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Food

TIC-PL-082-283

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PO-CON1458E

An LCMS Method for the Detection of Cocoa Butter Substitutes, Replacers, and Equivalents in Commercial Chocolate-like Products

ASMS 2014 ThP632

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Introduction

There is increasing demand for genuine cocoa butter (CB) in chocolate products in developed nations, however, this demand has created a shortage of CB and raised its costs. To overcome this, chocolate manufactures sometimes add vegetable-derived fats to some chocolate products to reduce costs while still maintaining desirable physical characteristics. It is of current interest to have a reliable method to detect, identify, and quantify the triacylglycerol (TAG) components of cocoa butter substitutes, replacers, and equivalents (CBEs) in chocolate products. Traditionally GC was used for this task, but due to the low volatility of triacylglycerides and their susceptibility to thermal decomposition, retention time is the only identifying factor for the TAGs and typical GC analyses of this type can take 40 minutes. LCMS is able to not only provide faster throughput, but also has the additional advantage of allowing characterization of the TAG, including qualitative regiospecific analysis. We have developed a single, UHPLC column-based LCMS method to analyze the TAG components in commercial chocolate and chocolate-like products. This analysis has a runtime of 17minutes, making it suitable for relatively high throughput. Additionally, the method was very repeatable, with an interday variability of <7% for the absolute area counts of the three major TAGs in CB (POP,POS,SOS).

Materials and Method

A Shimadzu Nexera UHPLC coupled to a Shimadzu LCMS-8040 triple quadrupole mass spectrometer was utilized for this analysis. A pure CB standard was used as a reference. Chocolate and chocolatey products were purchased in retail stores over a range of cocoa content.

Sample Preparation

For analysis, we slightly modified a sample preparation method originally used for algal oils. For analysis, 5mg of sample was weighed and then dissolved in a 3:1 Toluene-Isopropyl Alcohol solution. We then sonicated the mixture for 5 minutes. The solution was filtered through a Thomson filter vial (P/N 35538-100) to remove sugars and other insoluble materials and diluted 5-fold using 3:1 Toluene-IPA and injected into the UHPLC-MS system.

Chromatography

Instrument	: Shimadzu Nexera UHPLC system
Column	: Shimadzu Shim-Pack XR-ODSIII (200x2.1mm,)
Mobile Phase A	: LC/MS Acetonitrile
Mobile Phase B	: 1:1 Dichloromethane-Isopropyl Alcohol
Gradient Program	: 48% B (initially) – gradient to 51% B (0-8.0 min) – gradient to 54% B (8.0 – 11.0 min) – gradient to 74% B (11.0-14.0 min) – hold at 74% B (14.0-15.0 min) – reequilibrate at 48% B (15.1-17 min)
Flow Rate	: 0.33 mL/min
Column Temperature	: 30°C
Injection Volume	: 1 µL

Mass Spectrometry

Instrument	: Shimadzu LCMS-8040 Triple Quadrupole Mass Spectrometer
Ionization	: APCI
Polarity	: Positive
Scan Mode	: Q3 Scan

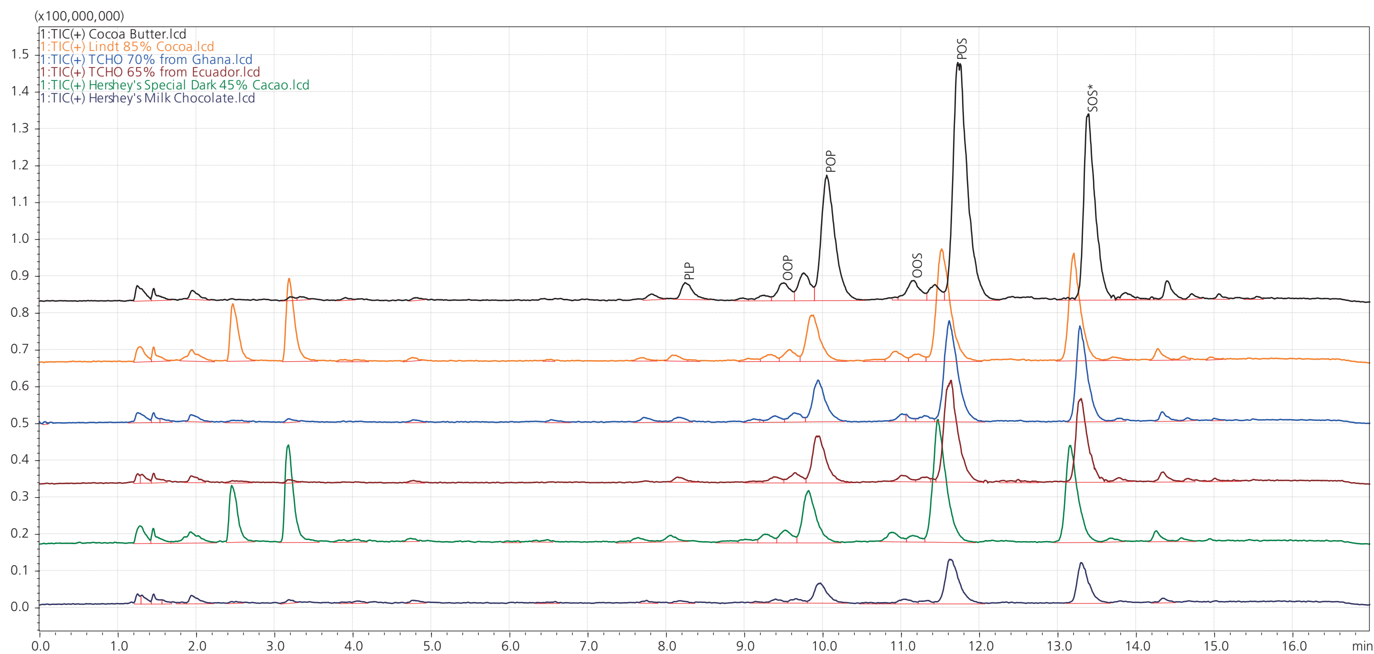
Results

Retail Chocolates from Hershey's, Lindt and Tcho, as well as a chocolatey candy - Charleston Chew - were compared against pure cocoa butter. The chocolates used were selected to cover a range of Cocoa content and purity. We specifically chose to use Hershey's Mr. Goodbar and Charleston Chews because they listed the use of vegetable oils in their ingredients list. As you can see in the chromatograms, the products that market themselves as pure chocolate have similar chromatograms in comparison to the pure CB.

We used an MS library that was provided to us by Dr. John Carney and Mona Koutchekinia to identify the types of TAGs contained in the chocolates using the spectral information captured in the Q3 scans. A minimum similarity of 70 was required for a result to be considered a match. In order to identify usage of CBEs, we applied the equation: $\%POP<44.025-0.733*\%SOS$, which was determined by the European Commission Joint Research Centre, which can detect around 2% CBE usage in CB content, or approximately 0.4% CBE content in chocolate. The chocolate products we tested all agreed with the expected results: All of the dark chocolate products we tested passed this specification, as well as Hershey's Milk Chocolate. The two products which had a higher %POP than is allowable, Mr. Goodbar and Charleston Chew, were selected specifically for the inclusion of vegetable oils. It may be informative to further test the accuracy of this testing method by adulterating cocoa butter with known quantities of CBEs. The data has been summarized in Table 1.

Table 1: Percentage of the major TAGs in CB in various chocolate products

Product	%POP	%POS	%SOS	%POP needs to be less than
Cocoa Butter	23.7%	46.9%	29.5%	43.8
Lindt 85% Cocoa	16.9%	46.4%	36.6%	43.8
TCHO 70% from Ghana	17.8%	46.1%	36.1%	43.8
TCHO 65% from Ecuador	20.9%	46.2%	32.9%	43.8
Hershey's Special Dark	20.0%	47.1%	32.9%	43.8
Hershey's Milk Chocolate	18.6%	46.6%	34.8%	43.8
Hershey's Mr Goodbar	44.8%	21.1%	34.1%	43.8
Charleston Chew	100.0%	0.0%	0.0%	44.0



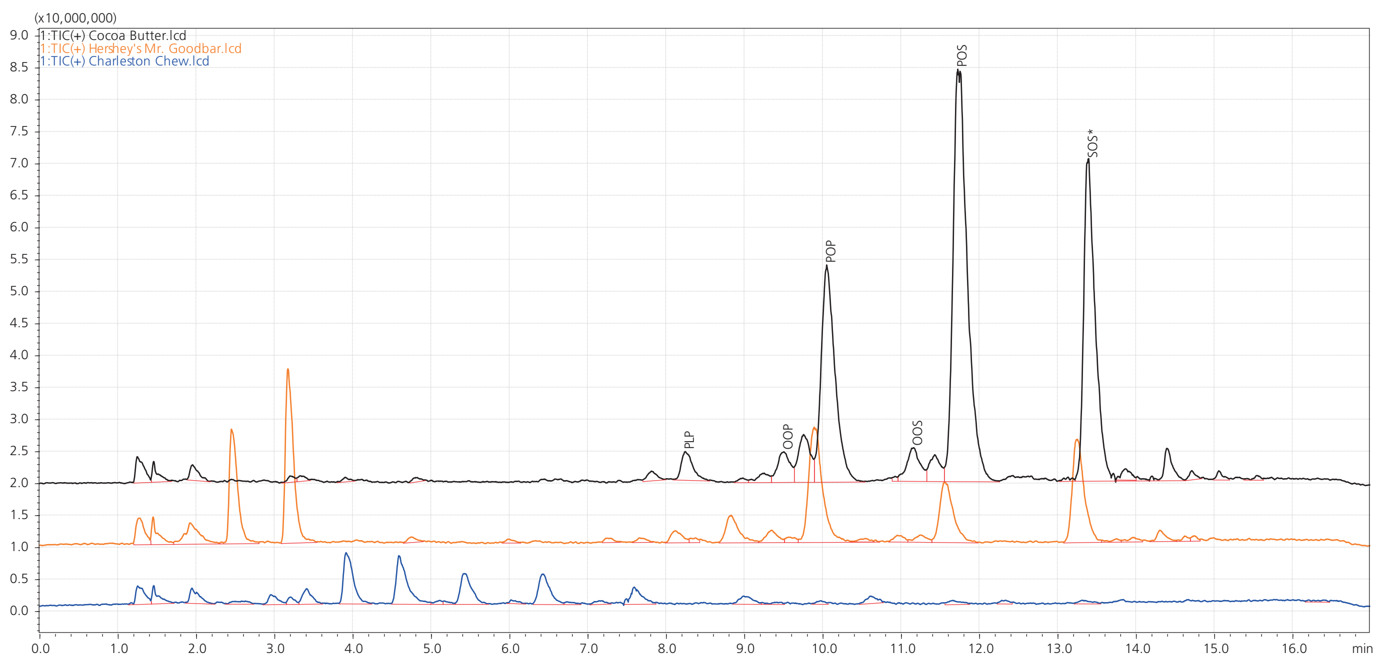


Figure 1. Chromatograms of the various chocolate products analyzed versus pure cocoa butter

Conclusions

We have developed a 17 minute method for the rapid determination of CBE usage in chocolate products by using a UHPLC column and Q3 ion scans to analyze samples and then matching spectral information with an MS library of ion ratios for identifying TAGs.
Further studies could add a calibration curve to enable quantification of TAGs. This method should also provide a base method which can be modified to support TAG analysis in other product types.

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Acknowledgements

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PO-CON1471E

Highly Sensitive and Robust LC/MS/MS Method for Quantitative Analysis of Artificial Sweeteners in Beverages

ASMS 2014 MP351

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Introduction

Artificial sweeteners described as intense, low-calorie and non-nutritive are widely used as sugar substitutes in beverages and foods to satisfy consumers' desire to sweet taste while concerning about obesity and diabetes. As synthetic additives in food, the use of artificial sweeteners must be approved by authority for health and safety concerns. For example, Aspartame, Acesulfame-K, Saccharin, Sucralose and Neotame are the FDA approved artificial sweeteners on the US market. However, there are also many other artificial sweeteners allowed to use in EU and many other countries (Table 2), but not in the US. In this regard, analysis of artificial sweeteners in beverages and foods has become essential due to the relevant regulations in protection of consumers' benefits and safety concerns in many countries [1, 2]. Recently, artificial sweeteners are found as emerging environmental contaminants in surface water and waste water [3]. Initially, HPLC analysis method with ELSD detection was adopted, because many artificial sweeteners are non-UV absorption compounds [2]. Recently, LC/MS/MS methods have been developed and used for identification and quantitation of artificial sweeteners in food and beverages as well as water for its high sensitivity and selectivity [3, 4]. Here we report a high sensitivity LC/MS/MS method for identification and quantitation of ten artificial sweeteners (Table 2) in beverage samples. An ultra-small injection volume was adopted in this study to develop a very robust LC/MS/MS method suitable for direct injection of beverage samples without any sample pre-treatment except dilution with solvent.

Experimental

Ten artificial sweeteners of high purity as listed in Table 2 were obtained from chemicals suppliers. Stock standard solutions and a set of calibrants were prepared from the chemicals with methanol/water (50/50) solvent as the diluent. Three brand soft-drinks and a mouthwash bought from local supermarket were used as testing samples in this study. The samples were not pretreated by any means except dilution with the diluent prior to injection into LCMS-8040 (Shimadzu Corporation, Japan), a triple quadrupole LC/MS/MS system. The front-end LC system connected to the LCMS-8040 is a high pressure binary gradient Nexera UHPLC. The details of analytical conditions of LC/MS/MS method are shown in Table 1.

Table 1: LC/MC/MS analytical conditions of artificial sweeteners on LCMS-8040

Column	Synergi, Polar-RP C18 (100 x 2 mm, 2.5µm)
Flow Rate	0.25 mL/min
Mobile Phase	A: water with 0.1% Formic acid - 0.03% TA B: MeOH with 0.1% FA - 0.03% Trimethylamine
Gradient program	B: 10% (0.01 to 0.5 min) → 95% (8 to 9 min) → 10% (9.01 to 11min)
MS mode	ESI, MRM, positive-negative switching
ESI condition	Nebulizing gas: 3L/min, Drying gas: 15L/min, Heating block: 400°C, DL: 250°C
Inj. Vol.	0.1uL, 0.5uL, 1uL, 5uL and 10uL

Results and Discussion

Method development

First, precursor selection and MRM optimization of the ten sweeteners studied was carried out using an automated MRM optimization program of the LabSolutions. Six compounds were ionized in negative mode and four in positive mode as shown in Table2. For each compound, two optimized MRM transitions were selected and used, with the first one for quantitation and the second one for confirmation.

