

Application Note

Determination of Trace Mono-Carbonyl Compounds in E-Cigarette Aerosols by LC-MS

Jeff Zhu, Aaron Heredia, Emily R Britton, Narendra Meruva

ITG Brands LLC, Waters Corporation



Abstract

This application note describes a simplified solution for the analysis of mono-carbonyls at low levels in complex aerosol sample matrices with good sensitivity and selectivity using a UPLC coupled to single quadrupole mass spectrometer (ACQUITY QDa Mass Detector).

Benefits

- Sensitive and selective detection using a single quadrupole mass spectrometer (ACQUITY QDa Mass Detector) of complex aerosol sample matrices with simple settings on the detector
- Simplified sample preparation procedure without phosphoric acid added to the DNPH extraction solution and without the use of internal standards
- Robust method with daily testing of up to 80 e-cigarette aerosol samples over a period of multiple weeks with minimal instrument maintenance and no loss in method performance metrics, such as system suitability, calibration curve, and S/N at LOQ
- Potential applications to other sample matrices with no or low carbonyl amounts such as e-liquid, 'heat not burn' products, and smokeless tobacco products

Introduction

E-cigarettes are battery-powered devices that deliver an aerosol to the user by heating an e-liquid of known chemical composition, which typically contains propylene glycol and/or glycerol, and may contain nicotine and flavorings. During the heating process, low levels of thermal degradation products such as mono-carbonyl compounds (formaldehyde, acetaldehyde, acrolein, and crotonaldehyde) may be formed. These mono-carbonyl compounds are of interest for e-vapor products testing based on recent guidance from FDA-CTP.¹ In recent years, various methods have been reported for the analysis of these compounds at low levels by LC-MS, LC-MS/MS, and GC-MS.²⁻⁴ Herein, we describe a simplified and robust LC-MS method developed and validated to quantitate mono-carbonyls in e-cigarette aerosols at low levels. Using this method, the free carbonyls are converted to DNPH-derivatives in solution during the aerosol collection. A UPLC system coupled with a single quadrupole mass spectrometer (ACQUITY UPLC H-Class PLUS System with ACQUITY QDa Mass Detector) is used for the analysis.

Analytical methods used to measure carbonyls in conventional tobacco cigarette smoke (e.g., CORESTA CRM 74) use high-performance liquid chromatography with ultraviolet detection (HPLC-UV). While UV detection is a robust, cost-effective, and simple method for analysis of carbonyls in conventional tobacco cigarettes, it has limited sensitivity and selectivity for detection of trace levels of carbonyls in e-cigarettes.

This new UPLC-MS method provides chromatographic separation and mass selective detection of these four carbonyl compounds from the interferences in the sample matrices so that the analysis can be performed with high accuracy, without the need for internal standards. This method has been validated following the 2005 International Conference on Harmonization (ICH) guideline, "Validation of Analytical Procedures: Text and Methodology Q2(R1),"⁵ and all validation results were well within internal acceptance criteria. The overall calibration range is generally 2 to 400 ng/mL for all four analytes, which covers most of the commercial e-cigarette samples tested, but can be extended down to 1 ng/mL if needed. The method limit of quantitation is 2 ng/mL as defined by spiking level during the validation or 2.9 ng/puff for 50 puffs for all four analytes. This is an improvement over current methodology in the literature with limits ranging from 5 to 15 ng/mL.

Experimental

Materials

Mono-carbonyls standards in the form of DNPH (2,4-dinitrophenylhydrazine) derivatives were purchased from Chem Service as neat material. LC-MS-grade acetonitrile (Optima) was purchased from Fisher and used for UPLC mobile phases. For standards/sample preparation and UPLC wash solutions, B&J carbonyl-free acetonitrile was used and purchased from Fisher Scientific. Ammonium acetate (HPLC-grade) was purchased from Fisher Scientific and DNPH hydrochloride salt was purchased from TCI. Tris-(hydroxymethyl)-aminomethane (Trizma base) was purchased from Sigma Aldrich.

Instrumentation

A Waters ACQUITY UPLC H-Class PLUS System with an ACQUITY QDa Mass Detector (performance) was used for the analysis. Cerulean CETI-8 e-cigarette testing instruments were used for aerosol collection.

