APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Determination of Coumarin in Electronic Cigarette Liquids by UHPLC Coupled with Isotope Dilution Tandem Mass Spectrometry

Introduction

Coumarin (1,2-benzopyrone) occurs as a natural component in some plants and is found as a flavoring ingredient in some foods, tobaccos and cosmetic products. Coumarin has also been identified and determined as a natural constituent in different types of tobaccos.[1] The high content of coumarin in some foods, tobacco products and other consumer products has received considerable attention due to its hepatotoxic effects found in animal experiments.[2] The European food safety authorities have set a maximum limit of 2 mg/kg for foods and beverages in general, and a maximum level of 10 mg/L for alcoholic beverages.[3] Coumarin has been banned as a flavor additive in food and other products in the United States.[4-5] The regulatory requirement for determining and reporting coumarin in tobacco products has been increased recently and coumarin was included by the U.S. Food and Drug Administration (FDA) on the established list as a harmful and potentially harmful constituent in tobacco and smokeless tobacco products.[6-9]

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Electronic cigarettes (E-cigs) are the battery-powered devices to heat liquid-based nicotine into an inhalable vapor. E-cigs are marketed as alternatives to traditional cigarettes because they can quell smokers' urges for nicotine without using cancer-causing tobacco. However, whether E-cig has less risk or more risk is still debatable because new toxic chemicals may be generated when heating the flavored E-liquid although it avoids the toxic chemicals from tobacco smoke. E-cigs are by far the most popular tobacco product among teens according to the 2017 national youth tobacco survey.[10] The teens are attracted to vaping by the various flavors in the E-cig liquids and U.S. FDA is weighing a ban on flavored E-cigs liquids. U.S. health officials are sounding the alarm about teenage use of e-cigarettes, calling the problem an "epidemic" and ordering manufacturers to reverse the trend or risk having their flavored vaping products pulled from the market.[11,12] More recently, San Francisco voters approved a proposition that would ban the sale of flavored tobacco products, including flavored vaping liquids.[13,14]

Although a variety of analytical methods such as thin layer chromatography (TLC), capillary electrophoresis (CE), gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled with different detection methods have been used for the determination of coumarin in plant extracts, various foods and fragrance products,[15-23] the most widely used method in the past was based on HPLC-UV with or without sample clean up and concentrations. However, HPLC-UV method suffered from its drawbacks of low selectivity and sensitivity. Because of the low selectivity, the method could easily give false positive results for coumarin due to matrix interfering components, especially for complex sample matrices such as food and tobacco samples, and therefore, it is required to use a longer analytical column and take longer runtime to separate coumarin from sample matrix components. Due to the low sensitivity of the method, extensive sample clean up and analyte concentration steps are often necessary to achieve good separation and sensitive response for coumarin analysis. The GC/MS method is more accurate and selective than the HPLC-UV method and has been used for coumarin analysis in tobacco samples[1,24-29] But, it still needs sample cleanup and analyte concentration to achieve good sensitivity. For instances, a GC/MS method was developed and applied for the analysis of natural levels of coumarin in different types of tobacco after sample purification and concentration by TLC or HPLC.[1] An automated solid-phase microextraction (SPME) method was developed in 1999 and had been applied for analysis of coumarin and other flavor-related compounds from tobacco by coupling SPME with GC/MS.[24-28] An ultrasound-assisted extraction followed by concentration with dispersive liquid-liquid microextraction was recently applied for coumarin analysis in tobacco additives by coupling with GC/MS.[29] Recently, LC/MS/MS method has been developed for coumarin analysis in food samples,[30-34] demonstrated much higher sensitivity and selectivity with simpler sample preparation. However, to our knowledge, no LC/MS/MS method has been applied for coumarin analysis in electronic cigarette liquid (E-liquid) samples. The aim of this study is to develop a simple, fast, selective and sensitive LC/MS/MS method for the analysis of coumarin in E-liquid samples. To obtain accurate results from the complex E-liquid sample matrices, multiple MS/MS transition pairs were evaluated for coumarin identification and quantification. Compared to the GC/MS method for tobacco analysis, this LC/MS/MS method is much simpler (no need for sample cleanup and analyte concentration), faster, more selective and sensitive. In addition, using a stable isotope labeled internal standard, the new method is more accurate and robust and can be easily applied as a turn-key solution to coumarin analysis in E-liquid samples in a routine commercial laboratory environment.

Experimental

Hardware/Software

Chromatographic separation of coumarin from potential interfering components was conducted by a PerkinElmer UHPLC System and determination of coumarin was achieved using a PerkinElmer QSight® 220 triple quadrupole mass detector with a dual ionization source. All instrument control, data acquisition and data processing were performed using Simplicity™ 3Q Software.

Method

Standards, Solvents and Sample Preparation

Coumarin (≥ 99% in purity; CAS No. 91-64-5) was obtained from Sigma-Aldrich and deuterium labelled coumarin- 5,6,7,8-d4 (98% in purity) was obtained from Toronto Research Chemical (Toronto, ON, Canada). The chemical structures of coumarin and coumarin-d4 are shown in Figure 1. E-liquid samples were obtained from a local E-Cigs/Vapor store (Waterloo, ON, Canada). LC/MS grade methanol (MeOH), formic acid, and water were obtained from Fisher Scientific™.

