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Use of Ion Mobility $^{TW}CCSN_2$ Values in Non-targeted Food Additives Screening

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APPLICATION BENEFITS

- A routine UPLC-IM-MS strategy has been used to generate positive ion and negative ion multi-parameter food additive libraries
- The combination of screening parameters such as retention time, collision cross section, precursor/product ion mobility accurate mass measurements can improve detection specificity in non-targeted food additive screening assays
- The food additives library described incorporates sweeteners, food colorings, antioxidants, and preservatives
- Compared to the expected values of the FA CCS libraries, FA's have been routinely detected with $^{TW}CCSN_2$ delta values of <2% using positive and negative ion modes
- Detections were obtained with the application of larger, as well as more flexible analytical post processed workflow tolerances, as afforded by the orthogonality of multi-factor mass spectrometry screening parameters

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Progenesis™ QI food additives

KEYWORDS

Food additives libraries, cumulative specificity reduces false detections, $HDMS^E$, $^{TW}CCSN_2$

INTRODUCTION

Although the use of food additives (FAs) is strictly regulated under various European Union (EU) acts,[1] national authorities have the responsibility to ensure effective controls are in place and monitor the consumption of food additives within their respective populations.[2] To fulfil these requirements, analytical methods are required to be able to quantify these substances in a wide variety of foodstuff types for a large number of items available in the marketplace. Many analytical applications are already successfully implemented but generally cover very few additives and/or few food matrices. Under these conditions, it is very challenging and expensive to monitor additive levels in foods, considering the large availability of products on the market. An approach to make the analytical process more efficient can be achieved through the development of more versatile, high-throughput multi-methods, which can flexibly cover the largest number of FAs in one analysis. Such methods will promote better coverage of additive composition for foods that are required to be controlled, and additionally can be used for exposure assessment to multiple FAs with a single analysis per sample.

According to the EU legislation, FAs are "any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value."[3] These substances are authorized for use in food by the European Commission after being subjected to a safety assessment by the European Food Safety Authority (EFSA). Authorization is dependent upon no observation of health hazards and whether use complies with EU legislation (e.g., technological need and the benefits for consumers). Enforcement of the legislation through implementation of national food control systems should ideally cover all food marketed within the country. Likewise, for risk assessment, the analysis of large numbers of products are necessary in order to obtain a representative estimate of the daily intake of FAs. To address the increasing number of sample matrices and the large number of FAs (authorized/unauthorized), it is essential to develop effective and reliable analytical methods.

We have investigated the utility of mass spectrometry libraries incorporating a $^{TW}CCS_{N_2}$ (travelling wave collision cross section against nitrogen buffer gas) metric. UPLC-IM-MS (UltraPerformance Liquid Chromatography ion mobility mass spectrometry) is comprised of ion mobility spectrometry (IMS; gas phase separation prior to MS analysis) coupled with UPLC (neutral species separation). The nested timescales of UPLC (seconds), IMS (~10 milliseconds), and time-of-flight MS (microseconds) are compatible with the requirement of high-throughput analysis of complex samples. IM separation of compounds results from gas phase ions being separated within a travelling wave ion mobility (TWIM) RF ion guide, located prior to the mass analyser of the instrument. Mobility separation is obtained by driving packets of ions through a low-pressure inert buffer gas (typically nitrogen) using a relatively weak electric field. The resultant separation depends on factors such as the mass, charge, and shape of the molecule. It provides an added dimension of separation to that of LC and MS, in addition to generating $^{TW}CCS_{N_2}$ as a complementary identification metric.

Pioneering strategies to incorporate ion mobility $^{TW}CCS_{N_2}$ in pesticide screening assays have been presented,[4-6] and the routine use of $^{TW}CCS_{N_2}$ for small molecule analysis has evolved across multiple areas of research, including pharma (metabolism, metabolomics, and lipids), forensic toxicology, food safety (veterinary drugs, mycotoxins, steroids, steviol glycosides, natural product screening, and natural toxins).[7-10] $^{TW}CCS_{N_2}$ searchable libraries have been produced, where the $^{TW}CCS_{N_2}$ value has been used as a screening parameter to improve the specificity of identification and decrease false detections. The approach has a chromatographic multi-additive method and MS library (incorporating $^{TW}CCS_{N_2}$), which has been utilized to perform investigations into non-targeted screening of FAs in "off the shelf" food commodities.

