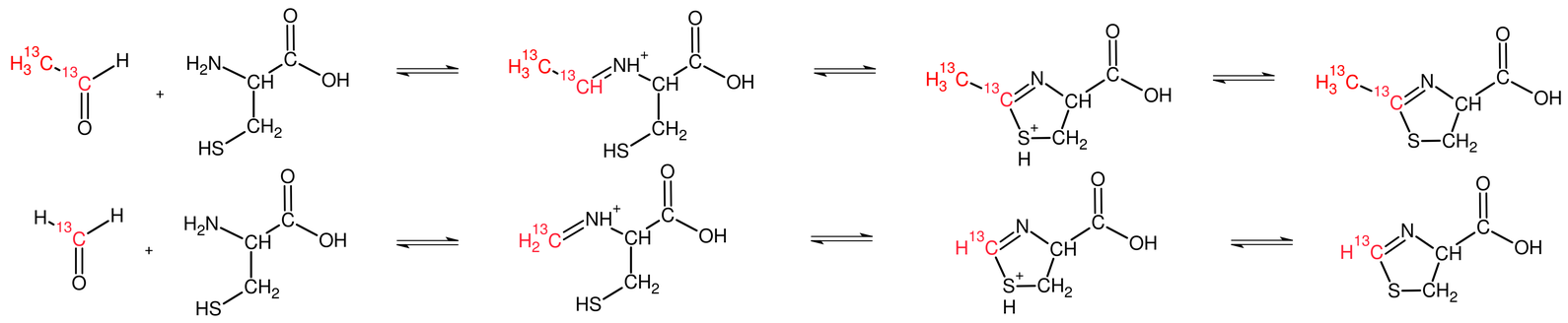

Figure 3: Biotransformation of formaldehyde and acetaldehyde to TCA (thiazolidine-4-carboxylic acid) and MTCA (methyl-thiazolidine-4-carboxylic acid). (Adapted from [2])

Analytical method

- LC-MS/MS: Sciex 6500$^+$Qtrap (MS); Shimadzu Nexera X2 (UPLC)
- Chromatographic separation (Figure 4):

  Phenomenex Kinetex EVO C18 (150 x 2.1 mm, 5 µm)

  Injection volume: 10 µL

  Eluents: 0.1% ammonium acetate buffer (A) – ACN with 0.1% formic acid (B);

  Flow rate: 0.7 mL/min

  Gradient: 0-1.0 min: 30% B; 1.0-5.0 min: 30-48% B; 5.0-5.5 min: 48% B; 5.5-6.0 min: 70% B, 6.0-8.0 min: 70% B, 8.0-11.0 min: 30% B

Adaption of an existing LC-MS/MS method to the analysis of $^{13}C_3$-labelled mercapturic acids (3-HPMA, 2-HPMA, DHPMA, HMPMA, HEMA)

Acrolein and glycidol as well as crotonaldehyde are of particular interest due to their possible pyrolytic formation from G and PG according to Figure 5. The aforementioned toxicants are detoxified mainly by formation and urinary excretion of mercapturic acids (MAs). With the precursors in Figures 5 and 7, the following MAs are to be expected:

Acrolein: *N*-Acetyl-*S*-(3-hydroxy-$^{13}C_3$-propyl)cysteine ($^{13}C_3$-3-HPMA);

Glycidol: *N*-Acetyl-*S*-(2,3-hydroxy-$^{13}C_3$-propyl)cysteine ($^{13}C_3$-DHPMA);

Crotonaldehyde: *N*-Acetyl-*S*-(3-hydroxy-$^{13}C_3$-propyl-1-methyl)-L-cysteine ($^{13}C_{2/4}$-HMPMA).

The adaption of the published methods [3] are shortly described:

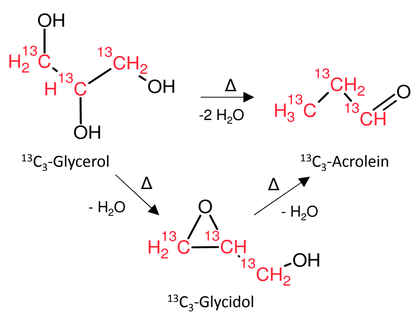

Figure 5: Selected thermal degradation products of G according to [3]. The corresponding MAs are given in brackets.