The ten compounds were well-separated as sharp peaks between 2 min and 8.2 min as shown in Figure 1. Linear calibration curves of wide concentration ranges were established with mixed standards in diluent as summarized in Table 2. We also investigated the performance of the LC/MS/MS method established by employing very small injection volumes (0.1, 0.5, 1 and 5 uL). This is because actual beverages usually contain very high contents of sweeteners (>>1ppm) to MS detection. Analysts normally dilute the samples before injection into LC/MS/MS. An alternative way is to inject a very small volume of samples even without dilution. Figs 2 & 3 show a chromatogram and calibration curves established with 0.1uL injection, which demonstrates the feasibility of an ultra-small injection volume combined with high sensitivity LC/MS/MS.

Table 2: Artificial Sweeteners, MRM transitions and calibration curves on LCMS-8040

Cat[1]	Compd. & Abbr. Name	MRM parameter					RT & Calibration Curve[4]		
		Trans. (m/z)	Pola. (+/-)	Q1 (V)	CE (V)	Q3 (V)	RT (min)	Conc. R. (ug/L)	R^2
A2	Acesulfame K (Ace-K)	161.9 >82.1	-	11	14	29	1.99	1 - 20000	0.9999
		161.9 >78.0	-	11	32	28			
A5	Cyclamate (CYC)[3]	178.3 >80.1	-	19	24	30	2.87	5 - 20000	0.9996
		178.3 >79.0	-	12	27	10			
A3	Saccharin (SAC)	181.9 >106.1	-	13	20	15	3.28	1 - 20000	0.9984
		181.9 >42.1	-	13	36	13			
A4	Sucralose[2] (SUC)	441.0 >395.1	-	20	11	25	4.61	5 - 20000	0.9983
		441.0 >359.1	-	20	15	23			
A1	Aspartame (ASP)	295.1 >120.1	+	-19	-25	-25	5.15	0.1 - 2000	0.9999
		295.1 >180.1	+	-19	-14	-20			
A6	Neotame (NEO)	379.3 >172.2	+	-18	-23	-20	7.51	0.05 - 1000	0.9998
		379.3 >319.3	+	-18	-18	-24			
B1	Alitame (ALI)	332.2 >129.1	+	-23	-19	-26	5.44	0.1 - 2000	0.9995
		332.2 >187.1	+	-23	-16	-21			
B3	Dulcin (DUL)	181.1 >108.1	+	-22	-25	-21	5.58	5 - 10000	0.999
		181.1 >136.1	+	-21	-18	-26			
B2	Neohespiridin Dihydrochalcone (NHDC)	611.3 >303.1	-	30	38	30	6.71	0.5 - 2000	0.9988
		611.3 >125.3	-	30	47	20			
C1	Glycyrrhi-Zinate (GLY)	821.5 >351.2	-	22	46	20	8.19	5 - 1000	0.9996
		821.5 >193.2	-	22	52	19			

1. *A1~A6: US FDA, EU and others approval; B1~B3: only EU and other countries approval. C1: natural sweetener, info not available.*
2. *Sucralose precursor ion m/z 441.0 is formic acid adduct ion.*
3. *Sodium cyclamate known as "magic sugar" was initially banned in the US in 2000. FDA lifted the ban in 2013.*
4. *Injection volume: 10 uL*

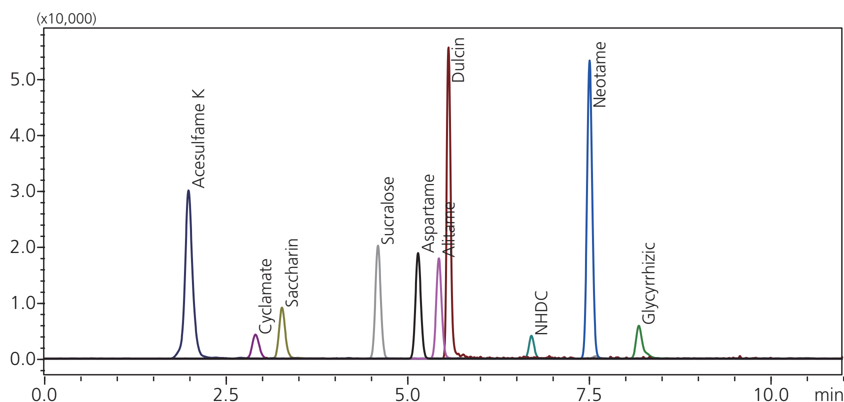

Figure 1: MRM Chromatogram of ten sweeteners by LC/MS/MS with 10uL injection:
Asp & Ali 1ppb, Neo 0.5ppb, Dul, Gly, Ace-K, Sac, Suc and Cyc 10ppb, NHDC 1ppb.

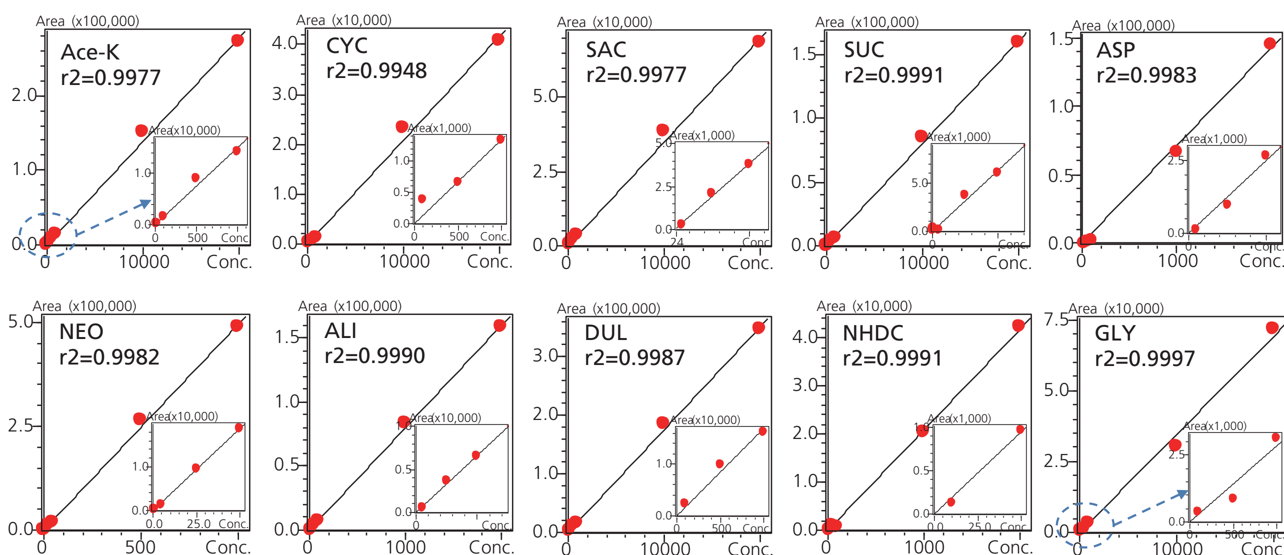

Figure 3: Calibration curves of artificial sweeteners on LCMS-8040 with
an ultra-small injection volume (0.1 uL) of same set of calibrants as shown in Table 2.

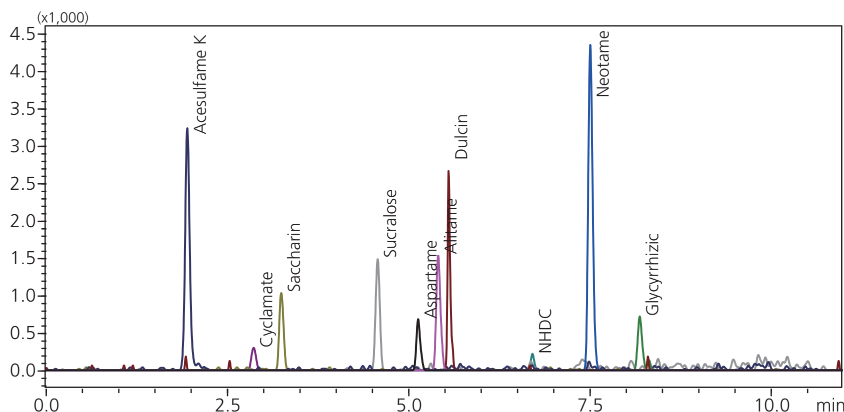

Figure 2: MRM Chromatogram of ten sweeteners by LC/MS/MS with 0.1uL injection:
Asp & Ali 0.1ppm, Neo 0.05ppm, Dul, Gly, Ace-K, Sac, Suc and Cyc 1ppm, NHDC 0.1ppm.

Method performance

Table 3 summarizes the results of repeatability and sensitivity of the method with mixed standards. The method was not evaluated with beverage spiked samples. However, because beverage samples are normally diluted many times, matrix effect and interferences can be ignored for high sensitivity LC/MS/MS analysis. The results indicate that the method with ultra-small injection volume exhibits good linearity, repeatability and sensitivity.

Table 3: Repeatability and Sensitivity of LC/MS/MS method of artificial sweeteners

Name	Repeatability (peak area), 10uL				Sensitivity (ug/L)					
	Conc. (ug/L)	RSD%	Conc. (ug/L)	RSD%	LOQ/LOD (0.1 µL inj)		LOQ/LOD (0.5 µL inj)		LOQ/LOD 10 (µL inj)	
Ace-K	20	5.1	100	5.2	200	50	40	10	4.0	1.33
CYC	20	11.7	100	8.1	800	500	200	90	14	4.5
SAC	20	8.0	100	5.8	250	100	50	20	4.5	1.5
SUC	20	7.5	100	2.7	200	100	50	15	2.4	0.8
ASP	2	7.8	10	3.0	80	20	20	4	0.5	0.17
NEO	1	5.3	5	1.0	5	3	2	1	0.03	N.A.
ALI	2	8.6	10	1.7	40	25	10	5	0.2	N.A.
DUL	20	7.5	100	3.1	160	50	30	10	1.4	0.5
NHDC	2	9.2	10	4.6	100	25	40	6	0.5	0.18
GLY	20	8.2	100	5.4	400	150	15	5	5.0	1.8

Analysis of beverage samples

The LC/MS/MS method established was applied for screening and quantitation of the targeted sweeteners in three brand beverages: S1, S2 and S3, and a mouthwash S4. The results are shown in Figure 4 and Table 4. It is interested to note that glycyrrizinate was found in the mouthwash.

Table 4: Screening and quantitation results for ten artificial sweeteners in beverages and mouthwash (mg/L)

Artificial Sweetener	S1	S2	S3	S4
ASP	116.9	127.9	ND	ND
Ace-K	143.9	165.9	97.2	ND
Saccharin	ND	ND	ND	208.7
SUC	55.1	ND	183.4	ND
GLY	ND	ND	ND	449.3
Others	ND	ND	ND	ND

1. *S2 was diluted 100 times, the rests were diluted 10 times. 1 uL injection.*
2. *ND = not detected.*

Highly Sensitive and Robust LC/MS/MS Method for Quantitative Analysis of Artificial Sweeteners in Beverages

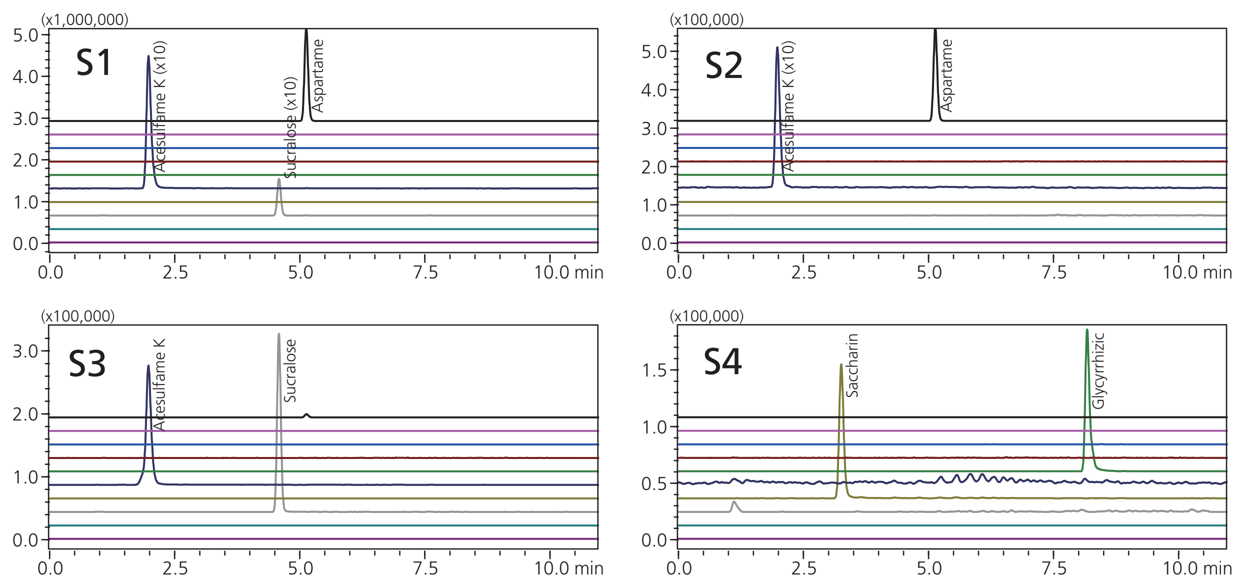

Figure 4: Screening and quantitation for 10 targeted artificial sweeteners in beverage and mouthwash samples by LC/MS/MS with 1uL injection.