EXPERIMENTAL

Sample description

Food commodities screened for food additives: Red fruits yogurt (YB); strawberry yogurt (YS); energy drink (D1); "zero" lemon drink (D2); "zero" strawberry and kiwi drink (D3); colorless tonic drink (D4); and sparkling lemonade drink (D5). (Note: zero equals no added sugars.)

Sample preparation

Soft drinks: Dilution 10:1 and 100:1 using H_2O

Yogurt extraction method: Yogurt samples (15 g) were weighed into Waters™ 50-mL screw-cap centrifuge tubes. A 10-mL volume of acetonitrile in 1% acetic acid was added as an extraction solvent and the tube then mixed vigorously for one minute using a vortex mixer. Anhydrous $MgSO_4$ (6 g) and sodium acetate (1.52 g) were added to the tube to induce phase separation. Samples were immediately shaken for one minute, and then centrifuged for five minutes at 1500 rcf at 4 °C. Dispersive-SPE (dSPE) of the samples was carried out by pouring the supernatant (8 mL) into a centrifuge tube (50 mL) containing $MgSO_4$ (1.2 g), PSA (410 mg), and C_{18} (404 mg). The sample was vortexed for one minute and centrifuged for five minutes at 1500 rcf at 4 °C.

Method conditions

LC conditions

LC system:	ACQUITY UPLC I-Class PLUS
Detection:	Ion mobility mass spectrometry
Vials:	LCMS Certified Clear Glass 12 × 32 mm Screw Neck Total Recovery Vial, with Cap and Pre-slit PTFE/Silicone Septa, 1 mL volume (p/n: 600000671CV)
Column:	ACQUITY UPLC HSS T3 100 mm × 2.1 mm, 1.8 µm (p/n: 186003539)
Column temp.:	45 °C
Sample temp.:	10 °C
Injection volume:	10 µL
Flow rate:	0.4 mL/min
Mobile phase A:	Water with 10 mM ammonium acetate (0.1% formic acid)
Mobile phase B:	Methanol/acetonitrile (1:1) with 10 mM ammonium acetate (0.1% formic acid)
Gradient:	0–0.5 min isocratic at (95:5(A:B)); 6.0 min (0:100); 9.0 min (0:100); 9.5 min (95:5) 11.0 min (95:5)

MS conditions

MS system:	SYNAPT G2-S*i*
Ionization mode:	ESI+ and ESI-
Capillary voltage:	3 kV(ESI+) and 2.2 kV(ESI-)
Cone voltage:	30 V
Desolvation temp.:	550 °C
Source temp.:	150 °C
Acquisition range:	*m/z* 50–1200
Acquisition rate:	10 spectra per second
Lock mass:	Leucine enkephalin ($C_{28}H_{37}N_5O_7$ (*m/z* 556.2766 +ve) and (*m/z* 554.2620 -ve))
Collision energy:	HDMS^E low collision energy 4 eV and high collision energy ramp (10–45 eV)
MS resolution:	20,000 resolution full width half maximum (FWHM) at *m/z* 556
IM resolution:	≈40 Ω/ΔΩ (FWHM)
IMS parameters:	Default IMS screening parameters include T-Wave™ Velocity Ramp = Start: 1000 m/s and End: 300 m/s; T-Wave Pulse Height = 40 V; and a gas flow of helium 180 mL and nitrogen 90 mL (buffer gas) for the respective gas cells was used, giving an IM cell pressure of ~3.2 mBar
Calibration:	IMS/ToF Calibration Kit (p/n: 186008113)

Data management

Chromatography software:	MassLynx v4.1 SCN 916/924
MS software:	MassLynx v4.1 SCN 916/924
Informatics:	MassLynx data post-processed using UNIFI v1.94

RESULTS AND DISCUSSION

Positive and negative ion mass spectrometry libraries for LC-MS-amenable food additives were developed using a standardized library generation protocol.[11] The strategy determines both precursor ion, ion mobility product ions, and collision cross section values. The generated library contains data for food additive classes such as colorings, preservatives, antioxidants, and sweeteners, including the banned sweetener glycyrrhizin. Examples of the classes of food additive characterized using ion mobility are shown in Figure 1.