The primary coumarin standard solution (10 mg/mL) and internal standard (IS) coumarin-d4 solution (1mg/mL) were prepared in methanol, separately. The secondary coumarin standard solution (10 µg/mL) and internal standard solution (IS spiking solution, 10 µg/mL) were prepared separately by diluting their primary standard solutions with 50% methanol solution (in LC/MS grade water, v/v). A tertiary coumarin standard (1.0 µg/mL) was prepared by diluting the secondary solution with 50% methanol solution (in water, v/v). Twelve levels of calibration standards containing coumarin at 0.05, 0.1, 0.5, 1, 5, 10, 20, 50, 100, 200, 500, and 1000 ng/mL, were prepared from the secondary and tertiary standard solutions by dilutions with the 50% methanol solution. Each calibration standard contains 100 ng/mL internal standard. Two zero standard solutions were also prepared: standard 01 was prepared by adding 50% of methanol solution directly into an auto sampler vial to check the background and potential contamination to the vials; standard 02 containing only the 100 ng/mL of IS, was prepared to check the isotope purity of the IS.

A 1.0 g of the E-liquid sample from a freshly opened source was spiked with 100 µL of the IS spiking solution (10 µg/mL) and then diluted and extracted with 10 mL of 50% methanol solution in a 50 mL centrifuge tube and agitated for 10 minutes on a shaker. The sample solution was analyzed directly by the LC/MS/MS method without further sample treatment.

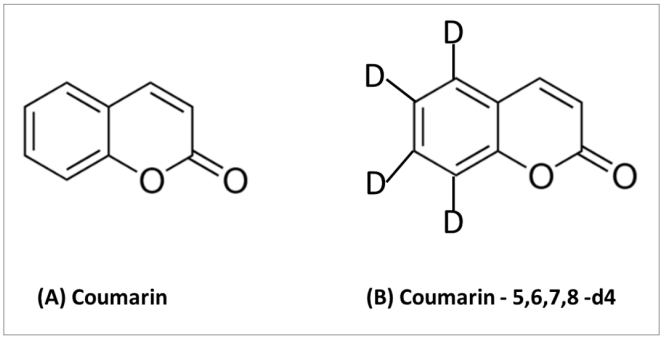

Figure 1. The chemical structures of coumarin and coumarin-5,6,7,8-d4.

LC Method and MS Source Conditions

The LC method and MS source parameters are shown in Table 1. Several available LC columns were tested initially; including Phenomenex Kinetex PFP, C8, C18 and XB-C18 columns (2.6 µm, 100 × 4.6 mm), Phenomenex Kinetex C18 column (2.6 µm, 100 × 2.1 mm), Agilent ZORBAX Eclipse XDB C8 and C18 columns (3.5 µm, 150 × 2.1 mm and 150 × 3.0 mm), Restek Raptor C18 column (2.7 µm, 100 × 4.6 mm), and PerkinElmer Brownlee SPP C18 (2.7 µm, 100 × 2.1 mm and 100 × 3.0 mm), The C8 and C18 columns with larger inner diameter (id) such as 3.0 mm or 4.6 mm provided equivalent results and were used for analyte separation during validation for method robustness test. Mobile phases were: A, 0.1% formic acid in water and B, 0.1% formic acid in methanol. The multiple reaction monitoring mode (MRM) transitions of coumarin and its internal standard and their optimized parameters are shown in Table 2. Multiple MRM transitions were monitored for both coumarin and its internal standard to evaluate potential interfering components for certain transitions in real samples, which will help confidently identify analyte from complex sample matrices, reduce false positive and negative in the method and increase the accuracy of analyte quantification.[35-36] Optimization of MS/MS parameters, such as collision energies (CE), entrance voltages (EV), the lens voltages prior to collision cell (CCL2) and so on, was done by infusion of standards and use of the software. Source conditions were optimized by flow injection (FIA) method. Dwell time for each transition was 85 ms.

Table 1. LC Method and MS Source Conditions.

LC Conditions	
LC Column	Brownlee, SPP C18, 100 x 3.0 mm, 2.7 µm (Cat#N9308410)
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in methanol
Mobile Phase Gradient (Flow Rate: 0.4 mL/min)	Start at 60% mobile phase B and perform isotactic run for 6 min, then increase B to 100% at 6.5 min and keep at 100% B for 2 mins to clean the column, finally equilibrate the column at initial condition for 3 min.
Column Oven Temperature	30 °C
Auto Sampler Temperature	5 °C
Injection Volume	5.0 µL
MS Source Conditions	
ESI Voltage (Positive)	2000 V
Drying Gas	120
Nebulizer Gas	400
Source Temperature	400 °C
HSID Temperature	260 °C
Detection mode	MRM

Table 2. Optimized MRM Parameters.

Compound Name	Polarity	Precursor ion	Product ion	CE	EV	CCL2
Coumarin 1	Positive	147.1	91.1	-33	25	-48
Coumarin 2	Positive	147.1	103.1	-25	25	-36
Coumarin 3	Positive	147.1	65.1	-48	25	-44
Coumarin 4	Positive	147.1	77.1	-36	25	-40
Coumarin 5	Positive	147.1	51.1	-63	25	-44
D4-Coumarin 1	Positive	151.1	95.1	-35	25	-56
D4-Coumarin 2	Positive	151.1	107.1	-25	25	-36
D4-Coumarin 3	Positive	151.1	68.2	-50	25	-44
D4-Coumarin 4	Positive	151.1	80.2	-38	25	-36
D4-Coumarin 5	Positive	151.1	69.1	-48	25	-44
D4-Coumarin 6	Positive	151.1	81.1	-37	25	-40
D4-Coumarin 7	Positive	151.1	54.1	-65	25	-44