Standards

Stock solutions were first made by accurately weighing the individual DNPH derivatives and dissolving in carbonyl-free acetonitrile at the concentrations of approximately 200 µg/mL as free carbonyls concentrations. Aliquots of the individual stock solutions were mixed and diluted to make intermediate solutions 1 and 2 at the concentrations of approximately 5 and 0.2 µg/mL each. These intermediate solutions were used to make calibration standards at the range of approximately 2 to 400 ng/mL for free carbonyls at eight varying concentrations. For the validation, the calibration range was extended down to 1 ng/mL in order to quantify the spiking results at LOQ (2 ng/mL).

DNPH Extraction Solution (6 mM)

The method was based on CORESTA Recommended Method CRM #74 Determination of Selected Carbonyls in Mainstream Cigarette Smoke by HPLC,⁶ with modifications made in terms of extraction solution, HPLC column, and detection to address the need for higher sensitivity and selectivity for e-cigarette aerosol analysis.⁷

Generally, there are higher levels of carbonyls in cigarette smoke than in e-cigarette aerosol. Therefore, 6 mM of DNPH extraction solution was used instead of 12 mM to reduce interferences from the DNPH. Furthermore, the use of commercially available DNPH hydrochloride salt enables the final solution to have the optimal pH without the addition of phosphoric acid. In addition, the background levels of formaldehyde in the extraction solution were largely reduced by using the

DNPH hydrochloride salt. DNPH Extraction Solution was made by adding 2,4-dinitrophenylhydrazine hydrochloride salt to a heated solution of acetonitrile and stirring until all solids were dissolved. Equal parts by volume of deionized water were then added to the solution to dilute to final volume. The solution was cooled to room temperature before use.

Aerosol Sample Preparation

Aerosol from e-cigarettes was collected using a Cerulean CETI-8 e-cigarette testing instrument directly into two serial glass impingers, each containing 35 mL of DNPH extraction solution. One port of the CETI-8 was used for air blank sample. An air blank sample is a sample collected with no e-cigarette in the collection port. The air blank is extracted and analyzed concurrent to study samples. The purpose is to determine background levels of analyte in the testing environment. After aerosol collection, the solutions containing derivatized carbonyls from two serial impingers were combined, and an aliquot of 7 mL was transferred to a scintillation vial, followed by the addition of 0.15 mL of 3.9% Trizma base aqueous solution to neutralize the solution. Subsequently, using a 0.2- μ m polypropylene filter, a filtered aliquot was transferred into an autosampler vial for analysis.

LC-MS Operating Parameters

LC-MS system:	ACQUITY UPLC H-Class PLUS with ACQUITY QDa Mass Detector
Software:	Empower 3
Column temp.:	50 °C
Column:	ACQUITY UPLC BEH Shield RP18, 1.7 µm, 2.1 x 100 mm
Flow rate:	0.6 mL/min
Mobile phase A:	1 mM aqueous ammonium acetate:acetonitrile (4:1)
Mobile phase B:	Acetonitrile
Mobile phase C:	0.1% formic acid in methanol/water 1:1 (for washing after the run)
Seal wash:	5% acetonitrile in water
Sample manager purge:	25:75 acetonitrile/water
Sample manager wash:	50:50 acetonitrile/water
Source temp.:	150 °C
Probe temp.:	600 °C
Capillary:	0.8 kV
Ionization mode:	Electrospray
Polarity:	Negative

Sampling rate:	5 points/sec
Divert to waste:	Before 3.8 min and after 9.5 min
Autosampler temp.:	Set at 5 °C*
Injection volume:	1 µL

*The actual reading may be variable depending on the cooling capacity of the instrument.

Time (min)	Mobile A (%)	Mobile B (%)	Curve
0	93	7	6
5	93	7	6
7	63	37	6
9.1	63	37	6
9.2	25	75	6
10.4	25	75	6
10.5	93	7	6
12	93	7	6

Table 1. Mobile phase gradient.

Compound	Mass (Da)	Cone voltage (V)	Time segment (min)
Formaldehyde-DNPH	209	2	3.8–6
Acetaldehyde-DNPH	223	2	6–7.3
Acrolein-DNPH	235	2	7.3–8.3
Crotonaldehyde-DNPH	249	2	8.3–9.5

Table 2. MS detector settings.