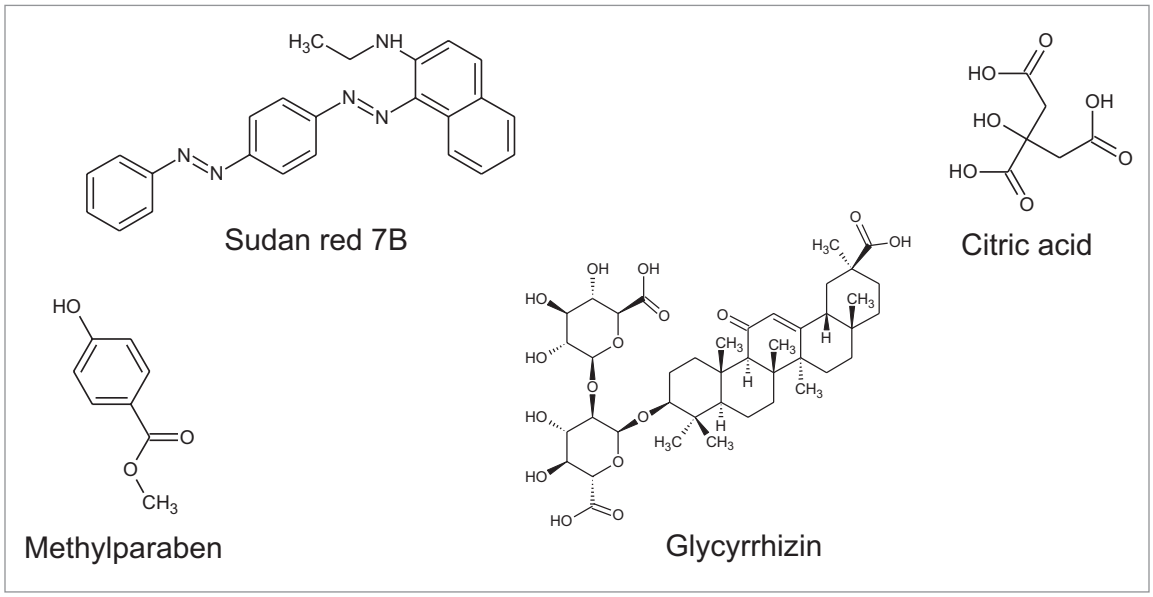

Figure 1. Example of food additive classes included in the MS library generated: colorings (Sudan red 7B), preservatives (methylparaben), antioxidants (citric acid), and sweeteners (glycyrrhizin).

Seven "off-the-shelf" food samples labelled as containing a variety of FAs, including sweeteners, preservatives, and food colorings, were purchased from Belgian supermarkets. The sample analysis performed using the FA multi-method was used to test the robustness of the $^{TW}CCS_{N_2}$ library generated. UPLC HDMS^E data were acquired in positive and negative ion modes, enabling comparison of the precursor/ion mobility product ions and $^{TW}CCS_{N_2}$ incorporated in the food additives libraries.

Using a "blind test" strategy for food commodity D2 (colorless lemon soft drink), two sweeteners (acesulfame E 969 and sucralose E 955), and a food preservative (citric acid E330) were detected and positively identified, with accurate mass measurement of 2 ppm and $^{TW}CCS_{N_2}$ Δ <2%. No food additive colorings were observed in sample D2. The corresponding negative ion HDMS^E precursor ion/mobility product ion spectra and CCS values for the food additives and natural constituent hesperidin identified in sample D2 are presented in Figure 2.