Sample preparation (MA Method 1)

- Urine sample spiked with internal standard ^{15}N, $^{13}C_3$-3-HPMA, DHBMA-d_7)
- Evaporation to dryness
- Reconstitution of residue in methanol

Analytical method (MA Method 1)

- LC-MS/MS: Sciex 6500$^+$Qtrap (MS); Shimadzu Nexera X2 (UHPLC)
- Chromatographic separation (Figure 6):

  Waters Acquity UPLC BEH HILIC (150 x 3 mm; 1.7 µm)

  Injection volume: 2 µL

  Eluents: ammonium acetate buffer (5mM) (A) – acetonitrile with 5 % ammonium acetate buffer (100 mM) (B)

  Gradient: 0-0.5 min: 1% A; 14 min: 1-7% A; 14.01-16 min: 50% A; 16.01-21 min: 95% A ;

  Flow rate: 0.7 mL/min

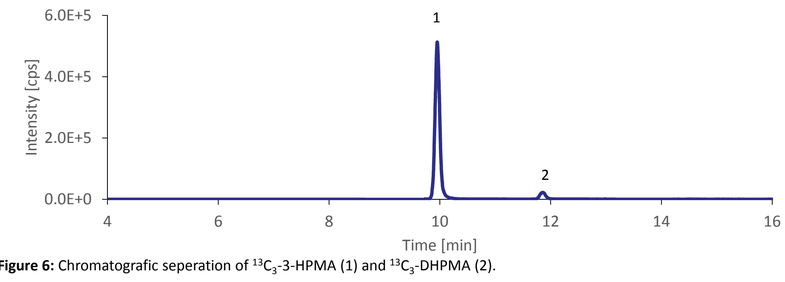

Figure 6: Chromatografic seperation of $^{13}C_3$-3-HPMA (1) and $^{13}C_3$-DHPMA (2).

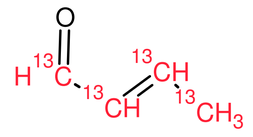

Figure 7: Structure of ^{13}C-labelled crotonaldehyde.

Sample preparation (MA Method 2)

- Urine sample spiked with internal standard (HEMA-d_3, HMPMA-d_3)
- Acidic hydrolysis
- Samples buffered (pH = 2.5)

Analytical method (MA Method 2)

- LC-MS/MS: Sciex API 5000 (MS); Agilent 1200 and 1100 (HPLC)
- Chromatographic separation:

  RAM-Phase: LiChrospher RP-8 ADS (25 x 4 mm; 25 µm)

  Phenomenex Luna C8 (150 x 4.6 mm; 3 µm)

  Injection volume: 50 µL

  Eluents: A & C: water with 0,1% formic acid, B: acetonitrile with 0.1% formic acid; D: 40% Eluent A; 60% Eluent B

  Gradient: 0-1.3 min: 88% A; 1.3-6 min: 88-82% A; 6-10 min: 82-52% A; 10-14 min: 52-5% A; 14-15 min: 5% A; 15-16.5 min: 0% A 10-14 min: 16.5-18 min: 88% A; RAM-Phase: 0-5 min: 100% C; 7-10 min: 0% C; 12-18 min: 100% C Flow rate: 0.7 mL/min

Conclusions

The methods have been developed and are suitable to differentiate between labelled and unlabeled aldehydes and therefore allowed for the analysis of aldehydes specifically formed due to the degradation of PG / G under vaping and smoking conditions as well as the excretion of the respective metabolites in urine.

References:

[1] Sleiman, M., *et al., Environ. Sci. Technol.* 50, 17, 9644-9651, **2016**; [2] Reischl, R., et al., Anal Bioanal Chem. Vol 404, , 1779-1787, **2012** ;
[3] Pluym, N., *et al., Anal Bioanal Chem*. Vol 407, 18, 5463-5476, **2015**.