Data Calculations

The primary calibration curve (Curve 1) consists of standard levels 1–6 (2–100 ng/mL). It has quadratic regression with intercept not forced to zero and 1/x weighting. To quantitate the samples at higher concentrations, the secondary calibration curve (Curve 2) can be used, which consists of standard levels 3–8 (10–400 ng/mL). It can have either linear or quadratic regression with intercept not forced to zero and 1/x weighting. In this case, a different quantitation method (for Curve 2) from

Empower Software can be used to process the same samples again. For samples at concentrations above the calibration range, a dilution can be performed on the sample solutions using a solution of acetonitrile:water 1:1.

For some analytes such as acetaldehyde and acrolein, the *E*- and *Z*- stereoisomers of the DNPH-derivatives (hydrazones) exist in samples and are separated under our chromatographic conditions with the first peak being smaller than the second. In cases such as these, the sum of both peak areas is used for quantitation.

Results and Discussion

The method has been validated and used for routine analysis of commercial e-cigarettes using two Waters ACQUITY UPLC H-Class PLUS-QDa systems at ITG Brands, LLC in Greensboro, NC.

Instrument Precision

Instrument precision is defined as the degree of reproducibility among several independent measures of the same sample with the same analyte. Six System Suitability Samples (SSS, as one of the mid-level standards) were injected back-to-back on one instrument. System suitability data for instrument precision in terms of concentrations and retention times are listed in Table 3 below.

Concentrations	Formaldehyde (ng/mL)	Acetaldehyde (ng/mL)	Acrolein (ng/mL)	Crotonaldehyde (ng/mL)
SSS -1	20.84	20.49	19.73	20.41
SSS -2	20.53	19.90	19.78	20.17
SSS -3	21.11	20.57	19.96	19.90
SSS -4	20.34	20.84	20.09	20.28
SSS -5	20.27	19.27	19.44	19.49
SSS -6	20.45	20.70	19.85	19.98
Avg	20.59	20.29	19.81	20.04
Std Dev	0.32	0.60	0.22	0.33
%RSD	1.6%	2.9%	1.1%	1.6%
Retention time	Formaldehyde (min)	Acetaldehyde (min)	Acrolein (min)	Crotonaldehyde (min)
SSS -1	4.449	6.884	7.916	8.747
SSS -2	4.445	6.883	7.917	8.747
SSS -3	4.444	6.881	7.916	8.746
SSS -4	4.442	6.881	7.915	8.745
SSS -5	4.443	6.881	7.915	8.745
SSS -6	4.443	6.880	7.914	8.743
Avg	4.444	6.882	7.916	8.746
Std Dev	0.003	0.002	0.001	0.002
%RSD	0.06%	0.02%	0.01%	0.02%

Table 3. System suitability data.

%RSD for concentrations of all analytes are all within 3% while for retention time are all within 0.1%, which are well within the range of 5% and 0.3% to be considered as good instrument precision.

Calibration Range/Linearity

Correlation coefficients (R^2) from both calibration curves are all above 0.999, which meet the internal acceptance criteria of above 0.990 and are considered as good.

All calibration points are within 10% of theoretical values, indicating high accuracy for all standards.

Figures 1–4 show the calibration curve 1 for all four analytes.