Conclusions

A MRM-based LC/MS/MS method was developed and evaluated for screening and quantitation of ten artificial sweeteners in beverages. This high sensitivity LC/MS/MS method combined with small or ultra-small injection volume (0.1~1.0 uL) was proven to be feasible and reliable in actual samples analysis of the targeted sweeteners in beverages, achieving high throughput and free of sample pre-treatment (except dilution). The method is expected to be applicable to surface water and drinking water samples. For wastewater and various foods, sample pre-treatment is usually required. However, the advantages of the method in high sensitivity and ultra-small injection volume are expected to enable it tolerates relatively simple sample pre-treatment procedures.

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Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching

ASMS 2014 MP345

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Introduction

Mycotoxins are toxic metabolites produced by fungal molds on food crops. For consumer food safety, quality control of food and beverages has to assay such contaminants. Depending on the potency of the mycotoxin and the use of the food, the maximum allowed level is defined by legislation. Baby food is particularly critical. For example, European Commission has fixed the maximum level of Aflatoxin B1 and M1 to 0.1 and 0.025 µg/kg, respectively, in baby food or milk.

Therefore, a sensitive method to assay mycotoxins in complex matrices is mandatory. In order to ensure productivity of laboratory performing such assays, a unique rapid method able to measure as much mycotoxins as possible independently of the sample origin is also needed. In this study, we tested three kind of samples: baby milk powder, milk thickening cereals (flour, rice and tapioca) and a vegetable puree mixed with cereals.

Materials and Methods

Sample preparation

Sample preparation was performed by homogenization followed by solid phase extraction using specific cartridges (Isolute® Myco, Biotage, Sweden) covering a large spectrum of mycotoxins.
Sample (5g) was mixed with 20 mL of water/acetonitrile (1/1 v/v), sonicated for 5 min and agitated for 30 min at room temperature. After centrifugation at 3000 g for 10 min, the supernatant was diluted with water (1/4 v/v).
Columns (60mg/3 mL) were conditioned with 2 mL of acetonitrile then 2 mL of water. 3 mL of the diluted supernatant were loaded at the lowest possible flow rate.
Then column was washed with 3 mL of water followed by 3 mL of water/acetonitrile (9/1 v/v). After drying, compounds were successively eluted with 2 mL of acetonitrile with 0.1% of formic acid and 2 mL of methanol.
The eluate was evaporated under nitrogen flow at 35 °C until complete drying (Turbovap, Biotage, Sweden).
The sample was reconstituted in 150 µL of a mixture of water/methanol/acetonitrile 80/10/10 v/v with 0.1% of formic acid.

LC-MS/MS analysis

Extracts were analysed on a Nexera X2 (Shimadzu, Japan) UHPLC system and coupled to a triple quadrupole mass spectrometer (LCMS-8050, Shimadzu, Japan). Analysis was carried out using selected reaction monitoring acquiring 2 transitions for each compound.

Table 1 – LC conditions

Analytical column	: Shimadzu GLC Mastro™ C18 150x2.1 mm 3µm
Mobile phase	: A = Water 2mM ammonium acetate and 0.5% acetic acid B = Methanol/Isopropanol 1/1 + 2mM ammonium acetate and 0.5% acetic acid
Gradient	: 2%B (0.0min), 10%B (0.01min), 55%B (3.0min), 80%B (7.0 -8.0min), 2%B (8.01min), Stop (11.0min)
Column temperature	: 50°C
Injection volume	: 10 µL
Flow rate	: 0.4 mL/min

Table 2 – MS/MS conditions

Ionization mode	: Heated ESI (+/-)
Temperatures	: HESI: 400°C Desolvation line: 250°C Heat block: 300°C
Gas flows	: Nebulizing gas (N2): 2 L/min Heating gas (Air): 15 L/min Drying gas (N2): 5 L/min
CID gas pressure	: 270 kPa (Ar)
Polarity switching time	: 5 ms
Pause time	: 1 ms
Dwell time	: 6 to 62 ms depending on the number of concomitant transitions to ensure a minimum of 30 points per peak in a maximum loop time of 200 ms (including pause time and polarity switching)

Table 3 – MRM transitions

Name	Ret. Time (min)	MRM Quan	MRM Qual
15-acetyldeoxynivalenol (15ADON) [M+H]+	3.37	339 > 297.1	339 > 261
3-acetyldeoxynivalenol (3ADON) [M+H]+	3.37	339 > 231.1	339 > 231.1
Aflatoxine B1 (AFB1) [M+H]+	3.78	312.6 > 284.9	312.6 > 240.9
Aflatoxine B2 (AFB2) [M+H]+	3.57	315.1 > 259	315.1 > 286.9
Aflatoxine G1 (AFG1) [M+H]+	3.46	329.1 > 242.9	329.1 > 199.9
Aflatoxine G2 (AFG2) [M+H]+	3.26	330.9 > 244.9	330.9 > 313.1
Aflatoxine M1 (AFM1) [M+H]+	3.30	329.1 > 273	329.1 > 229
Alternariol [M-H]-	4.78	257 > 214.9	257 > 213.1
Alternariol monomethyl ether [M-H]-	5.81	271.1 > 255.9	271.1 > 228
Beauvericin (BEA) [M+H]+	8.03	784 > 244.1	784 > 262
Citrinin (CIT) [M+H]+	4.16	251.3 > 233.1	251.3 > 205.1
D5-OTA (ISTD)	5.22	409.2 > 239.1	N/A
Deepoxy-Deoxynivalenol (DOM-1) [M-H]-	3.02	279.2 > 249.3	279.2 > 178.4
Deoxynivalenol (DON) [M-CH3COO]-	2.61	355.3 > 295.2	355.3 > 265.1
Deoxynivalenol 3-Glucoside (D3G) [M+CH3COO]-	2.45	517.5 > 457.1	517.5 > 427.1
Deoxynivalenol 3-Glucoside (D3G) [M+CH3COO]-	2.45	517.5 > 457.1	517.5 > 427.1
Diacetoxyscirpenol (DAS) [M+NH4]+	1.20	384 > 283.3	384 > 343
Enniatin A (ENN A) [M+H]+	8.51	699.2 > 682.2	699.3 > 210
Enniatin A1 (ENN A1) [M+H]+	8.22	685.3 > 668.3	685.3 > 210.1
Enniatin B (ENN B) [M+H]+	7.57	657 > 640.4	657 > 195.9
Enniatin B1 (ENN B1) [M+H]+	7.92	671.2 > 654.2	671.2 > 196
Fumagillin (FUM) [M+H]+	6.16	459.2 > 131.1	459.2 > 338.7
Fumonisine B1 (FB1) [M+H]+	4.10	722.1 > 334.2	722.1 > 352.2
Fumonisine B2 (FB2) [M+H]+	4.71	706.2 > 336.3	706.2 > 318.1
Fumonisine B3	4.38	706.2 > 336.2	706.2 > 688.1
Fusarenone-X (FUS-X) [M+H]+	2.84	355.1 > 247	355.1 > 175
HT2 Toxin [M+Na]+	4.58	446.9 > 344.9	446.9 > 285
Moniliformin (MON) [M-H]-	1.16	97.2 > 40.9	N/A
Neosolaniol (NEO) [M+NH4]+	2.90	400.2 > 215	400.2 > 185
Nivalenol (NIV) [M+CH3COO]-	2.41	371.2 > 280.9	371.2 > 311.1
Ochratoxin A (OTA) [M+H]+	5.53	404.2 > 239	404.2 > 358.1
Ochratoxin B (OTB) [M+H]+	4.83	370.2 > 205.1	370.2 > 187
Patulin (PAT) [M-H]-	2.35	153 > 81.2	153 > 53
Sterigmatocystin (M+H]+	5.60	325.3 > 310	325.3 > 281.1
T2 Tetraol [M+CH3COO]-	1.64	356.8 > 297.1	356.8 > 59.1
T2 Toxin [M+NH4]+	4.94	484.2 > 215	484.2 > 305
Tentoxin [M-H]-	4.77	413.1 > 140.9	413.1 > 271.1
Tenuazonic acid (TEN) [M-H]-	4.51	196.1 > 138.8	196.1 > 112
Wortmannin (M-H)	3.95	426.9 > 384	426.9 > 282.1
Zearalanol (alpha) (ZANOL) [M-H]-	5.17	321.3 > 277.2	321.3 > 303.2
Zearalanol (beta) (ZANOL) [M-H]-	4.85	321.3 > 277.2	321.3 > 303.1
Zearalanone (ZOAN) [M-H]-	5.43	319 > 275.1	319 > 301.1
Zearalenol (alpha) (ZENOL) [M-H]-	5.25	319.2 > 275.2	319.2 > 160.1
Zearalenol (beta) (ZENOL) [M-H]-	4.94	319.2 > 275.2	319.2 > 160.1
Zearalenone (ZON) [M-H]-	5.52	316.8 > 174.9	316.8 > 131.1

Results and discussion

Method development

LC conditions were transferred from a previously described method (Tamura et al., Poster TP-739, 61st ASMS). In particularly, the column was chosen to provide very good peak shape for chelating compounds like fumonisins thanks to its inner PEEK lining.

Small adjustments in the mobile phase and in the gradient program were made to handle more mycotoxins, especially the isobaric ones. These modifications are reported in the Table 1.

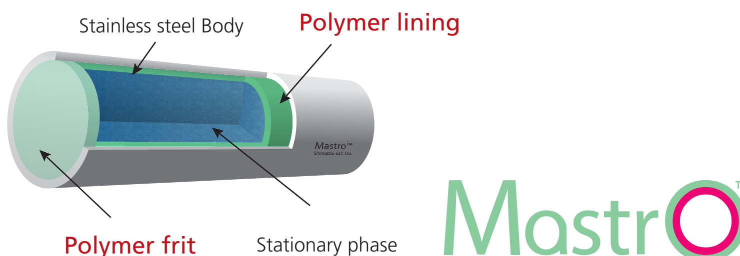

Figure 1 – Structure of the Mastro™ column

Also, autosampler rinsing conditions were kept to ensure carry-over minimisation of some difficult compounds. Electrospray parameters (gas flows and temperatures) were cautiously optimized to find the optimal combination for the most critical mycotoxins (aflatoxins). Since these parameters act in a synergistic way, a factorial design experiment is needed to find it. Manually testing all combinations in the chromatographic conditions is very time consuming. Therefore, new assistant software (Interface Setting Support) was used to generate all possible combinations and generate a rational batch analysis. Optimal combination was found in chromatographic conditions. The difference observed between optimum and default or worst parameters was of 200 and 350%, respectively.

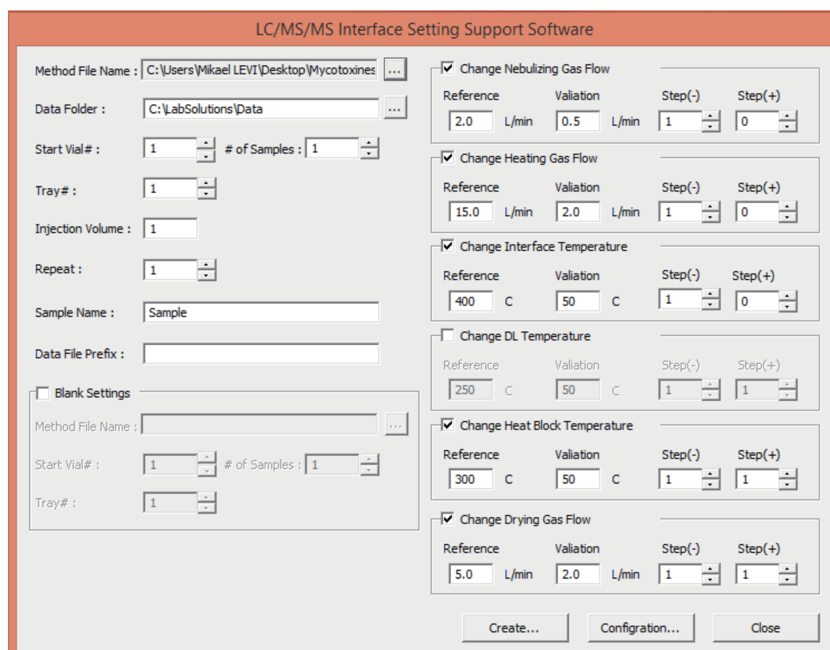

Figure 2 – Parameters selection view in the Interface Setting Support Software

Results

Extraction and ionisation recovery for aflatoxins was measured in the three matrices by comparing peak areas of the raw sample extract to extract spiked at 50 ppb after or before extraction and to standard solution. Results in table 4 showed that the total recovery was quite acceptable to ensure accurate quantification. Results from other matrices were not significatively different.

Table 4 – Extraction and ionisation recoveries in puree

	AFB1	AFB2	AFG1	AFG2	AFM1
Extraction recovery	101%	109%	104%	114%	118%
Ionisation recovery	49%	90%	96%	106%	91%
Total recovery	49%	98%	100%	121%	108%

Repeatability was evaluated at low level for aflatoxins. Figure 3 shows an overlaid chromatogram (n=4) for aflatoxins.