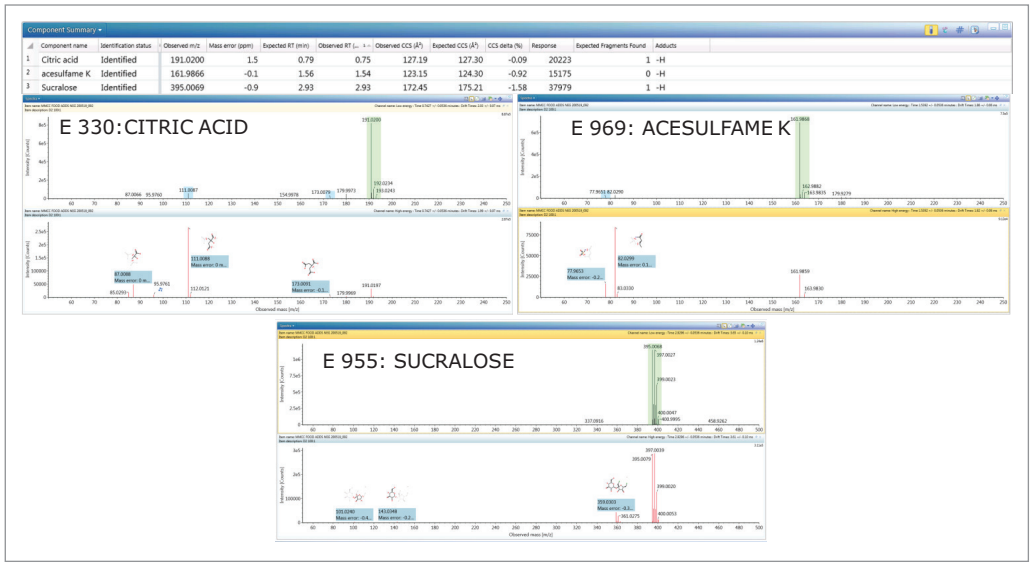

Figure 2. Negative ion HDMS^E precursor/product ion spectra for sweeteners and antioxidant FAs detected in a lemon soft drink (D2).

The value of the $^{TW}CCS_{N_2}$ libraries generated is illustrated in Figure 3 for food coloring (sodium cyclamate E 952), antioxidant (citric acid E 330), and sweetener (aspartame E 951 and acesulfame K E 950) agents identified in food commodity D3 (strawberry and kiwi drink), with the corresponding negative ion HDMS^E ion mobility precursor/ion mobility spectra for aspartame. The highly specific non-targeted retention time/drift time aligned ion mobility product ion spectrum obtained for aspartame shows product ion mass accuracy within 1 mDa (*m/z* 97.0404 =-4.12 ppm, *m/z* 146.0606=-3.4 ppm, *m/z* 200.0712=-2.5 ppm, *m/z* 261.0878=-1.14 ppm). For the food additives identified, delta $^{TW}CCS_{N_2}$ Δ <2% were observed. Moreover, no false detections were obtained for the food commodities screened using the food additive $^{TW}CCS_{N_2}$ MS library.