Quality Control Sample Preparation

To test possible interference or contamination from reagents or materials used and from the sample preparation processes, a Laboratory Reagent Blank (LRB) was prepared per day or per each work shift. The values of LRB should be close to zero or at least less than the LOQ of the method. Otherwise, an investigation on the source of contamination must be carried out. A LRB sample was prepared by following the same procedures as for E-liquid sample preparation described above, using 0.0 gram of E-liquid sample. To study possible analyte loss or contamination during sample preparations, a Laboratory Fortified Blank (LFB) sample was prepared per day or per work shift. A LFB sample can be prepared by following the same E-liquid sample preparation procedures as described above, using 0.0 gram of E-liquid sample spiked with a known amount of analyte solution. During method validation, LFB samples were prepared by spiking the analyte in three different concentration levels as shown in Table 3 and three replicates of the LFB samples at each level were prepared on three separate days. To evaluate sample matrix effects and analyte recovery from E-liquid sample matrix, a Laboratory Fortified Matrix sample (LFM) was prepared per day or per work shift. A LFM sample can be prepared by following the same E-liquid sample preparation procedures as described above, using 1.0 gram of a E-liquid sample spiked with a known amount of analyte. The percent recovery is calculated by comparing the difference of the spiked (LFM sample) and non-spiked E-liquid sample results and the expected (spiked) value. During method validation, the LFM samples were prepared using two different E-liquid sample matrices (with different nicotine contents and flavor components) and three different concentration levels of analyte were spiked onto each sample matrix. This LFM study was repeated on three separate days.

Table 3. The Coumarin Amounts Spiked in QC Samples and the Recovery Results.

Sample ID	Spiked (ng/g)	Recovered (ng/g)	Recovery (%)
LRB	0	0	0
LFB1	20	20.3 (2.6)*	88.5 – 114.4
LFB2	50	49.7 (1.9)	95.6 – 103.2
LFB3	100	103.1 (3.7)	99.4 – 106.8
S1-LFM1	20	18.7 (3.1)	78.0 – 109.0
S1-LFM2	50	47.9 (5.6)	84.6 – 107.0
S1-LFM3	100	106.3 (11.8)	94.5 – 118.1
S6-LFM1	20	17.9 (2.4)	77.5 – 101.5
S6-LFM2	50	51.3 (7.1)	88.4 – 116.8
S6-LFM3	100	93.6 (13.4)	80.2 – 107.0

**The values in parentheses are standard deviations (n = 3).*

Results and Discussion

UHPLC/MS/MS Method Optimization

For mass detection of coumarin, both positive and negative electrospray ionization (ESI) modes were evaluated initially. The results showed that the analyte gave better sensitivity and better signal to noise ratio under positive mode and therefore positive ESI detection was used in this study. For coumarin and the IS coumarin-d4, several product ions were generated at certain collision energies as shown in Figure 2 and thus. multiple MS/MS transitions could be formed. The optimized MRM parameters were listed in Table 2 in the order of signal intensity. To separate coumarin from interfering components in different tobacco sample matrices, several reversed phase LC columns available in our laboratory were evaluated. The Phenomenex Kinetex pentafluorophenyl propyl (PFP) column was confirmed not suitable for this study because very broad and sometimes splitting analyte peaks were found with this column. All the tested C8 and C18 columns, including Raptor C18 and Brownlee SPP C18 columns, could be applied to the analysis of coumarin, but it was found that the columns with smaller inner diameters (i.e. 2.1 mm in this study) have difficulty to separate coumarin from interfering components. The C8 and C18 columns with larger column inner diameters (4.6 mm or 3.0 mm) provided much better separation efficiency due to higher column capacity and therefore were used during method validation and robustness study. Mobile phase compositions of methanol/water and acetonitrile /water with or without acid were evaluated and it was found that methanol/water with 0.1% acid provided better analyte signal (more sensitive) than acetonitrile/water compositions. Formic acid and acetic acid were tested as the additives in mobile phases and no difference was found between these two acids and any one of the acids could be used in the mobile phases.

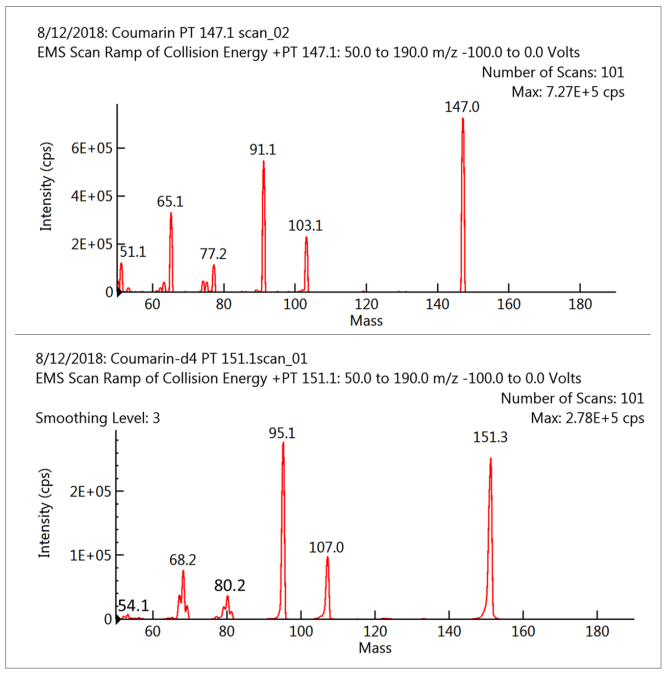

Figure 2. Product ion mass spectra of coumarin (upper with m/z 147.1) and coumarin-d4 (lower with m/z 151.1) obtained by scanning from 50 to 190 m/z with collision energy ramp from -100 to 0 Volts.