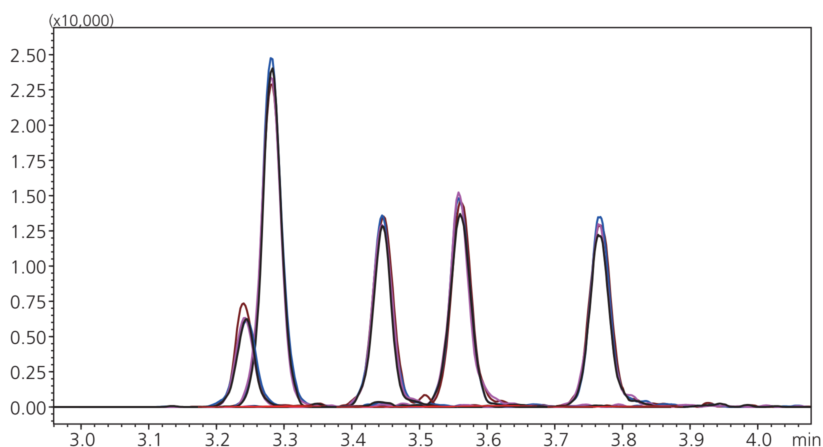

Figure 3 – Chromatogram of aflatoxins at 0.1 ppb in milk thickening cereals

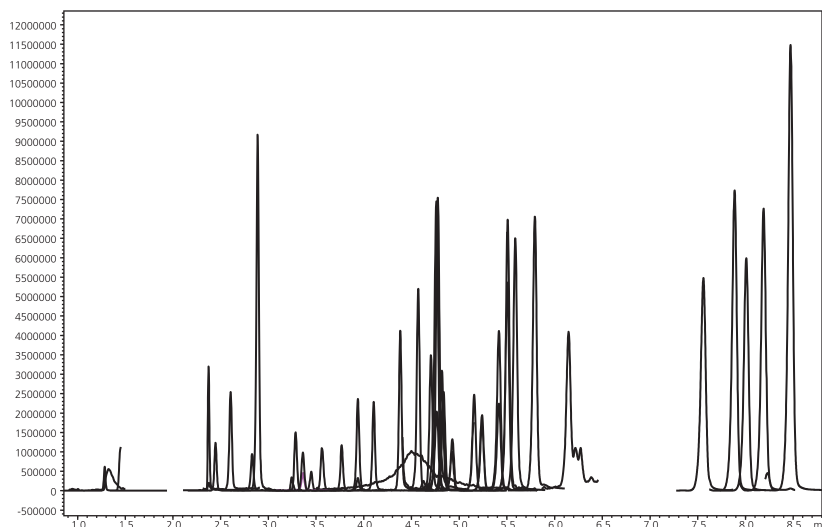

Figure 4 – Chromatogram of the 45 mycotoxins in standard at 50 ppb (2 ppb for aflatoxins and ochratoxines)

Conclusion

- A very sensitive method for multiple mycotoxines was set up to ensure low LOQ in baby food sample,
- Thanks to high speed polarity switching, a high number of mycotoxines can be assayed using the same method in a short time,
- The extraction method demonstrate good recoveries to ensure accurate quantification.

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High Sensitivity Analysis of Acrylamide in Potato Chips by LC/MS/MS with Modified QuEChERS Sample Pre-treatment Procedure

ASMS 2014 MP342

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Introduction

Acrylamide was found to form in fried foods like potato-chips via the so-called Maillard reaction of asparagine and glucose (reducing sugar) at higher temperature (120°C) in 2002 [1,2]. The health risk of acrylamide present in many processing foods became a concern immediately, because it is known that the compound is a neurotoxin and a potential carcinogen to humans [3]. Various analytical methods, mainly LC/MS/MS and GC/MS based methods, were established and used in analysis of acrylamide in foods in recent years [4]. We present a novel LC/MS/MS method for quantitative determination of acrylamide in potato chips with using a modified QuEChERS procedure for sample extraction and clean-up, achieving high sensitivity and high recovery. A small sample injection volume (1uL) was adopted purposely to reduce the potential contamination of samples to the interface of MS system, so as to enhance the operation stability in a laboratory handling food samples with high matrix contents.

Experimental

Acrylamide and isotope labelled acrylamide-d3 (as internal standard) were obtained from Sigma-Aldrich. The QuEChERS kits were obtained from RESTEK. A modified procedure of the QuEChERS was optimized and used in the sample extraction of acrylamide (Q-sep Q100 packet, original unbuffered) in potato chips and clean-up of matrix with d-SPE tube (Q-sep Q250, AOAC 2007.01). Acrylamide and acrylamide-d3 (IS) stock solutions and diluted calibrants were prepared using water as the solvent.

Method development and performance evaluation were carried out using spiked acrylamide samples in the extracted potato chip matrix. A LCMS-8040 triple quadrupole LC/MS/MS (Shimadzu Corporation, Japan) was used in this work. A polar-C18 column of 2.5µm particle size was used for fast UHPLC separation with a gradient elution method. Table 1 shows the details of analytical conditions on LCMS-8040 system,.

Table 1: LC/MS/MS analytical conditions of LCMS-8040 for acrylamide

LC condition

Column	Phenomenex Synergi 2.5u Polar-Rp 100A (100 x 2.00mm)
Flow Rate	0.2 mL/min
Mobile Phase	A: water B: 0.1% formic acid in Methanol
Elution Mode	Gradient elution, B%: 1% (0 to 1 min) → 80% (3 to 4.5 min) → 1% (5.5 to 10min)
Oven Temp.	40°C
Injection Vol.	1.0 µL

MS Interface condition

Interface	ESI
MS mode	Positive, MRM, 2 transitions each compound
Block Temp.	400°C
DL Temp.	200°C
CID Gas	Ar (230kPa)
Nebulizing Gas Flow	N2, 1.5L/min
Drying Gas Flow	N2, 10.0L/min

Results and Discussion

QuEChERS Sample Pre-treatment

The details of a modified QuEChERS procedure for potato chips are shown in Figure 1. Hexane was used to defat potato chips, removing oils and non-polar components. In the extraction step with Q-sep Q100Packet extraction salt (contain 4g $MgSO_4$ & 0.5g NaCl), additional 4g of $MgSO_4$ was added to absorb the water completely (aqueous phase disappeared). Acrylamide is soluble in both aqueous and organic phases. With this modification, high recovery of acrylamide was obtained. It is believed that this is because complete removal of water in the mixed extract solution could promote acrylamide transferring into the organic phase. Dispersive SPE tube was used as PSA to remove organic acids which may decompose acrylamide in the process.

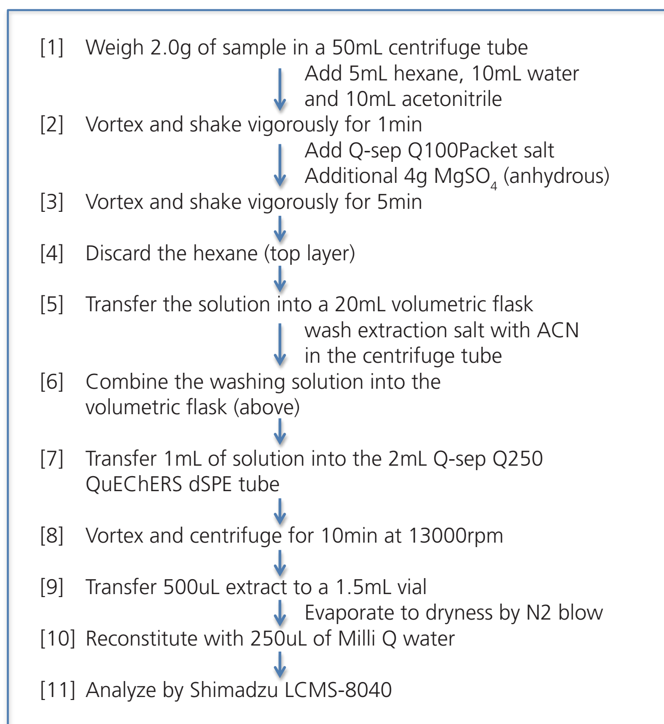


Figure 1: Flow chart of sample pre-treatment with modified QuEChERS.

Method Development

As acrylamide is a more polar compound, a Polar-RP type column was selected. Isotope labeled internal standard (acrylamide-d_3) was used to compensate the variation of acrylamide peak area caused by system fluctuation and inconsistency in sample preparation of different batches.

The precursor ions of acrylamide and acrylamide-d_3 (IS) were their protonated ions (m/z72.1 and m/z75.1). The MRM optimization was carried out using an automated program of the LabSolutions workstation, which could generate a list of all MRM transitions with optimized CID voltages accurate to (+/-) 1 volt in minutes. Two MRM transitions of acrylamide and acryl-amide-d_3 were selected as quantifier and confirmation ion as shown in Table 2.

The obtained extract solution of potato chips was used as “blank” and also matrix for preparation of post-spiked calibrants for establishment of calibration curve with IS (acrylamide-d_3). To obtain reliable results, the blank and each post-spiked calibrant as shown in Table 3 were injected three times and the average peak area ratios were calculated and used.

Table 2: MRM transitions and CID voltages

Name	MRM (m/z)	CID Voltage (V)		
		Q1	CE	Q3
Acrylamide-d_3	75.1 > 58.0*	-29	-15	-22
	75.1 > 30.1	-29	-24	-30
Acrylamide	72.1 > 55.0*	-17	-16	-24
	72.1 > 27.1	-17	-22	-30

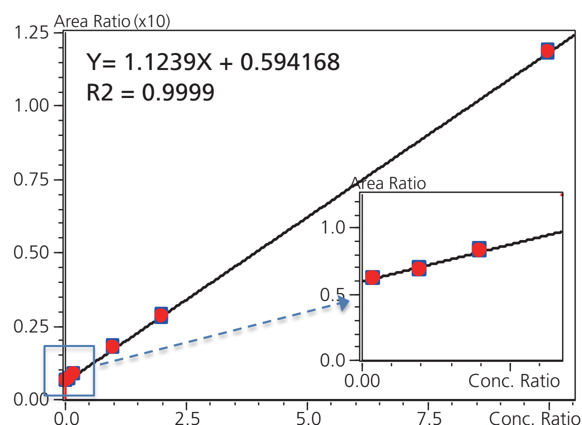
**MRM transition as quantifier*

Table 3: Acrylamide spiked samples and peak area ratios of measured by IS method

Acrylamide post-spiked	IS post-spiked	Conc. Ratio Calculated	Area Ratio measured*
L0, Blank	50ppb	0	0.6033
L1, 1ppb		0.02	0.6120
L2, 5ppb		0.10	0.6786
L3, 10ppb		0.20	0.8239
L4, 50ppb		1.00	1.7686
L5 100ppb		2.00	2.8196
L6, 500ppb		10.00	11.8330

**= Area (acrylamide) / Area (IS)*

It was found that the potato chips used as "blank" in this study was not free of acrylamide. Instead, it contained 27.1 ng/mL of acrylamide in the extract solution. A linear calibration curve was established with an intercept of 0.594 at zero spiked concentration (L0) as shown in Figure 2. Good linearity with correlation coefficient (R2) greater than 0.9999 across the range of 1.0 ng/mL– 500.0 ng/mL was obtained.



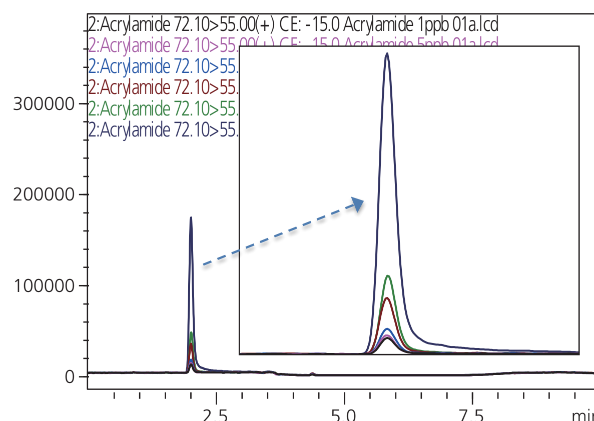


Figure 2: Calibration curve (left) and MRM peaks (right) of acrylamide spiked into potato chips matrix, 1-500 ppb with 50 ppb IS added.

Method Performance Evaluation

It was hard to estimate the LOD and LOQ of the analytical method due to the presence of acrylamide (27.1 ng/mL) in the "blank" (extract of potato chips). However, as reported also by other researchers, it is difficult to obtain potato chips free of acrylamide actually. To obtain actual concentration, it is normally subtracting the background content of acrylamide of a "blank" sample used as reference from a measurement of testing sample. The same way was used to estimate actual S/N value in this work. As a result, the LOD and LOD of acrylamide of this method with 1ul injection volume were estimated to be lower than 1ng/mL and 3ng/mL, respectively. This is consistence with the results estimated with the IS.
The repeatability of the method was evaluated with L2 and L4 spiked samples. The results are shown in Table 4 and Figure 3. The peak area %RSD of acrylamide and IS were below 4%.
The matrix effect (M.E.), recovery efficiency (R.E.) and process efficiency (P.E.) of the method were determined with a duplicate set of spiked samples of 50 ng/mL level except for the non-spiked sample. The chromatograms of "set 2", i.e., non-spiked extract, pre-spiked, post-spiked and the standard in neat solution are shown in Figure 4. Noted that, the existing acrylamide in the extract of the potato chips used as reference was accounted for 27.1 ng/mL, corresponding to 135.5 ng per gram of potato chips. The average R.E, M.E and P.E of the method for extraction and analysis of acrylamide obtained are shown in Table 6.

Table 4: Repeatability Test Results (n=6)

spiked Sample	Compound	Conc. (ng/mL)	%RSD
L2	Acrylamide	5	3.5
	Acrylamide-d_3	50	3.8
L4	Acrylamide	50	3.9
	Acrylamide-d_3	50	3.6

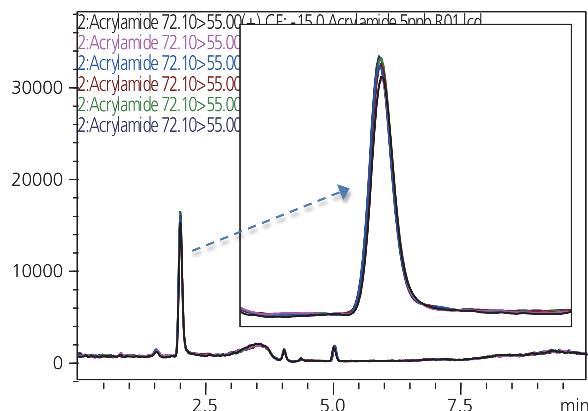


Figure 3: Overlay MRM chromatograms of 5 ng/mL acrylamide spiked in potato chips extract (total: 27.1+5 = 32.1 ng/mL)

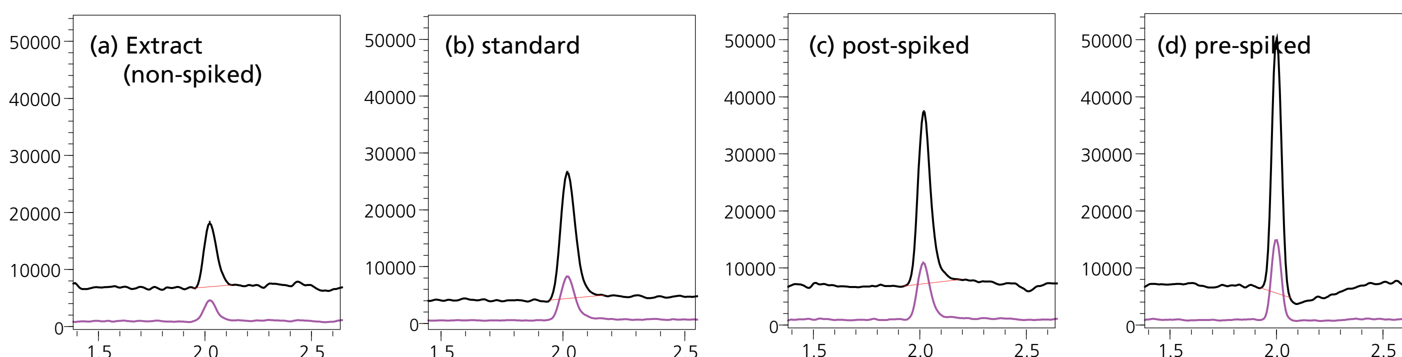


Figure 4: The MRM peaks of acrylamide detected in "blank" extract of potato chips (a), neat standard of 50ppb (b) post-spiked sample of 50ppb (c) and pre-spiked sample of 50ppb.