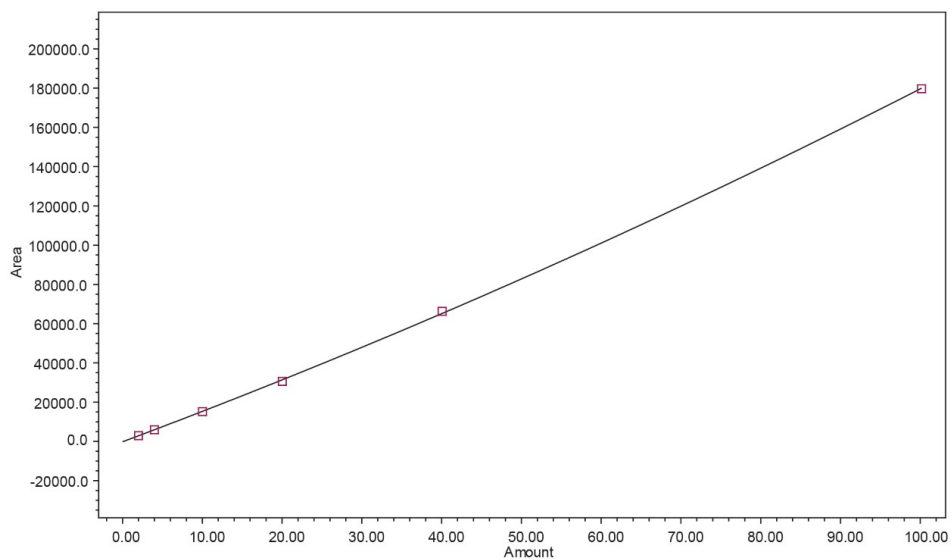


Figure 1. Calibration curve 1 for formaldehyde.

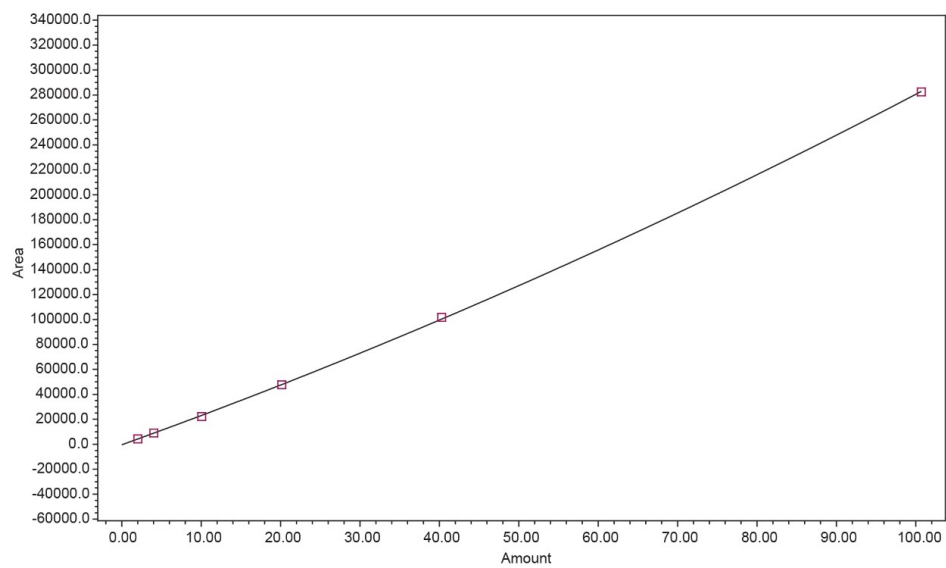


Figure 2. Calibration curve 1 for acetaldehyde.

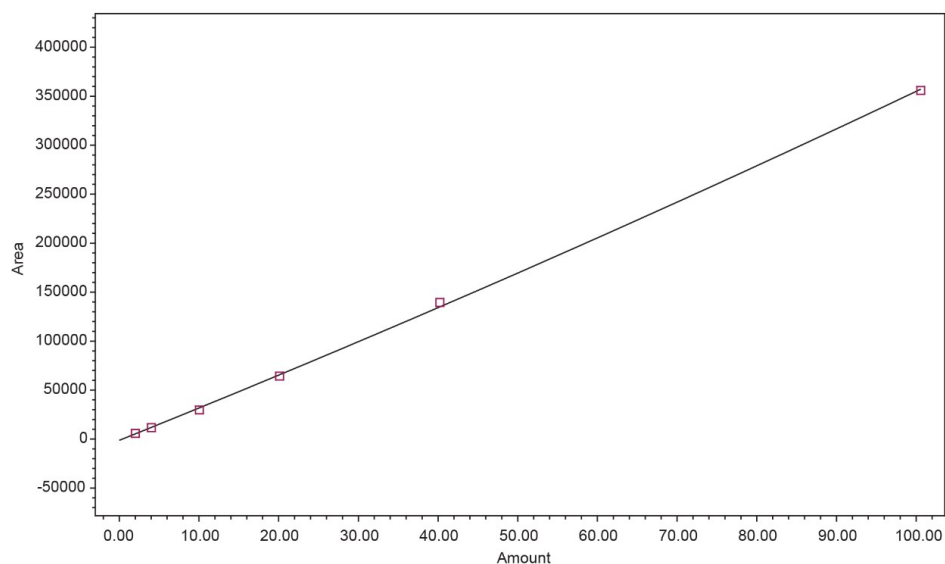


Figure 3. Calibration curve 1 for acrolein.