Calibration Curves and Determination of LOD and LOQ

Several sets of calibration curves with concentration levels ranging from 0.05 ng/mL to 1000 ng/mL were generated on separate days for all the five coumarin MS/MS transition pairs. All the calibration curves show good linearity with correlation coefficients (R^2) greater than 0.99 as shown in Figure 3. Therefore, all of the five MS transitions could be used for coumarin quantification if no interfering components in the peaks (see Method Validation section for details). The accuracies for most of the calibration points evaluated by the RSD% of the residuals are less than 15% (it is less than 20% for the lowest standard). The limit of detection (LOD) and limit of quantification (LOQ) for the LC/MS/MS analysis of coumarin were determined based on signal to noise ratio (S/N = 3 for LOD and S/N = 10 for LOQ) of the highest intensity 147.1/91.1 peak (quantifier). Although the instrument has a LOD of 0.02 ng/mL and LOQ of 0.05 ng/mL for coumarin standard solutions, the LOD and LOQ of the method for real E-liquid samples are 2 ng /g of coumarin/ sample (corresponding to 0.2 ng/mL of coumarin in final solution) and 5 ng/g (which corresponds to 0.5 ng/mL of coumarin in final solution) due to sample matrix effects (mainly ion suppression) in E-liquid samples.

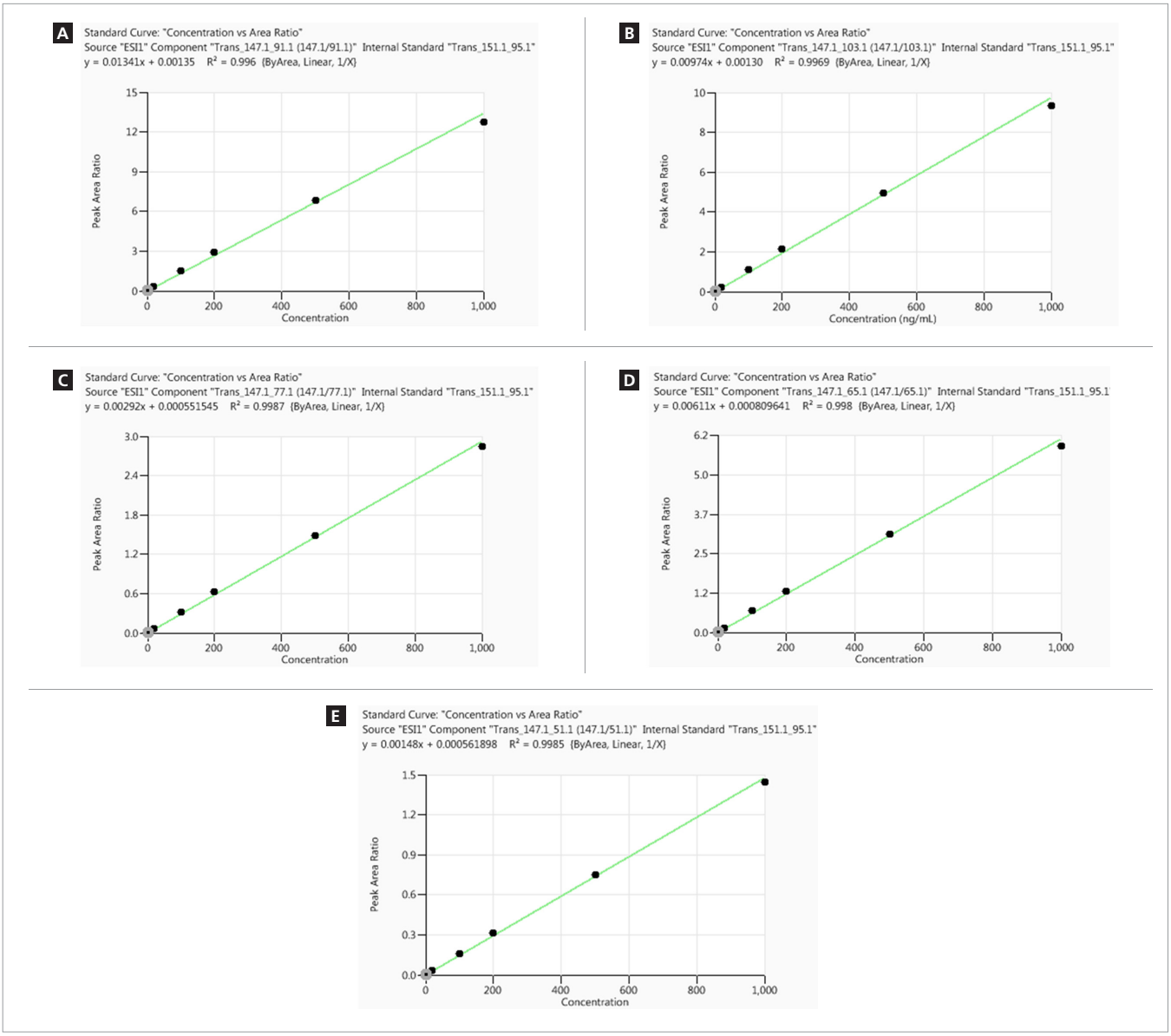

Figure 3. Calibration curves for the five MS/MS transitions of coumarin.