Table 6: Method evaluation of at 50.0ng/mL concentration in potato chips matrix

Parameter	Set 1	Set 2	Average
R.E.	104.7%	112.0%	108.4%
M.E.	96.5%	84.6%	90.5%
P.E.	100.8%	94.5%	97.6%

Conclusions

Acrylamide is formed unavoidably in starch-rich food in cooking and processing at high temperature like potato chips, French fries, cereals and roasted coffee etc. The analysis method established in this work can be used to monitor the levels of acrylamide in processing food accurately and reliably. The QuEChERS method is proven to be fast and effective in extraction of acrylamide from potato chips. The excellent performance of the method in terms of sensitivity, linearity, repeatability and recovery are related to the outstanding performance of the LC/MS/MS used which features ultra fast mass spectrometry (UFMS) technology. The high sensitivity of the method allows the analysis to be performed with a very small injection volume (1µL or below), which would be a great advantage in running heavily food samples with high matrix contents and strong matrix effects. Maintenance of the interface of a mass spectrometer could also be reduced significantly.

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Determination of Benzimidazole Residues in Animal Tissue by Ultra High Performance Liquid Chromatography Tandem Mass Spectrometry

ASMS 2014 TP 281

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Determination of Benzimidazole Residues in Animal Tissue by Ultra High Performance Liquid Chromatography Tandem Mass Spectrometry

Introduction

Benzimidazoles are broad-spectrum, high efficiency, low toxicity anthelmintic. Because some benzimidazoles and their metabolites showed teratogenic and mutagenic effects in animal and target animal safety evaluation experiment, many countries have already put benzimidazoles and metabolites as the monitoring object.

This poster employed a liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method to determinate 16 benzimidazole residues in animal tissue. The method is simple, rapid and high sensitivity, which meets the requirements for the analysis of veterinary drug residue in animal tissue.

Method

Sample Preparation

(1) Animal tissue samples were extracted with ethyl acetate-50% potassium hydroxide-1% BHT
(2) The samples were treated with n-hexane for defatting and further cleaned-up on MCX solid phase (SPE) cartridge.
(3) The separation of benzimidazoles and their metabolites was performed on LC-MS/MS instrument.

LC/MS/MS Analysis

The analysis was performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pumps, a CTO-30A column oven, a DGU-30A5 degasser, and an SIL-30AC autosampler. The separation was carried out on a Shim-pack XR-ODS III (2.0 mmI.D. x 50 mmL., 1.6 µm, Shimadzu) with the column temperature at 30 °C. A triple quadrupole mass spectrometer (Shimadzu LCMS-8040, Kyoto, Japan) was connected to the UHPLC instrument via an ESI interface.

Analytical Conditions

UHPLC (Nexera system)

Column	: Shim-pack XR-ODS III (2.0 mmI.D. x 50 mmL., 1.6 µm)
Mobile phase A	: water with 0.1% formic acid
Mobile phase B	: acetonitrile
Gradient program	: as in Table 1
Flow rate	: 0.4 mL/min
Column temperature	: 30 °C
Injection volume	: 20 µL

Table 1 Time program

Time (min)	Module	Command	Value
0.01	Pumps	Pump B Conc.	5
3.50	Pumps	Pump B Conc.	80
4.00	Pumps	Pump B Conc.	80
4.01	Pumps	Pump B Conc.	5
6.00	Controller	Stop	

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MS/MS (LCMS-8040 triple quadrupole mass spectrometer)

Ionization	: ESI
Polarity	: Positive
Ionization voltage	: +4.5 kV
Nebulizing gas flow	: 3.0 L/min
Heating gas pressure	: 15.0 L/min
DL temperature	: 200 °C
Heat block temperature	: 350 °C
Mode	: MRM

Table 2 MRM parameters of 16 benzimidazoles (*: for quantitation)

Compound	Precursor *m/z*	Product *m/z*	Dwell Time (ms)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Fenbendazole	300.10	268.05*	50	-15.0	-21.0	-18.0
		159.05	50	-15.0	-36.0	-30.0
Albendazole sulfoxide	282.00	240.10*	10	-14.0	-12.0	-17.0
		208.05	10	-14.0	-23.0	-22.0
Thiabendazole	202.00	175.10*	10	-30.0	-24.0	-18.0
		131.15	10	-30.0	-31.0	-25.0
Thiabendazole-5-hydroxy	218.00	191.05*	50	-30.0	-23.0	-13.0
		147.10	50	-30.0	-32.0	-27.0
Oxfendazole	316.20	159.15*	20	-11.0	-34.0	-30.0
		191.15	20	-11.0	-22.0	-20.0
Albendazole	266.30	234.10*	8	-30.0	-19.0	-25.0
		191.10	8	-30.0	-33.0	-20.0
Albendazole -2-aminosulfone	240.30	133.20*	50	-15.0	-27.0	-24.0
		198.10	50	-15.0	-18.0	-21.0
Albendazole sulfone	298.30	159.10*	20	-13.0	-37.0	-30.0
		224.05	20	-13.0	-27.0	-23.0
Mebendazole	296.30	264.15*	10	-13.0	-21.0	-27.0
		105.25	10	-13.0	-35.0	-19.0
Mebendazole-amine	238.30	105.20*	10	-15.0	-26.0	-20.0
		133.20	10	-15.0	-36.0	-25.0
5-Hydroxymebendazole	298.30	266.10*	10	-30.0	-22.0	-18.0
		160.15	10	-30.0	-35.0	-30.0
Flubendazole	314.30	282.15*	10	-14.0	-22.0	-19.0
		123.15	10	-14.0	-35.0	-24.0
2-Aminoflubendazole	256.30	123.20*	10	-16.0	-26.0	-22.0
		95.20	10	-16.0	-41.0	-18.0
Cambendazole	303.20	217.15*	5	-30.0	-28.0	-23.0
		261.10	5	-30.0	-17.0	-28.0
Oxibendazole	250.30	218.15*	5	-30.0	-17.0	-23.0
		176.15	5	-30.0	-27.0	-18.0
Oxfendazole	332.20	300.10*	10	-15.0	-22.0	-21.0
		159.05	10	-15.0	-39.0	-30.0

Results and Discussion

A liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method has been developed to identify and quantify trace levels of 16 benzimidazoles residue (fenbendazole, albendazole sulfoxide, thiabendazole, thiabendazole- 5-hydroxy, oxfendazole, albendazole, albendazole-2-aminosulfone, albendazole sulfone, mebendazole, mebendazole-amine, 5-hydroxymebendazole, flubendazole, 2-aminoflubendazole, cambendazole, oxibendazole, oxfendazole) in animal tissue. The MRM chromatograms of 16 drugs mixture are presented in Fig.1. The correlation coefficients for 16 drugs (0.5 – 50 ng/mL) were found to 0.9993~0.9999. MRM chromatograms of pork samples and pork samples spiked with standards are shown in Fig.2. By analyzing 16 drugs at three levels including 0.5 ng/mL, 5 ng/mL, 50 ng/mL, excellent repeatability was demonstrated with the %RSD being better than 5% for all the compound within six injections as shown in Table 3. Results of recovery test were good as shown in Table 4.

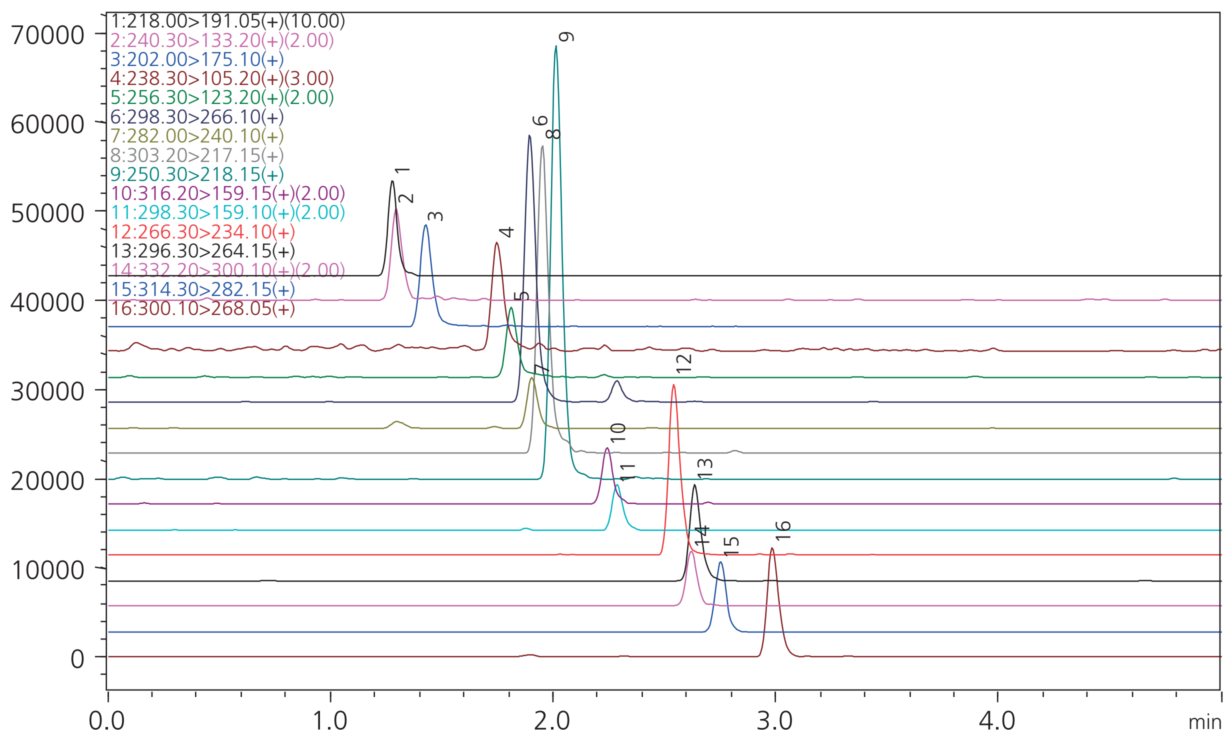

Figure 1 MRM chromatograms of standard 16 drugs (1 ng/mL)
(1: Thiabendazole-5-hydroxy; 2: Albendazole -2-Aminosulfone; 3: Thiabendazole; 4: Mebendazole-amine; 5: 2-Aminoflubendazole;6: 5-Hydroxymebendazole; 7: Albendazole Sulfoxide; 8: Cambendazole; 9: Oxibendazole; 10: Oxfendazole; 11: Albendazole sulfone; 12: Albendazole; 13: Mebendazole; 14: Oxfendazole; 15: Flubendazole; 16: Fenbendazole)

Determination of Benzimidazole Residues in Animal Tissue by Ultra High Performance Liquid Chromatography Tandem Mass Spectrometry

Table 3 Repeatability of 16 drugs in pork sample (n=6)

Compound	%RSD (0.5 ng/mL)		%RSD (5.0 ng/mL)		%RSD (50 ng/mL)	
	R.T.	Area	R.T.	Area	R.T.	Area
Fenbendazole	0.059	3.01	0.064	1.48	0.082	0.34
Albendazole Sulfoxide	0.202	4.26	0.084	2.86	0.153	0.92
Thiabendazole	0.272	4.52	0.180	2.85	0.132	2.58
Thiabendazole-5-hydroxy	0.526	4.44	0.249	3.91	0.158	1.41
Oxfendazole	0.121	2.71	0.089	2.91	0.105	0.97
Albendazole	0.073	2.07	0.090	1.29	0.099	0.92
Albendazole -2-Aminosulfone	0.392	4.36	0.162	2.08	0.177	1.72
Albendazole sulfone	0.103	3.95	0.126	0.63	0.113	0.64
Mebendazole	0.093	4.95	0.095	1.69	0.094	0.74
Mebendazole-amine	0.363	3.95	0.149	2.72	0.243	0.94
5-Hydroxymebendazole	0.091	2.31	0.099	0.79	0.140	1.17
Flubendazole	0.107	4.22	0.058	1.52	0.091	1.00
2-Aminoflubendazole	0.339	4.30	0.177	2.53	0.166	1.43
Cambendazole	0.150	4.90	0.123	3.38	0.121	1.87
Oxibendazole	0.091	3.46	0.108	1.31	0.125	1.20
Oxfendazole	0.170	3.23	0.044	3.09	0.084	0.80