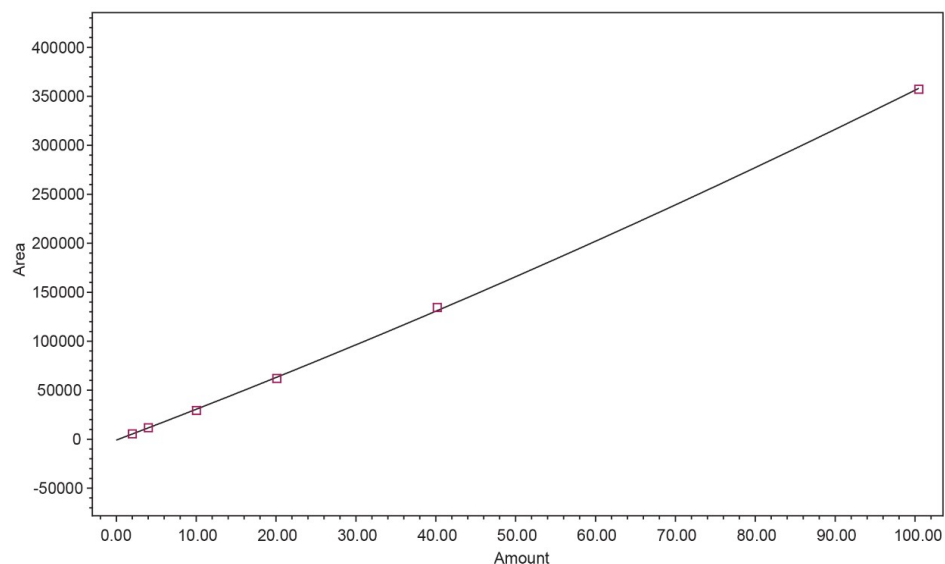


Figure 4. Calibration curve 1 for crotonaldehyde.

Spike Recovery

Aerosol sample solutions were spiked at low, medium, and high levels in each calibration range as well as at LOQ levels (2 ng/mL) in three replicates. Since it is known that carbonyls concentrations in aerosols are highly variable among individual e-cigarettes, the solutions in impingers from different ports were pooled together during the validation process, and aliquots were taken out of these solutions to be processed as un-spiked and spiked samples. The aerosol was collected under two different conditions (Standard Conditions Reference CRM #81:⁸ 55 mL puff volume – 3-second puff duration – 30-second puff frequency; Intense Condition: 95-5-30) with square puff profile.

Tables 4 and 5 show the spike recovery data and %RSD for sample replicates precision:

	Formaldehyde		Acetaldehyde		Acrolein		Crotonaldehyde	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
LOQ spike*	114.2%	3.2%	108.0%	2.0%	106.5%	2.2%	101.7%	2.5%
Low spike - Curve 1	101.9%	1.6%	99.4%	1.4%	94.1%	0.7%	100.8%	1.5%
Mid spike - Curve 1	96.1%	0.7%	99.5%	0.7%	97.1%	1.4%	97.8%	0.8%
High spike - Curve 1	100.4%	2.2%	98.9%	3.3%	99.9%	2.0%	98.2%	2.1%
Low spike - Curve 2	101.9%	0.6%	99.4%	0.6%	94.1%	1.3%	100.8%	0.7%
Mid spike - Curve 2	96.1%	2.4%	99.5%	3.6%	97.1%	2.1%	97.8%	2.3%
High spike - Curve 2	100.4%	1.4%	98.9%	1.5%	99.9%	1.4%	98.2%	1.2%

Table 4. Recoveries for aerosol collected under standard conditions.