Extraction of Coumarin from E-Liquid Samples

The method extraction efficiency for the targeted analytes is one of the important parameters that will influence the method's precision, accuracy and robustness. The extraction efficiency depends on the solubility of the analytes in a certain solvent or a mixture of solvents, the extraction time, extraction temperatures and extraction methods. For coumarin extraction from E-liquid samples, since both coumarin and the E-liquid solvents (glycerin and propylene glycol) are soluble in water and methanol, water, methanol and the mixture of water and methanol with different ratios were studied as extraction solutions for extraction of coumarin from samples. The extraction results shown no significant differences between these solutions and therefore, a 50% methanol aqueous solution was finally used as an extraction solution to closely match the initial LC mobile phase components. The most convenient extraction method of agitating sample solutions on a Vertex Mixer at room temperature was applied in this study and the extraction time was kept at 10 minutes for all samples.

Method Validation

As shown in Table 3, no interference or contamination from reagents or glassware was observed in this study as demonstrated by the LRB sample results. Good recoveries were obtained for LFB samples, indicating no analyte loss or contamination during sample preparations. The method's selectivity and analyte confirmation from E-liquid samples were evaluated by comparing the analyte retention time and mass spectra information between reference coumarin standard and E-liquid samples. According to the regulatory guidance on analytical method validation, at least two MS/MS transition ion pairs should be used in a method.[35-36] In this study, five MS/MS ion pairs were examined, and their ion peak area ratios of qualifier/quantifier were compared between standard and samples. As illustrated in Table 4 and Figure 4-5 using sample 6 (S6) as an example, although the second transition ion pair 147.1/103.1 of coumarin in standard solution is the second intense peak among all the ion pairs (Figure 4B), it suffered from heavy matrix effects in the E-liquid sample (Figure 5B), which could not be compensated for by the corresponding isotope labeled internal standard peak (because internal standard peaks were not affected as much as this ion pair of coumarin by the sample matrix) and thus lead to much lower coumarin result and lower ion ratio value of 103.1/91.1, while all other transition ion pairs gave consistent results for both coumarin content in the sample and ion ratio values between standard and sample. These results demonstrated the advantages of having multiple MS/MS transition ion pairs of an analyte in a LC/MS/MS method, which could help compounds confirmation and achieve more accurate results. In addition, the results also show that the isotope labeled internal standard sometimes could not overcome the sample matrix effects because they might not be affected by the sample matrix in the same way or to the same degree.

Method precision was assessed based on replicate analyses of a standard and a E-liquid sample (seven replicates) on three days. The precision was then calculated based on the coefficient of variation (RSD%) of the collected data. The within-day RSDs were 3.1% for the low level standard (at 1.0 ng/mL), 2.6% for the middle level standard (at 100 ng/mL), 5.8% for the higher level standard (at 1000 ng/mL) and 9.7% for the E-liquid sample 6, respectively; the between-day RSDs were 4.9% for the low level standard (at 1.0 ng/mL), 3.9% for the middle level standard (at 100 ng/mL), 3.0% for the higher level standard (at 1000 ng/mL) and 11.3% for the E-liquid sample 6, respectively. Method accuracy assesses how close the experimental value is to the expected value. Method accuracy was evaluated by the recovery of a known amount of analyte spiked to a E-liquid sample (LFM samples). As shown in Table 3, the recoveries of coumarin from the spiked samples were between 78% and 118%, demonstrating good accuracy of the method. Figure 6 gave the overlapped chromatograms of coumarin in sample 1, sample 6 and their three spiked LFM samples.

Method robustness is the capacity of a method to remain unaffected by small, deliberate changes in method parameters. In this study, these parameters include solvent composite ratio in the extraction solution, mobile phase compositions and equivalent HPLC columns from different suppliers. The results from this study show that the performances of the method were not affected by the small variations in these parameters and thus confirmed the robustness of the method. In addition, the method was validated on two QSight 220 LC/MS/MS systems with equivalent results obtained.

Table 4. MRM Ion Pairs, Ion Peak Area Ratio of Qualifier/Quantifier in Standard and in Sample 6 and the Coumarin Results in Sample 6 Calculated from Each MS/MS Ion Pair.

MRM Ion Pair	Qualifier Ion/ Quantifier Ion	Area Ratio (Standard)	Area Ratio (Sample 6)	Coumarin Found (ng/mL)
147.1/91.1	91.1/91.1	1.00	1.00	11.07
147.1/103.1	103.1/91.1	0.74	**0.095**	**1.31**
147.1/65.1	65.1/91.1	0.45	0.39	9.04
147.1/77.1	77.1/91.1	0.22	0.24	12.25
147.1/51.1	51/91.1	0.12	0.14	14.24