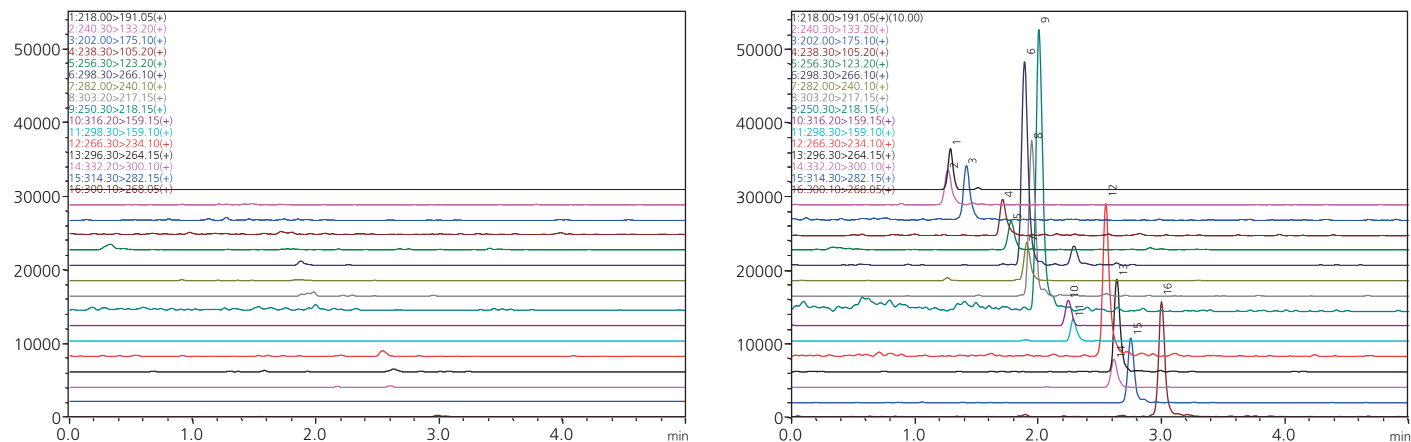

Figure 2 MRM chromatograms of pork sample (left) and spiked pork sample (right)
(1: Thiabendazole-5-hydroxy; 2: Albendazole -2-Aminosulfone; 3: Thiabendazole;
4: Mebendazole-amine; 5: 2-Aminoflubendazole;6: 5-Hydroxymebendazole;
7: Albendazole Sulfoxide; 8: Cambendazole; 9: Oxibendazole; 10: Oxfendazole;
11: Albendazole sulfone; 12: Albendazole; 13: Mebendazole; 14: Oxfendazole;
15: Flubendazole; 16: Fenbendazole)

Table 4 Recovery of 16 drugs in pork sample

Compound	Sample Conc. (µg/kg)	Spike Conc. (µg/kg)	Measured Conc. (µg/kg)	Recovery (%)
Fenbendazole	N.D.	10.0	9.5	94.5
Albendazole Sulfoxide	N.D.	10.0	8.1	80.9
Thiabendazole	N.D.	10.0	9.8	98.2
Thiabendazole-5-hydroxy	N.D.	10.0	10.0	99.8
Oxfendazole	N.D.	10.0	11.4	113.8
Albendazole	N.D.	10.0	9.6	96.3
Albendazole -2-Aminosulfone	N.D.	10.0	9.6	96.1
Albendazole sulfone	N.D.	10.0	11.8	118.5
Mebendazole	N.D.	10.0	11.3	112.8
Mebendazole-amine	N.D.	10.0	11.8	118.3
5-Hydroxymebendazole	N.D.	10.0	9.8	97.8
Flubendazole	N.D.	10.0	10.4	103.6
2-Aminoflubendazole	N.D.	10.0	9.3	92.6
Cambendazole	N.D.	10.0	10.8	107.8
Oxibendazole	N.D.	10.0	9.6	96.1
Oxfendazole	N.D.	10.0	9.1	90.7

Conclusion

The sensitive and reliable LC/MS/MS technique was successfully applied for determination of 16 benzimidazoles residue. The calibration curves of 16 benzimidazoles ranging from 0.5 to 50 ng/mL were established and the correlation coefficients were 0.9993~0.9999. The LODs of the 16 benzimidazoles were 1 -2.2 µg/kg. The recoveries were in the range of 80.9%~118.5% for pork samples, with relative standard deviations less than 5%.

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PO-CON1459E

High Sensitivity Quantitation Method of Dicyandiamide and Melamine in Milk Powders by Liquid Chromatography Tandem Mass Spectrometry

ASMS 2014 TP275

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Introduction

Melamine was found to be used as a protein-rich adulterant first in pet-food in 2007, and then in infant formula in 2008 in China [1]. The outbreak of the melamine scandal that killed many dogs and cats as well as led to death of six infants and illness of many had caused panic in publics and great concerns in food safety worldwide. Melamine was added into raw milk because of its high nitrogen content (66%) and the limitation of the Kjeldahl method for determination of protein level indirectly by measuring the nitrogen content. In fact, in addition to melamine and its analogues (cyanuric acid etc), a number of other nitrogen-rich compounds was reported also to be potentially used as protein-rich adulterants, including amidinourea, biuret, cyromazine, dicyandiamide, triuret and urea [2]. Recently, low levels of dicyandiamide (DCD) residues were found in milk products from New Zealand [3]. Instead of addition directly as an adulterant, the trace DCD found in milk products was explained to be relating to the grass “contaminated by DCD”. Dicyandiamide has been used to promote the growth of pastures for cows grazing. We report here an LC/MS/MS method for sensitive detection and quantification of both dicyandiamide (DCD) and melamine in infant milk powder samples.

Experimental

High purity dicyandiamide (DCD) and melamine were obtained from Sigma Aldrich. Amicon Ultra-4 (MWCO 5K) centrifuge filtration tube (15 mL) obtained from Millipore was used in sample pre-tretment. The milk powder sample was pre-treated according to a FDA method [1] with some modification as illustrated in Figure 1. The final clear sample solution was injected into LC/MS/MS for analysis. Stock solutions of DCD and melamine were prepared in pure water.

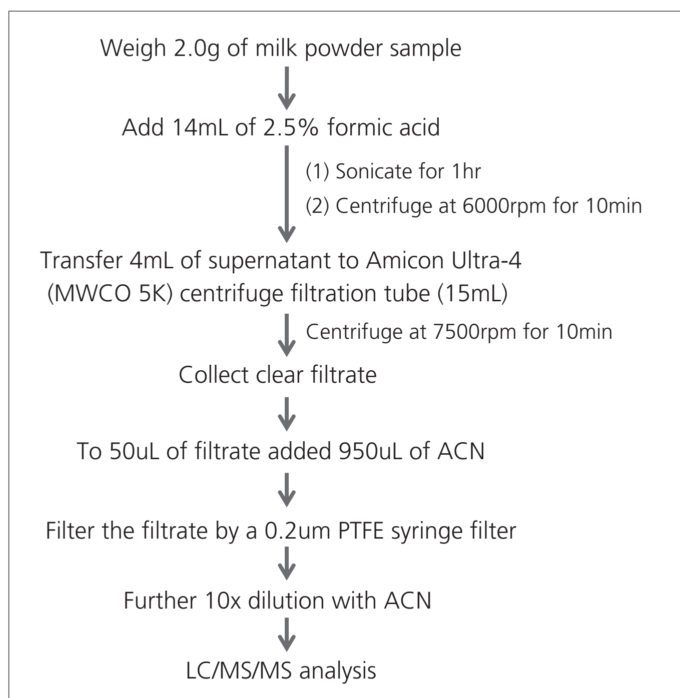

Fig 1: Sample pre-treatment workflow

Table 1: Analytical conditions of DCD and melamine in milk powders on LCMS-8040

LC conditions

Column	Alltima HP HILIC 3μ, 150 x 2.10mm
Flow Rate	0.2 mL/min
Mobile Phase	A: 0.1 % formic acid in H_2O/ACN (5:95 v/v) B: 20mM Ammonium Formate in H_2O/ACN (50:50 v/v)
Elution Mode	Gradient elution: 5% (0.01 to 3.0 min) → 95% (3.5 to 5.0 min) → 5% (5.5 to 9.0 min)
Oven Temperature	40°C
Injection Volume	5 μL

MS conditions

Interface	ESI
MS mode	Positive
Block Temperature	400°C
DL Temperature	300°C
CID Gas	Ar (230kPa)
Nebulizing Gas Flow	N2, 2.0L/min
Drying Gas Flow	N2, 15.0L/min

An LCMS-8040 triple quadrupole LC/MS/MS (Shimadzu Corporation, Japan) was used in this work. The system is consisted of a high pressure binary gradient Nexera UHPLC coupled with a LCMS-8040 MS system. An Alltima HP HILIC column was used for separation of DCD and melamine with a gradient program developed (Table 1). The details of the LC and MS conditions are shown in Table 1. A set of calibrants (0.5, 1.0, 2.5, 5 and 10 ppb) was prepared from the stock solutions using of ACN/water (90/10) as diluent.

Results and Discussion

MRM optimization

MRM optimization of DCD and melamine were performed using an automated MRM optimization program of the LabSolutions. The precursors were the protonated ions of DCD and melamine. Two optimized MRM transitions of each compound were selected and used for quantitation and confirmation. The MRM transitions and parameters are shown in Table 2.

Table 2: MRM transitions and optimized parameters

Name	RT (min)	Transition (m/z)	Voltage (V)		
			Q1 Pre Bias	CE	Q3 Pre Bias
DCD	2.55	85.1 > 68.1	-15	-21	-26
		85.1 > 43.0	-15	-17	-17
MEL	6.29	127.1 > 85.1	-26	-20	-17
		127.1 > 68.1	-26	-27	-26

Method Development

A LC/MS/MS method was developed for quantitation of DCD and melamine based on the MRM transitions in Table 2. Under the HILIC separation conditions (Table 1), DCD and melamine eluted at 2.55 min and 6.29 min as sharp peaks (see Figures 4 & 5). Figures 2 and 3 show the calibration curves of DCD and melamine standard in neat solutions and in milk matrix solutions (spiked). The linearity with correlation coefficient (R2) greater than 0.997 across the calibration range of 0.5~10.0 ng/mL was obtained for both compounds in both neat solution and matrix (spiked).

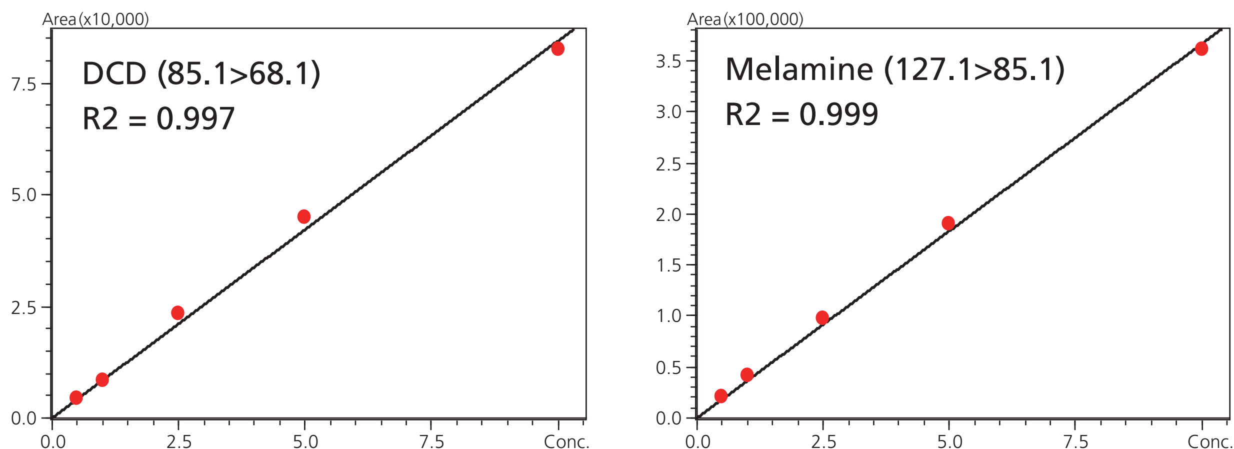

Figure 2: Calibration curves of DCD and melamine in neat solution

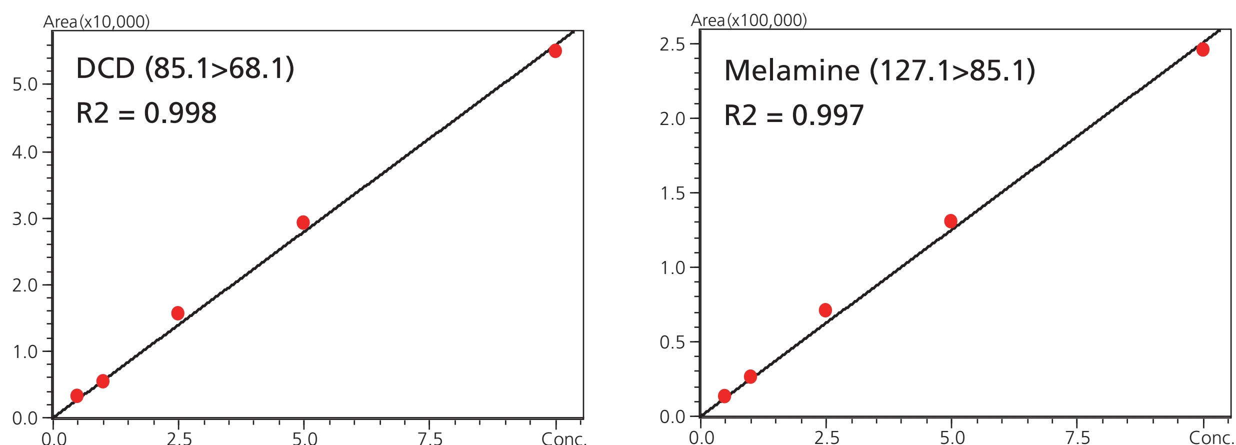

Figure 3: Calibration curves of DCD and melamine spiked in milk powder matrix

Performance Evaluation

The repeatability of the method was evaluated at the levels of 0.5 ng/mL and 1.0 ng/mL. Figures 4 & 5 show the MRM chromatograms of DCD and melamine of six consecutive injections of 0.5 ng/mL level with and without matrix. The peak area %RSD for the two analytes were lower than 9.2% (see Table 3).