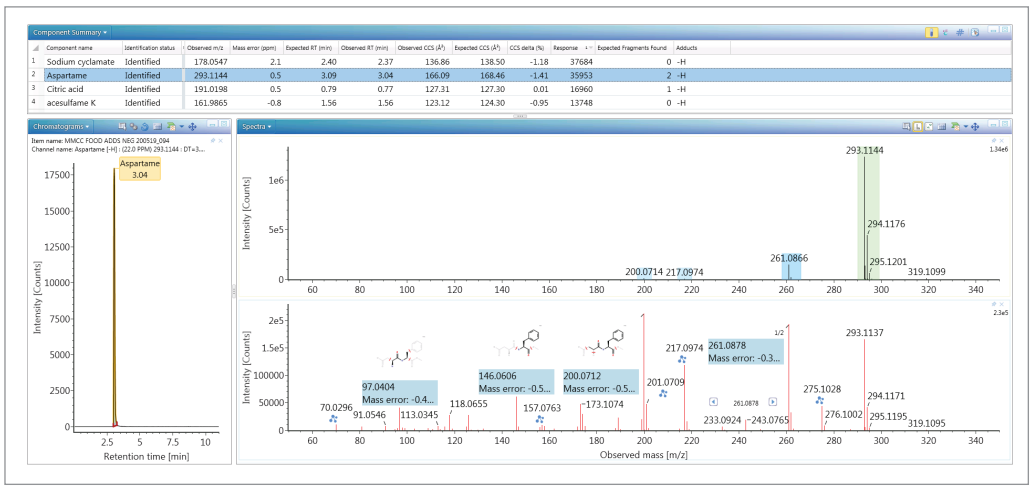

Figure 3. Negative ion HDMS^E precursor/product ion spectra for aspartame detected in a strawberry and kiwi soft drink (D3). Coloring and sweeteners identified.

To evaluate the robustness of the library when used in screening, several food commodities were also spiked with a series of food additive colorings and sweeteners. Food commodity D4 is a colorless tonic drink for which it would be expected that that no food coloring additives would be detected, as can be seen from the results shown in Figure 4, where the negative ion HDMS^E precursor/product ion spectra for additive acesulfame K (E 950) is shown, as well as the detection of compounds E 330 and E 955. The tonic drink D4 was spiked with a series of additional sweeteners and colorings for which the component summary detection results are shown in Figure 5, illustrating that the unauthorized sweeteners, alitame and glycyrrhizin were correctly detected. The ion mobility trace and HDMS^E precursor/product ion spectra for detection glycyrrhizin (E 958) are shown in Figure 6, where for glycyrrhizin $^{TW}CCSN_2$=286.2 Å^2 ($^{TW}CCSN_2$ Δ = -0.3%), was obtained. In Figure 7, the positive ion HDMS^E precursor and ion mobility product ion spectra for food additive library constituent alitame (E 956) is presented, with the +ve and -ve mobility traces and respective 172.3 Å^2/176.4 Å^2 values measured. The combined specificity of these values of these characterized $^{TW}CCSN_2$ values can be used to confirm detection at trace levels where only monisotopic information is determined. The obtained results confirm the robustness of the FAs libraries, where expected and unexpected food additives can be screened for by using the combination of retention time, precursor/ion mobility product ion *m/z*, and CCS values. When compared to the MS library, CCS measurements are routinely determined with delta values <2% (compared to typically accepted MRM ratio screening tolerances of 20%), providing confidence that $^{TW}CCSN_2$ can be utilised as a robust and reliable identification metric, in conjunction with retention time and *m/z*, in the application of flexible screening data processing workflows within critically important food safety research studies.