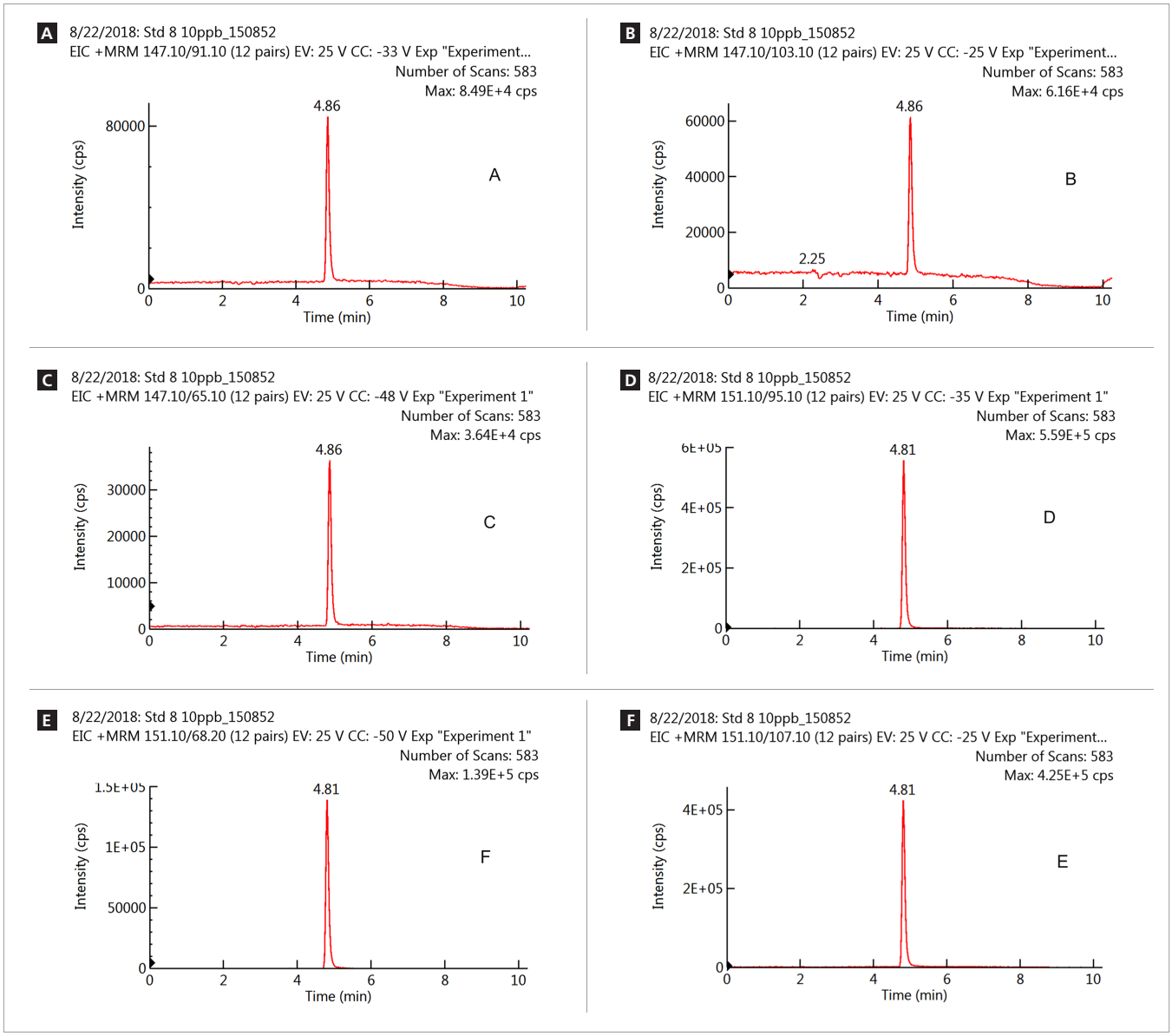

Figure 4. MRM Chromatograms of coumarin (A, B, C) and corresponding internal standard coumarin-d4 (D, E, F) in a calibration standard solution (10 ppb).