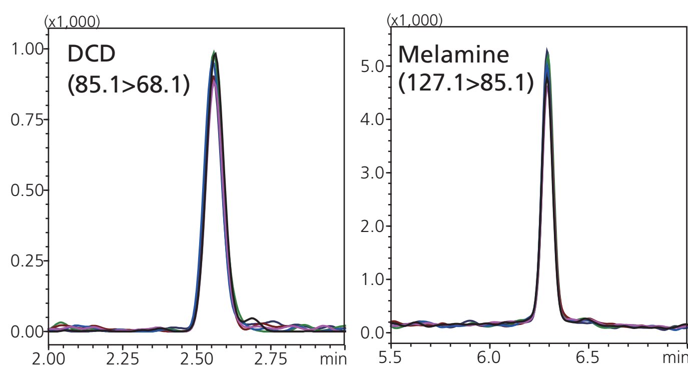

Figure 4: Overlapping of six MRM peaks of 0.5 ng/mL DCD and melamine in neat solution

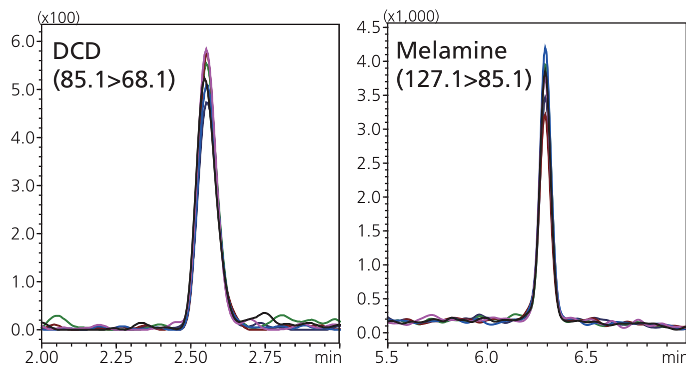

Figure 5: Overlapping of six MRM peaks of 0.5 ng/mL DCD and melamine in milk powder matrix

Table 3: Results of repeatability and sensitivity evaluation of DCD and melamine (n=6)

Sample	Compd.	Conc. (ng/mL)	%RSD	LOD (ng/mL)	LOQ (ng/mL)
In solvent	DCD	0.5	5.9	0.03	0.10
		1.0	5.3		
	MEL	0.5	5.5	0.03	0.09
		1.0	2.6		
In matrix	DCD	0.5	5.9	0.05	0.16
		1.0	8.2		
	MEL	0.5	9.2	0.05	0.15
		1.0	2.4		

High Sensitivity Quantitation Method of Dicyandiamide and Melamine in Milk Powders by Liquid Chromatography Tandem Mass Spectrometry

The LOD and LOQ were estimated from the results of 0.5 ng/mL in both neat and matrix solution. The LOD and LOQ results were summarized in Table 3. The method achieved LOQs (in matrix) of 0.16 and 0.15 ng/mL (ppb) for DCD and melamine, respectively. Tables 4 & 5 show the results of matrix effect and recovery of the method. The matrix effects for DCD and melamine in the whole concentration ranges were at 64%~70% and 62%~73%, respectively.

The recovery was determined by comparing the results of pre-spiked and post-spiked mixed samples of DCD and melamine in the milk powder matrix (2.5 ng/mL each compound). The chromatograms of these samples are shown in Figure 6. The recovery of DCD and melamine were determined to be 103% and 105% respectively.

Table 4: Matrix effect (%) of DCD and melamine in milk powder matrix

Conc. (ng/mL)	0.5	1	2.5	5	10
DCD	70.4	65.4	66.9	64.8	66.6
MEL	62.2	62.5	73.1	68.9	68.0

Table 5: Recovery of DCD and melamine determined with spiked sample of 2.5 ng/mL

Compound	Pre-spiked Area	Post-spiked Area	Recovery (%)
DCD	14,393	13,987	102.9
MEL	65,555	62,659	104.6

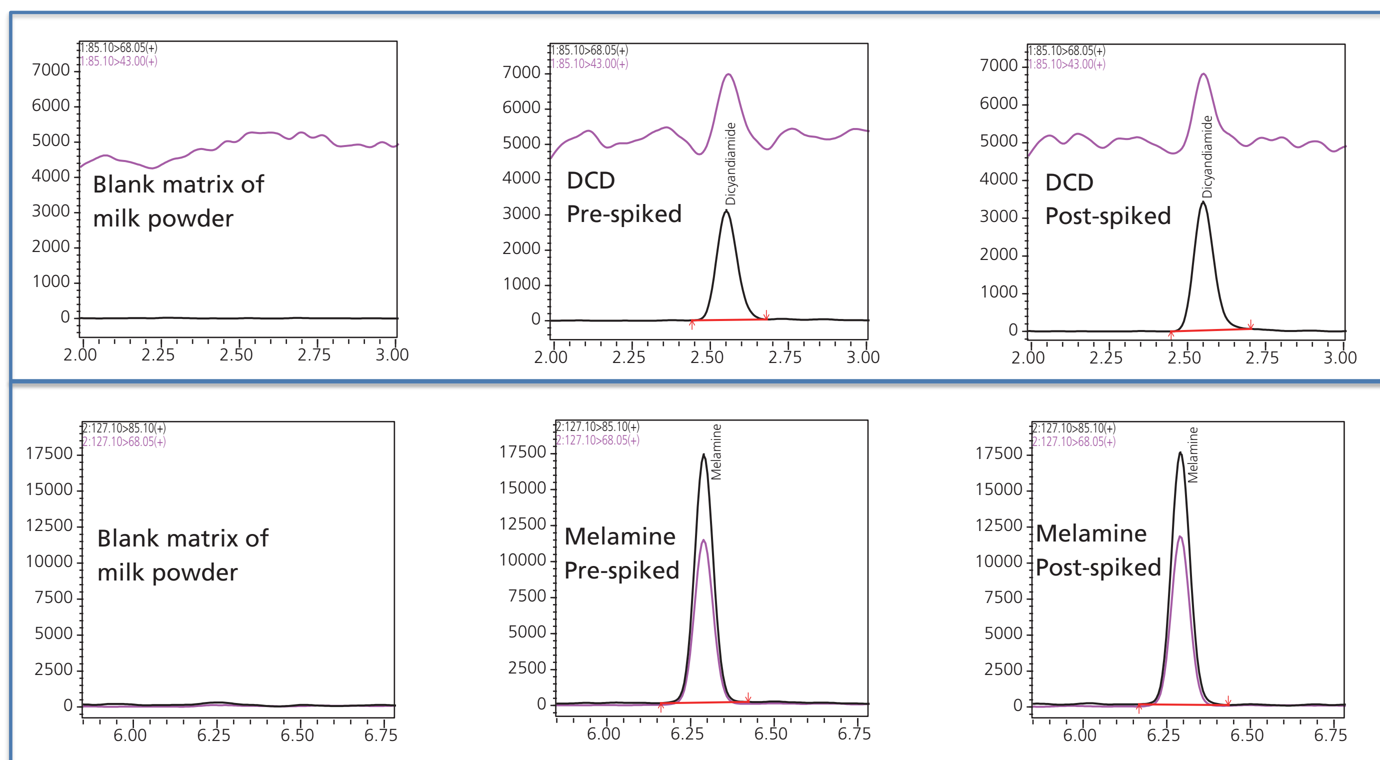

Figure 6: MRM peaks of DCD and melamine in pre- and post-spiked samples of 2.5 ng/mL (each). DCD and melamine were not detected in blank matrix of milk powder.

Conclusions

A high sensitivity LC/MS/MS method was developed on LCMS-8040 for detection and quantitation of dicyandiamide (DCD) and melamine in milk powders. The method performance was evaluated using infant milk powders as the matrix. The method achieved LOQ of 0.16 ng/mL for both compounds in the matrix, allowing its application in simultaneous analysis of melamine, a protein adulterant in relatively high concentration, and dicyandiamide residue in trace level in milk powders samples.

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Multiresidue pesticide analysis from dried chili powder using LC/MS/MS

ASMS 2014 WP350

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Introduction

Pesticide residues in foodstuffs can cause serious health problems when consumed. LC/MS/MS methods have been increasingly employed in sensitive quantification of pesticide residues in foods and agriculture products. However, matrix effect is a phenomenon seen in Electro Spray Ionization (ESI) LC/MS/MS analysis that impacts the data quality of the pesticide analysis, especially for complex matrix like spice/herb.
Chili powder is one such complex matrix that can exhibit matrix effect (either ion suppression or enhancement). A calibration curve based on matrix matched standards can demonstrate true sensitivity of analyte in presence of matrix. Therefore, this approach was used to obtain more reliable and accurate data as compared to quantitation against neat (solvent) standards[1].
Multiresidue, trace level analysis in complex matrices is challenging and tedious. Feature of automatic MRM optimization in LCMS-8040 makes method development process less tedious. In addition, the lowest dwell time and pause time along with ultra fast polarity switching (UFswitching) enables accurate, reliable and high sensitive quantitation. UFsweeper™ II technology in the system ensures least crosstalk, which is very crucial for multiresidue pesticide analysis.

Method of Analysis

Sample Preparation

Commercially available red chili was powdered using mixer grinder. To 1 g of this chili powder, 20 mL water:methanol (1:1 v/v) was added and the mixture was sonicated for 10 mins. The mixture was centrifuged and supernatant was collected. This supernatant was used as diluent to prepare pesticide matrix matched standards at concentration levels of 0.01 ppb, 0.02 ppb, 0.05 ppb, 0.1 ppb, 0.2 ppb, 0.5 ppb, 1 ppb, 2 ppb, 5 ppb, 10 ppb and 20 ppb. Each concentration level was then filtered through 0.2 µ nylon filter and used for the analysis.

LC/MS/MS Analytical Conditions

Pesticides were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8040 triple quadrupole system (Shimadzu Corporation, Japan), shown in Figure 1. The details of analytical conditions are given in Table 1.

Table 1. LC/MS/MS analytical conditions

Parameter	Condition
• Column	: Shim-pack XR-ODS (75 mm L x 3 mm I.D.; 2.2 µm)
• Guard column	: Phenomenex SecurityGuard ULTRA Cartridge
• Mobile phase	: A: 5 mM ammonium formate in water:methanol (80:20 v/v) B: 5 mM ammonium formate in water:methanol (10:90 v/v)
• Flow rate	: 0.2 mL/min
• Oven temperature	: 40 °C
• Gradient program (B%)	: 0.0–1.0 min → 45 (%); 1.0–13.0 min → 45-100 (%); 13.0–18.0 min → 100 (%); 18.0–19.0 min → 100-45 (%); 19.0–23.0 min → 45 (%)
• Injection volume	: 15 µL
• MS interface	: ESI
• Polarity	: Positive and negative
• Nitrogen gas flow	: Nebulizing gas 2 L/min; Drying gas 15 L/min
• MS temperature	: Desolvation line 250 °C; Heat block 400 °C
• MS analysis mode	: Staggered MRM

Figure 1. Nexera with LCMS-8040 triple quadrupole system by Shimadzu

Results

LC/MS/MS method was developed for analysis of 80 pesticides belonging to different classes like carbamate, organophosphate, urea, triazines etc. in a single run[2]. LOQ was determined for each pesticide based on the following criteria – **(1)** % RSD for area < 16 % (n=3), **(2)** % Accuracy between 80-120 % and **(3)** Signal to noise ratio (S/N) > 10.

LOQ achieved for 80 pesticides have been summarized in Table 2 and results for LOQ and linearity for each pesticide have been given in Table 3. Representative MRM chromatogram of pesticide mixture at 1 ppb level is shown in Figure 2. Representative MRM chromatograms at LOQ level for different classes of pesticides are shown in Figure 3.

Table 2: Summary of LOQ achieved

LOQ (ppb)	0.01	0.02	0.05	0.1	0.2	0.5	1
Number of pesticides	1	1	3	8	17	24	26

Table 3. Results of LOQ and linearity for pesticide analysis

Sr. No.	Name of compound	MRM Transition	Polarity	LOQ (ppb)	Linearity (R^2)
1	Spinosyn D	746.20>142.10	Positive	0.01	0.9987
2	Fenpyroximate	421.90>366.10	Positive	0.02	0.9915
3	Bifenazate	301.00>198.00	Positive	0.05	0.9947
4	Spinosyn A	732.20>142.10	Positive	0.05	0.9974
5	Spiromesifen	371.00>273.10	Positive	0.05	0.9957
6	Acetamiprid	222.90>126.00	Positive	0.1	0.9910
7	Carbofuran	221.70>123.00	Positive	0.1	0.9971
8	Dimethoate	229.80>198.90	Positive	0.1	0.9970
9	Dimethomorph I	387.90>301.00	Positive	0.1	0.9991
10	Dimethomorph II	387.90>301.00	Positive	0.1	0.9992
11	Isoproturon	207.00>72.10	Positive	0.1	0.9984
12	Pirimiphos methyl	305.70>108.00	Positive	0.1	0.9997
13	Trifloxystrobin	408.90>186.00	Positive	0.1	0.9989