	Formaldehyde		Acetaldehyde		Acrolein		Crotonaldehyde	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
LOQ spike*	119.1%	0.3%	114.4%	1.9%	93.7%	3.3%	104.9%	1.7%
Low spike - Curve 1	102.3%	0.9%	103.6%	1.1%	92.1%	0.8%	101.9%	1.7%
Mid spike - Curve 1	108.8%	1.3%	109.9%	3.4%	100.3%	3.2%	100.1%	3.7%
High spike - Curve 1	101.1%	NA**	99.8%	1.4%	100.0%	1.3%	98.8%	1.3%
Low spike - Curve 2	93.7%	1.3%	96.9%	3.3%	97.1%	3.0%	99.4%	3.4%
Mid spike - Curve 2	99.3%	1.1%	95.5%	1.5%	96.5%	1.4%	95.3%	1.3%
High spike - Curve 2	103.3%	0.8%	100.3%	0.9%	97.8%	1.7%	97.5%	0.7%

Table 5. Recoveries for aerosol collected under intense condition.

*Results quantitated using a different calibration curve with an additional calibration standard as sub-level 1 (1S at 1 ng/mL) included.

**Results were around the highest standard (level 6) in Curve 1. Only two replicates were quantifiable within the calibration range of Curve 1.

All the recoveries were within the range of 92.1–119.1%, which meet the internal acceptance criteria of $100 \pm 30\%$ and are considered as good for the sample matrix tested.

MLOQ

Method Limits of Quantitation (MLOQ) are determined by analyzing samples at known concentrations and establishing the minimum level at which the analyte can be reliably quantified with an S/N criteria of ≥ 10 . Table 6 below shows the S/N Ratio >10 for Standard Levels 1S/1.

Standard	Formaldehyde	Acetaldehyde	Acrolein	Crotonaldehyde
Std 1S (1 ng/mL)	21.9	27.7	21.3	66.4
Std 1 (2 ng/mL)	49.2	70.4	77.9	90.6

Table 6. S/N Ratio for standard levels 1S/1.

Figures 5–8 show the chromatograms of a commercial e-cigarette sample spiked at 2 ng/mL.

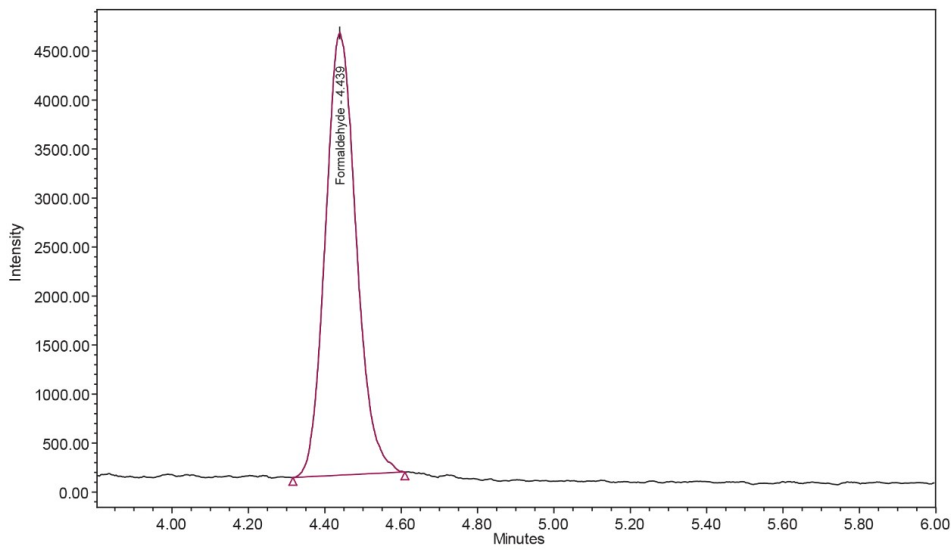


Figure 5. One commercial e-cigarette sample spiked at 2 ng/mL – formaldehyde.