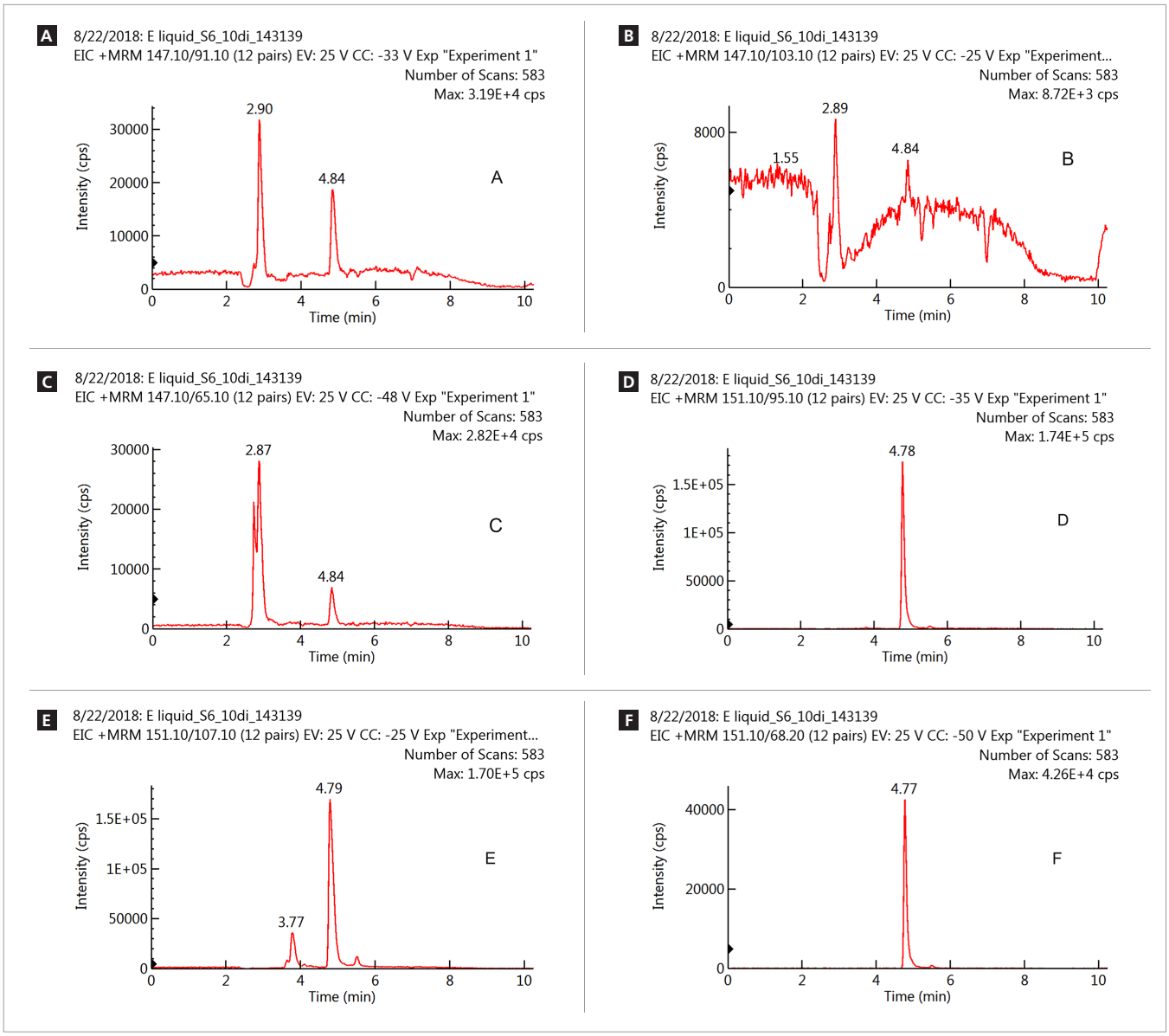

Figure 5. MRM Chromatograms of coumarin (A, B, C) and corresponding internal standard coumarin-d4 (D, E, F) in a E-liquid sample solution (sample S6).

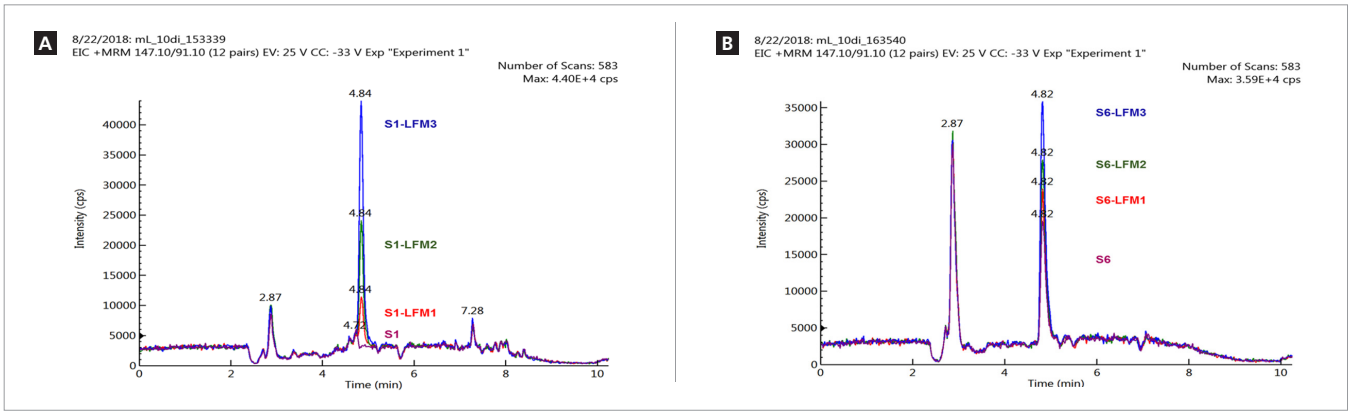

Figure 6. The overlapped chromatograms of coumarin in sample 1 (A), sample 6 (B) and their three spiked LFM samples.

Determination of Coumarin in E-Liquid Samples

The method has been successfully applied to the determination of coumarin from six different electronic cigarette liquid samples as shown in Table 5.

Table 5. The Labeled Nicotine Content, Sample Weight, Extraction Solution Final Volume and Coumarin Results Found in the E-Liquid Samples.

Sample ID	Nicotine Content (mg/g)	Sample Weight (g)	Extraction Solution (mL)	Coumarin Found (ng/mL)	Coumarin Found (ng/g)
S1	6	1.0740	10	0	0
S2	25	1.0062	10	0	0
S3	15	1.0346	10	0	0
S4	0	1.0043	10	0	0
S5	3	1.0586	10	0	0
S6	8	1.0129	10	11.07	110.7

Stability of Standards and Samples

According to the recommendation from the suppliers, the coumarin stock may be stored at room temperature or at -20 °C for up to three years. The primary standard solutions are stable for 12 months if kept in a dark container in a freezer. Secondary standards are stable for three months if kept in a dark container in a freezer after preparation. Tertiary standard and calibration standards prepared from secondary standards are stable for three weeks if kept in a dark container in a refrigerator after preparation. Sample extracts are stable at least for a week if kept in a dark container in a refrigerator after preparation.

Conclusions

The objective of this study is to develop a simple, fast, sensitive, selective, and robust analytical method for the determination of coumarin from electronic cigarette liquids (E-liquids). This goal was realized by coupling UHPLC with tandem mass spectrometry. The method is fully validated with two E-liquid samples containing different nicotine contents and flavor components. The method can be applied to the analysis of coumarin in E-liquid samples with good linearity, precision and accuracy. Compared to the HPLC/UV methods, this LC/MS/MS method is more sensitive, selective and accurate. Compared with GC/MS method, this method is simpler, faster, and without the need for sample cleanup and analyte concentration. This method can be easily extended to the analysis of coumarin in other sample matrices such as cigarette tobacco and smokeless tobacco products as well as various food samples.