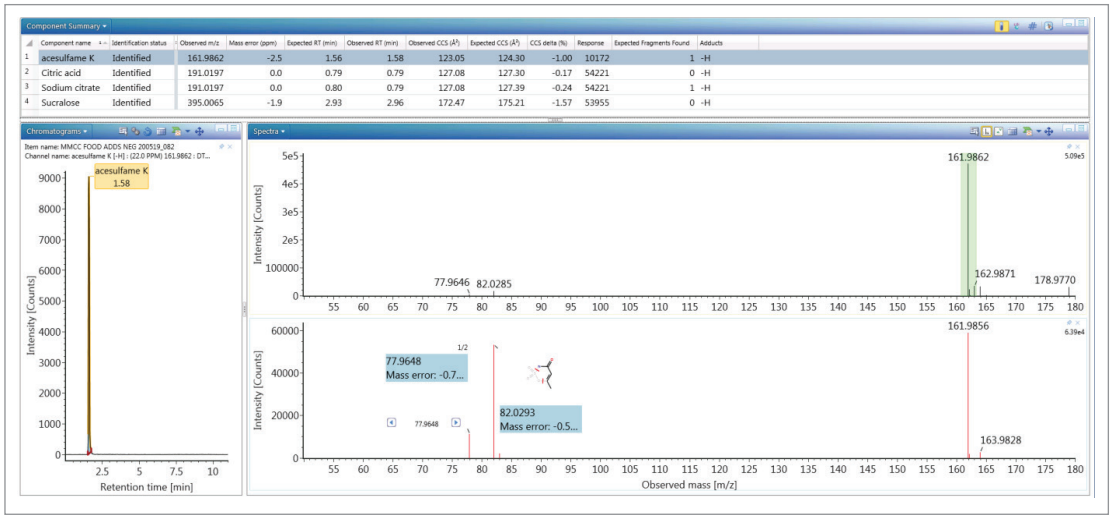

Figure 4. Negative ion HDMS^E precursor/product ion spectra for acesulfame K detected a colorless tonic drink (D4). Additives E 330, E 955 and E 950 identified.

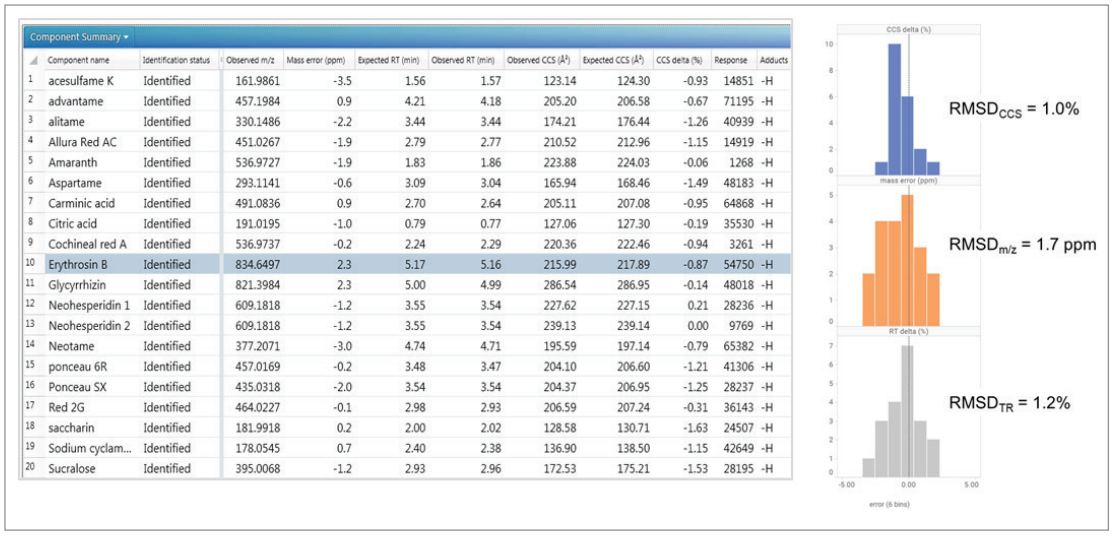

Figure 5. Negative ion HDMS^E detections for additional authorized/ unauthorized food additives spiked into a colorless tonic drink (D4), illustrating retention time, accurate mass measurement, and expected/observed $^{TW}CCSN_2$.