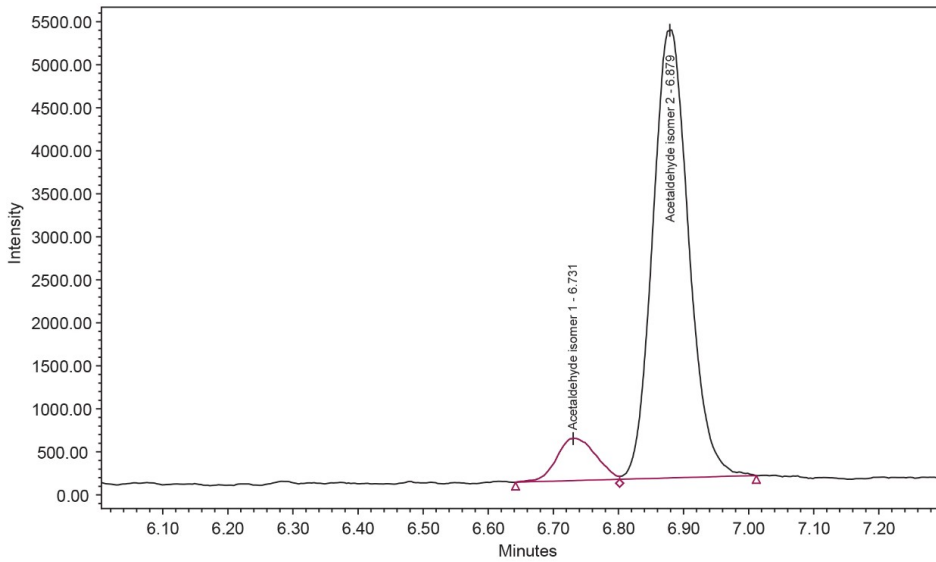


Figure 6. One commercial e-cigarette sample spiked at 2 ng/mL – acetaldehyde.

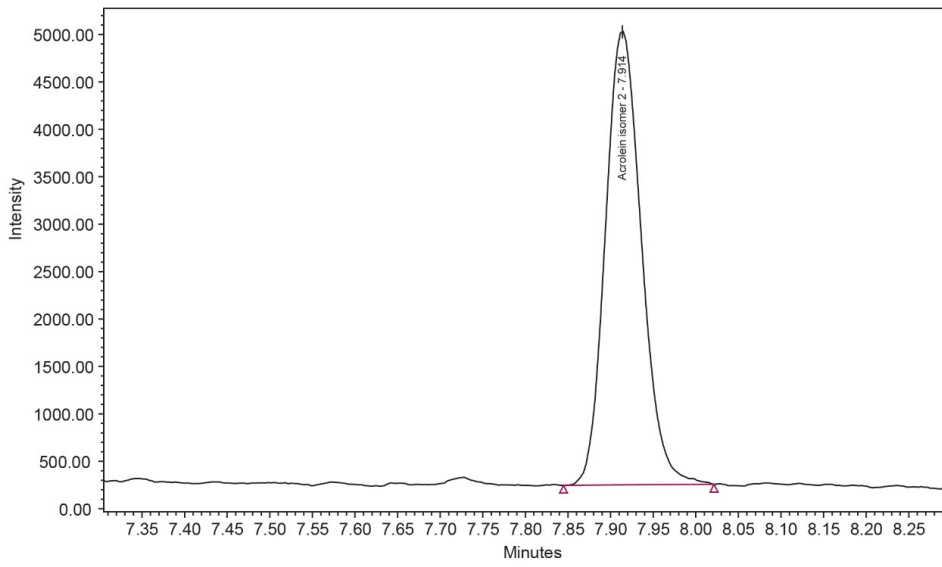


Figure 7. One commercial e-cigarette sample spiked at 2 ng/mL – acrolein.