References

1. A. Christakopoulos, K. Feldhusen, H. Norin, A. Palmqvist and I. Wahlberg, *J. Agric. Food Chem.*, 1992, 40, 1358.
2. B. G. Lake, *Food Chem. Toxicol.* 1999, 37, 423.
3. European Council, Council Directive (EEC) No. 88/388 on the approximation of the laws of the Member States relating to flavorings for use in foodstuffs and to source materials for their production. Off. *J. Europ. Comm.,* 1988, L184, 61.
4. U. S. Food and Drug Administration (FDA), *Fed. Regist.* 1954, 19, 1239.
5. Code of Federal Regulations, Title 21, Chapter 1, Part 189, April 2001.
6. Harmful and Potentially Harmful Constituents (HPHCs) in Tobacco Products and Tobacco Smoke: Established list. U.S. Food and Drug Administration (FDA), March 2012: https://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/ucm297786.htm.
7. Guidance for Industry Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke Under Section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act: https://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297828.pdf.
8. A. Rodgman, *Beitr. Tabakforsch. Int.* 2012, 24, 258.
9. World Health Organization (WHO) Technical Report series 951, 2008: The scientific basis of tobacco product regulation. http://www.who.int/tobacco/global_interaction/tobreg/publications/9789241209519.pdf.
10. Centers for disease Control and Prevention (CDC) Press Release, Youth tobacco use drops during 2011-2017. https://www.cdc.gov/media/releases/2018/p0607-youth-tobacco-use.html.
11. Mike Strobe for **PBS News Hours, 2018,** September. "FDA calls teen vaping an 'epidemic,' weighs bans on flavored e-cigarettes" https://www.pbs.org/newshour/nation/fda-calls-teen-vaping-an-epidemic-weighs-bans-on-flavored-e-cigarettes.
12. Jayne O'Donnell, Ken Alltucker and Josephine Chu, "Teens hooked by vaping: FDA weighing a ban on flavored e-cigarette liquids". **U.S.A TODAY, 2018,** August 13. https://www.U.S.atoday.com/story/news/nation/2018/08/13/teen-vaping-fda-weighs-ban-flavored-e-cigarette-liquid/890218002/.
13. "San Francisco Voters Uphold Ban on Flavored Vaping Products". ***The New York Times, 2018***. June 6. https://www.nytimes.com/2018/06/06/health/vaping-ban-san-francisco.html.

14. Madison Park, Roni Selig, "San Francisco bans sales of flavored tobacco products". **CNN. 2018,** June 6. https://www.cnn.com/2018/06/06/health/san-francisco-flavored-cigarettes-proposition-e/index.html.

15. M. T. Belay and C. F. Poole, *Chromatographia,* 1993, 37, 365.

16. R. J. Ochocka, D. Rajzer, P. Kowalski and H. Lamparczyk, *J. Chromatogr. A.,* 1995, 709, 197.

17. M. J. Scotter, D. P. T. Roberts and G. O. Rees, *Anal. Methods,* 2011, 3, 414.

18. C. Sproll, W. Ruge, C. Andlauer, R. Godelmann and D. W. Lachenmeier, *Food Chem.,* 2008, 109, 462.

19. R. M. S. Celeghini, J. H. Y. Vilegas and F. M. Lancas, *J. Braz. Chem. Soc.,* 2001, 12, 706.

20. Z. D. He, C. F. Qiao, Q. B. Han, C. L. Cheng, H. X. Xu, R. W. Jiang, P. P. H. But and P. C. Shaw, *J. Agric. Food Chem.,* 2005, 53, 2424.

21. R. D. Thompson and T. Hoffmann, *J. Chromatogr.,* 1988, 438, 369.

22. E. Martino, I. Ramaiola, M. Urbano, F. Bracco and S. Collina, *J. Chromatogr. A*. 2006, 127, 147.

23. H. H. Wisneski, *J. AOAC International,* 2001, 84, 689.

24. S. B. Stanfill and D. L. Ashley, *J. Chromatogr. A.,* 1999, 858, 79.

25. S. B. Stanfill, A. M. Calafat, C. R. Brown, G. M. Polzin, J. M. Chiang, C. H. Watson, D. L. Ashley, *Food Chem. Toxicol.* 2003, 41, 303.

26. S. B. Stanfill and D. L. Ashley, *J. Agric. Food Chem.,* 2000, 48, 1298.

27. S. B. Stanfill, C. R. Brown, X. Yan, C. H. Watson, and D. L. Ashley, *J. Agric. Food Chem.,* 2006, 54, 8580.

28. G. M. Polzin, S. B. Stanfill, C. R. Brown, D. L. Ashley and C. H. Watson, *Food Chem. Toxicol.* 2007, 45, 1948.

29. P. Li, X. Zhu, S. Hong, Z. Tian and J. Yang, *Anal. Methods,* 2012, 4, 995.

30. L. S. De Jager, G. A. Perfetti and G. W. Diachenko, *Food Chem.,* 2008, 107, 1701.

31. L. S. De Jager, G. A. Perfetti and G. W. Diachenko, *J. Chromatogr. A,* 2007, 1145, 83.

32. M. Raters and R. Matissek, *Eur. Food Res. Technol.,* 2008, 227, 637.

33. M. Rychlik, *J. Agric. Food Chem.,* 2008, 56, 796.

34. M. Vierikova, R. Germuska and J. Lehotay, *J. liquid Chromatogr. and Rel. Technol.,* 2009, 32, 95.

35. European Commission, SANCO. 2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed, SANTE/11945/2015 https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_11945.pdf.

36. Us FDA, Bioanalytical Method Validation Guidance for industry, 2018. https://www.fda.gov/downloads/drugs/guidances/ucm070107.Pdf.

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