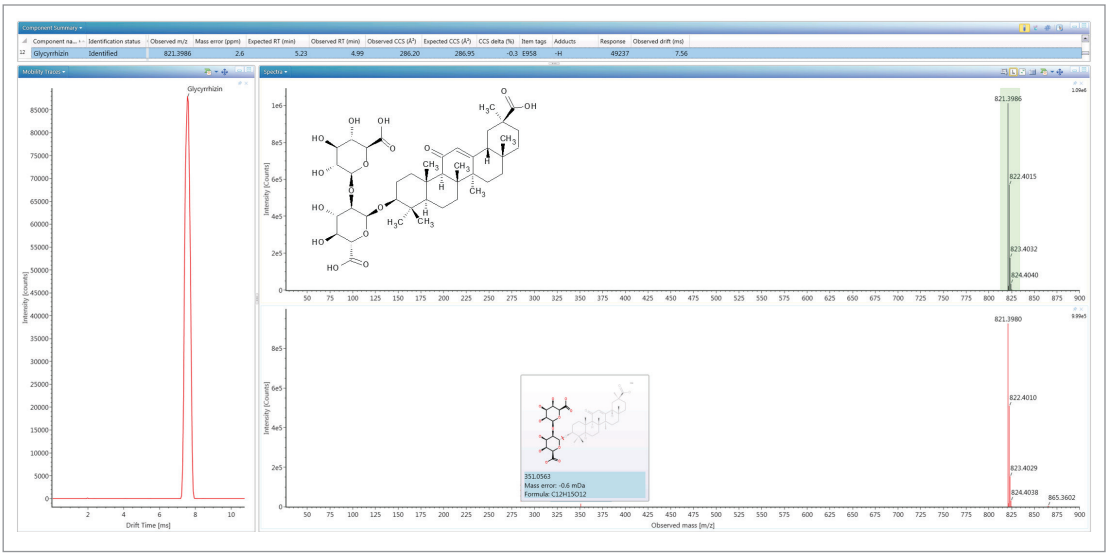

Figure 6. Negative ion HDMS^E precursor and ion mobility product ion spectra for unauthorized glycyrrhizin food additive spiked into a colorless tonic drink (D4). Observed $^{TW}CCSN_2$=286.2 Å^2 (CCS Δ = -0.3%).

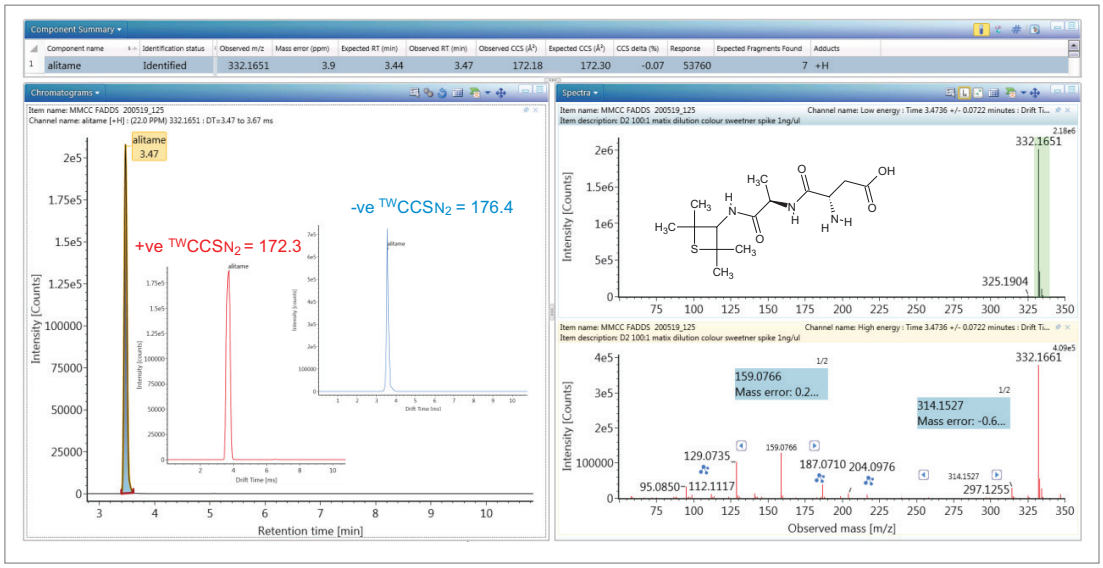

Figure 7. Positive ion HDMS^E precursor and ion mobility product ion spectra for food additive library constituent alitame, illustrating +ve and -ve $^{TW}CCSN_2$ values.