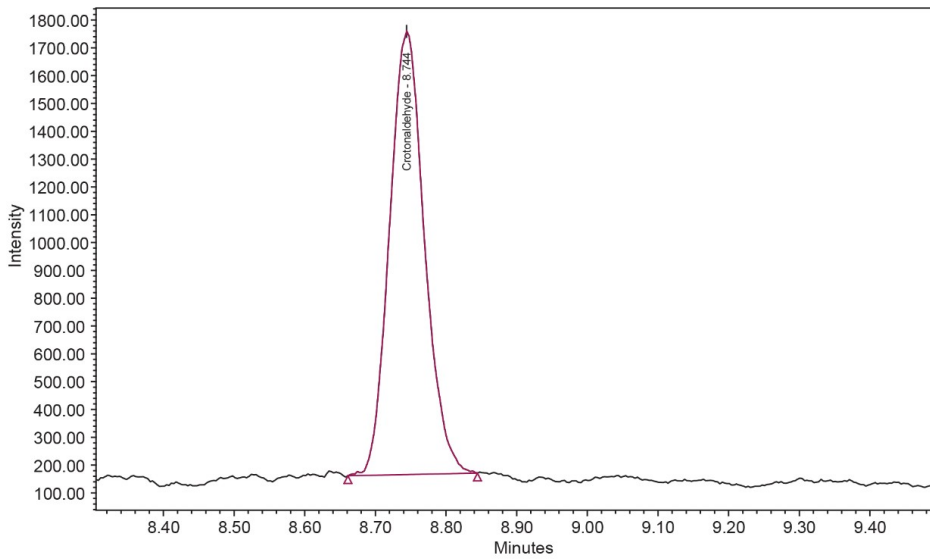


Figure 8. One commercial e-cigarette sample spiked at 2 ng/mL – crotonaldehyde.

Conclusion

This method provides a simplified solution for the analysis of mono-carbonyls at low levels in complex aerosol sample matrices with good sensitivity and selectivity using a UPLC coupled to a single quadrupole mass spectrometer (ACQUITY QDa Mass Detector). A UPLC column (ACQUITY UPLC BEH Shield RP18, 1.7 μm , 2.1 mm x 100 mm) was used to improve the separation and shorten runtime over the conventional 5 μm HPLC column specified in the CRM. A mass spectrometric detector was used instead of UV detection to improve sensitivity and selectivity. A modifier (ammonium acetate) has been added to further improve the sensitivity for the MS detection. The use of a diverter valve in the ACQUITY QDa detector reduces the unnecessary load from sample matrices to the mass detector for decreased routine maintenance.

Preliminary results have shown that the method can be used for the analysis of e-liquid with good recoveries and LOQ at 2 ng/mL. The method may be applicable to smokeless and heated tobacco product analyses, which also have trace or non-detectable levels of mono-carbonyl compounds.

References

1. Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems - Guidance for Industry, FDA Center for Tobacco Products, June 2019.
2. Flora, J. W.; Wilkinson, C. T.; Wilkinson, J. W.; Lipowicz, P. J.; Skapars, J. A.; Anderson, A.; Miller, J. H. Method for the Determination of Carbonyl Compounds in E-Cigarette Aerosols. *J Chromatogr Sci*, 2017, 55: 142–148.
3. Qian, N.; Wilkins, J.; Seamans, A.; Adams, C. J.; Chouchane, S. Validated Method for the Quantitative Determination of Carbonyls in Electronic Cigarette Aerosol and Liquid. *71st Tobacco Science Research Conference*, Nov. 2017, Bonita Springs, FL.
4. Bao, M.; Joza, P.; Rickert, W. Analysis of 21 Carbonyl Compounds in E-Liquids and E-Aerosols by Gas Chromatography-Mass Spectrometry after PFBHA Derivatization. *70th Tobacco Science Research Conference*, Sept. 2016, Palm Beach Gardens, FL.
5. ICH. (2005) International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline Q2(R1): Validation of Analytical Procedures: Text and Methodology Q2(R1), https://database.ich.org/sites/default/files/Q2_R1_Guideline.pdf.
6. CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) Recommended Method No. 74 – Determination of Selected Carbonyls in Mainstream Cigarette Smoke by HPLC, last updated in August 2019.
7. Zhu, J.; Heredia, A. A Simplified Method for the Analysis of Mono-Carbonyl Compounds in E-Cigarette Aerosols by LC-MS. *73rd Tobacco Science Research Conference*, Sept. 2019, Leesburg, VA.
8. CORESTA Recommended Method No. 81 – Routine Analytical Machine for E-Cigarette Aerosol Generation and Collection - Definitions and Standard Conditions.

Featured Products

[ACQUITY UPLC H-Class PLUS System](#)

[ACQUITY QDa Mass Detector](#)

[Empower Chromatography Data System](#)

720006979, July 2020