CONCLUSIONS

- A robust mass spectrometry library building strategy has been applied to generate positive ion and negative ion FA libraries comprising sweeteners, preservatives, antioxidants, and food colorings. Data collected included retention time, exact mass, retention/drift time precursor/ion mobility product ion *m/z* values, and CCS values.
- The library building strategy, incorporating the measurement and use of $^{TW}CCS_{N_2}$ values has been shown to be robust and the generated library used to perform non-targeted screening for food additives in everyday food commodities. Positive identification of authorized/unauthorized food additives have routinely been determined within a 2% CCS tolerance, which compares favorably to typically accepted MRM ratio screening tolerances of 20%.
- The use of CCS as a screening parameter offers the potential to reduce the initial specificity of the applied post-acquisition workflow screening parameters and reduce false detections in complex matrices where generic extraction methods have been utilized.
- Combined positive and negative ion $^{TW}CCS_{N_2}$ values create a highly specific fingerprint that can be used to enhance specificity at trace detection levels.
- A multi-additive method to enable the analysis of a large number of FAs has been developed to enable better coverage of foods that are required to be controlled and simultaneously facilitate exposure assessment to multiple FAs.
- $^{TW}CCS_{N_2}$ can be utilized as a robust and reliable identification metric in conjunction with retention time and *m/z*, in application of flexible screening data processing workflows, to increase confidence in identification in non-targeted food additive screening within critically important food safety research studies.

References

1. Regulation (EC) No 882/2004.2. Directives 94/35/CE (Article 8), 94/36/CE (Article 6), and 95/2/CE (Article 7).
2. Directives 94/35/CE (Article 8), 94/36/CE (Article 6), and 95/2/CE (Article 7).
3. https://ec.europa.eu/food/safety/food_improvement_agents/additives.
4. Goscinny, S.; McCullagh, M. A Novel Approach to the Reduction of False Positive and Negative Identifications in Screening of Pesticide Residues in Food Analysis, Proceedings of the 61st ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis, MN, 2013.
5. McCullagh, M.; Goscinny, S. Discovery of Pesticide Protomers Using Routine Ion Mobility Screening. McCullagh, M.; Goscinny, S. Waters Corporation Application Note 720005028EN. 2014.
6. Goscinny, S.; McCullagh, M.; Far, J.; De Pauw, E.; Eppe, G. Towards the Use of Ion Mobility Mass Spectrometry Derived Collision Cross Section as a Screening Approach for Unambiguous Identification of Targeted Pesticides in Food. *Rapid Commun. Mass Spectrom.* **2019**, p. 1–15.
7. McCullagh, M.; Wood, M.; Mistry, N.; Goscinny, S.; Douce, D.; Dalsgaard, P. Investigations into Cross-Platform and Long-Term Robustness of a CCS metric, 67th ASMS Conference on Mass Spectrometry and Allied Topics, Atlanta, GA, 2019.
8. McCullagh, M.; Giles, K.; Richardson, K.; Stead, S.; Palmer, M. Investigations into the Performance of Travelling Wave Enabled Conventional and Cyclic Ion Mobility Systems to Characterise Protomers of Fluoroquinolone Antibiotic Residues. *Rapid Commun Mass Spectrom.* **2019**; p. 1–11.
9. McCullagh, M.; Pereira, C.A.M; Yariwake, J.H. Use of Ion Mobility Mass Spectrometry to Enhance Cumulative Analytical Specificity and Separation to Profile 6-C/8-Cglycosylflavone Critical Isomer Pairs and Known–Unknowns in Medicinal Plants. *Phytochemical Analysis*. **2019**; p. 1–13.
10. McCullagh, M.; Douce, D.; Van Hoeck, E.; Goscinny, S. Exploring the Complexity of Steviol Glycosides Analysis Using Ion Mobility Mass Spectrometry. *Anal. Chem.* **2018**; *90*, p. 4585–4595.
11. Goshawk, J.; Barknowitz, G.; McCullagh, M. The Development of a Natural Products Library Using Ion-Mobility Enabled Mass Spectrometry. 67th ASMS Conference on Mass Spectrometry and Allied Topics, Atlanta, GA, 2019.

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