Sr. No.	Name of compound	MRM Transition	Polarity	LOQ (ppb)	Linearity (R^2)
14	Anilophos	367.70>198.85	Positive	0.2	0.9974
15	Atrazine	215.90>174.00	Positive	0.2	0.9985
16	Carboxin	235.90>143.00	Positive	0.2	0.9952
17	Cyazofamid	324.85>108.10	Positive	0.2	0.9971
18	Edifenphos	310.60>111.00	Positive	0.2	0.9997
19	Ethion	384.70>198.80	Positive	0.2	0.9957
20	Fipronil	434.70>330.00	Negative	0.2	0.9973
21	Linuron	248.80>159.90	Positive	0.2	0.9945
22	Metolachlor	283.90>252.00	Positive	0.2	0.9966
23	Oxycarboxin	267.90>174.90	Positive	0.2	0.9995
24	Phosalone	367.80>181.90	Positive	0.2	0.9987
25	Phosphamidon	299.90>173.90	Positive	0.2	0.9997
26	Thiacloprid	252.90>126.00	Positive	0.2	0.9976
27	Thiobencarb	257.90>125.10	Positive	0.2	0.9977
28	Thiodicarb	354.90>88.00	Positive	0.2	0.9906
29	Triadimefon	293.90>196.90	Positive	0.2	0.9994
30	Tricyclazole	189.90>162.90	Positive	0.2	0.9977
31	Aldicarb	208.10>116.05	Positive	0.5	0.9962
32	Benfuracarb	411.10>190.10	Positive	0.5	0.9981
33	Bitertanol	338.00>99.10	Positive	0.5	0.9935
34	Buprofezin	305.70>201.00	Positive	0.5	0.9933
35	Clodinafop propargyl	349.90>266.00	Positive	0.5	0.9978
36	Chlorantraniliprole	483.75>452.90	Positive	0.5	0.9994
37	Diclofop methyl	357.90>280.80	Positive	0.5	0.9976
38	Flufenacet	363.70>193.90	Positive	0.5	0.9997
39	Flusilazole	315.90>247.00	Positive	0.5	0.9983
40	Hexaconazole	313.90>70.10	Positive	0.5	0.9996
41	Hexythiazox	352.90>227.90	Positive	0.5	0.9909
42	Iodosulfuron methyl	507.70>167.00	Positive	0.5	0.9971
43	Iprobenfos	288.70>205.00	Positive	0.5	0.9981
44	Malaoxon	314.90>99.00	Positive	0.5	0.9996
45	Malathion	330.90>284.90	Positive	0.5	0.9997
46	Mandipropamid	411.90>356.20	Positive	0.5	0.9952
47	Metalaxyl	280.00>220.10	Positive	0.5	0.9996
48	Methabenzthiazuron	221.70>150.00	Positive	0.5	0.9957
49	Methomyl	162.90>88.00	Positive	0.5	0.9988
50	Oxadiazon	362.15>303.00	Positive	0.5	0.9963
51	Penconazole	283.90>70.10	Positive	0.5	0.9992
52	Phorate	260.80>75.00	Positive	0.5	0.9987
53	Phorate sulfoxide	276.80>96.90	Positive	0.5	0.9991
54	Thiophanate methyl	342.90>151.00	Positive	0.5	0.9996
55	Avermectin B1a	890.30>305.10	Positive	1	0.9990
56	Carpropamid	333.70>139.00	Positive	1	0.9985

Multiresidue pesticide analysis from dried chili powder using LC/MS/MS

Sr. No.	Name of compound	MRM Transition	Polarity	LOQ (ppb)	Linearity (R^2)
57	Clomazone	241.90>127.00	Positive	1	0.9967
58	Clorimuron ethyl	415.30>186.00	Positive	1	0.9965
59	Cymoxanil	198.90>128.10	Positive	1	0.9949
60	Diafenthiuron	385.00>329.10	Positive	1	0.9961
61	Diflubenzuron	310.80>158.00	Positive	1	0.9982
62	Dodine	228.10>60.00	Positive	1	0.9980
63	Emamectin benzoate	886.30>158.10	Positive	1	0.9983
64	Fenamidone	311.90>236.10	Positive	1	0.9997
65	Fenarimol	330.70>268.00	Positive	1	0.9900
66	Fenazaquin	306.95>57.10	Positive	1	0.9992
67	Flonicamid	229.90>202.70	Positive	1	0.9971
68	Flubendiamide	680.90>254.05	Negative	1	0.9993
69	Forchlorfenuron	247.90>129.00	Positive	1	0.9956
70	Kresoxim methyl	331.00>116.00	Positive	1	0.9996
71	Paclobutrazol	293.90>70.10	Positive	1	0.9974
72	Pencycuron	328.90>125.00	Positive	1	0.9943
73	Pendimethalin	281.90>212.10	Positive	1	0.9932
74	Profenofos	372.70>302.70	Positive	1	0.9966
75	Propargite	368.00>231.10	Positive	1	0.9950
76	Propoxur	209.90>110.90	Positive	1	0.9987
77	Pyrazosulfuron ethyl	414.90>182.00	Positive	1	0.9992
78	Pyriproxyfen	321.90>96.10	Positive	1	0.9975
79	Simazine	201.90>103.90	Positive	1	0.9992
80	Thiomethon	246.80>89.10	Positive	1	0.9989

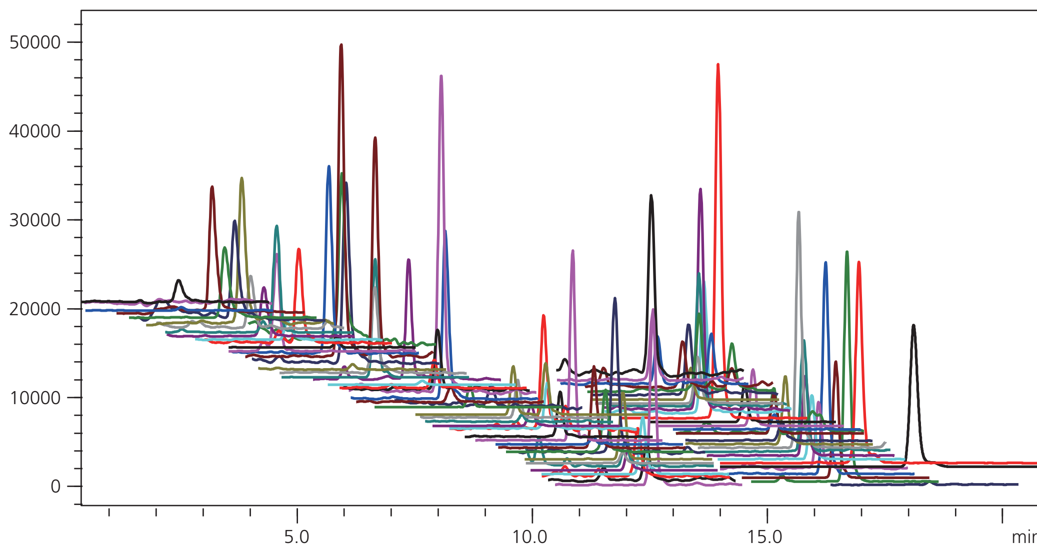

Figure 2. MRM chromatogram of pesticide mixture at 1 ppb level

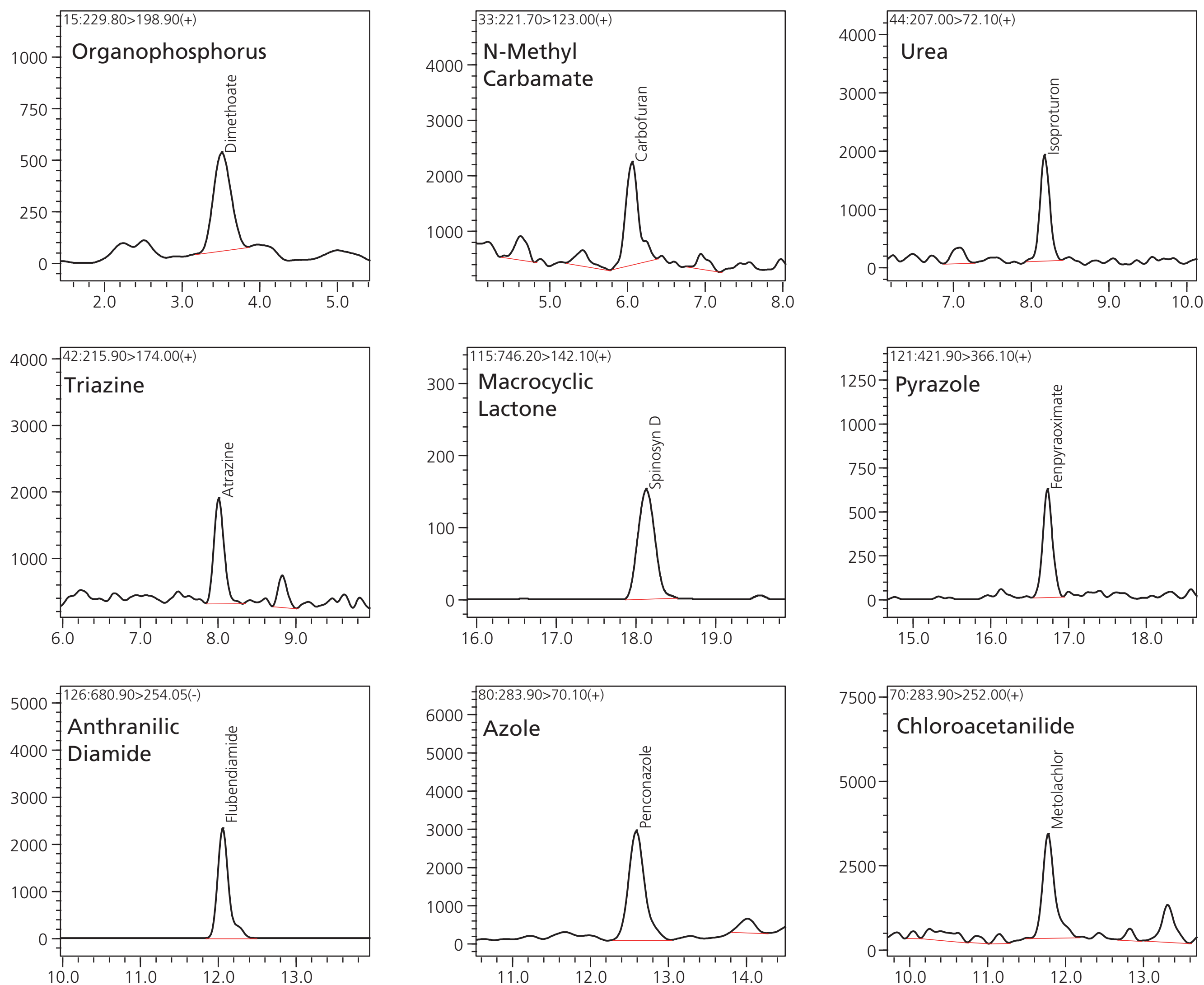

Figure 3. Representative MRM chromatograms at LOQ level from different classes of pesticides

Conclusion

- A highly sensitive method was developed for analysis of 80 pesticides belonging to different classes, from dried chili powder in a single run.
- Ultra high sensitivity, ultra fast polarity switching (UFswitching), low pause time and dwell time along with UFsweeper™ II technology enabled sensitive, selective, accurate and reproducible multiresidue pesticide analysis from complex matrix like dried chili powder.

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PO-CON1463E

Multi pesticide residue analysis in tobacco by GCMS/MS using QuEChERS as an extraction method

ASMS 2014 TP762

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Introduction

India is the world's second largest producer (after China) and consumer (after Brazil) of tobacco with nearly $ 1001.54 million revenue generated annually from its export.[1] In countries like India, with tropical-humid climate, the incidences of insect attacks and disease infestations are frequent and application of pesticides for their management is almost obligatory. Like any other crop, tobacco (*Nicotiana tabacum Linn.*), one of the world's leading high-value crops, is also prone to pest attacks, and the farmers do apply various pesticides as a control measure.

The residues of pesticides applied on tobacco during its cultivation may remain in the leaves at harvest that may even sustain post harvest processing treatments and could appear in the final product. Thus, monitoring of pesticide residues in tobacco is an important issue of critical concern from public health and safety point of view demanding implementation of stringent regulatory policies.[2]

To protect the consumers by controlling pesticide residue levels in tobacco, the Guidance Residue Levels (GRL) of 118 pesticides have been issued by the Agro-Chemical Advisory Committee (ACAC) of the Cooperation Center for Scientific Research Relative to Tobacco (CORESTA).

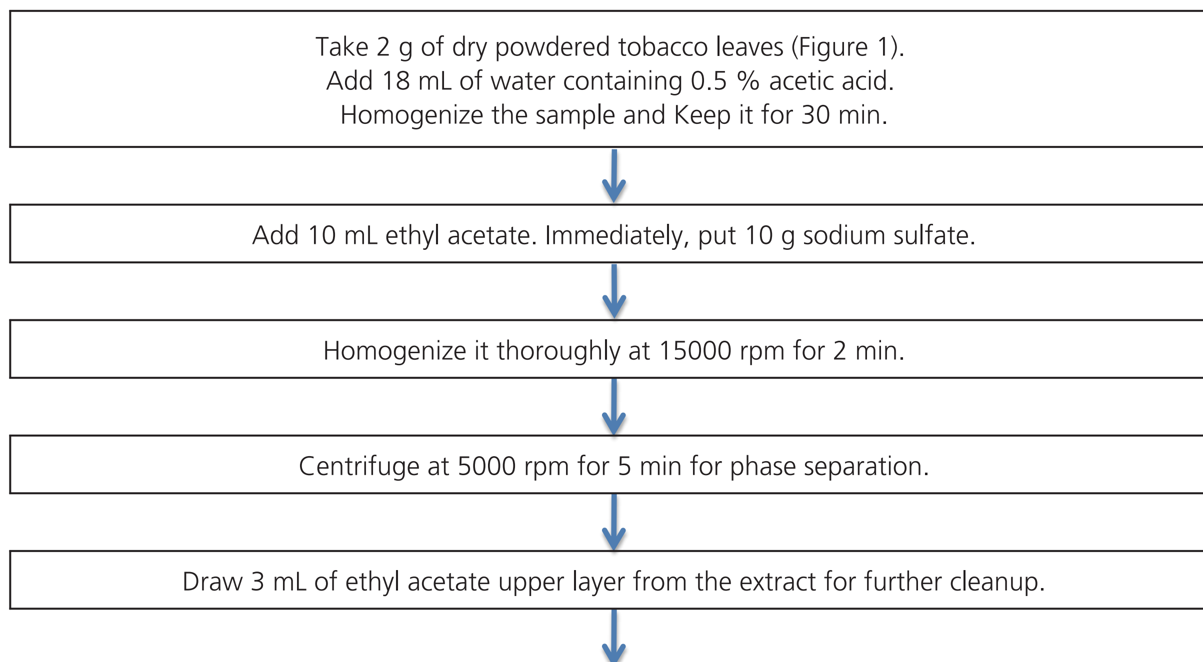
Tobacco is a complex matrix and hence requires selective extraction and extensive cleanup such as QuEChERS (Quick Easy Cheap Effective Rugged Safe) to ensure trace level detection with adequate precision and accuracy. The objective of the present study was to develop an effective, sensitive and economical multi-pesticide residue analysis method for 203 pesticides in tobacco as listed in Table 1.

Figure 1. Dried tobacco

Method of Analysis

Extraction of pesticides from tobacco

Extraction of pesticides was done using QuEChERS method, as described below.[3]



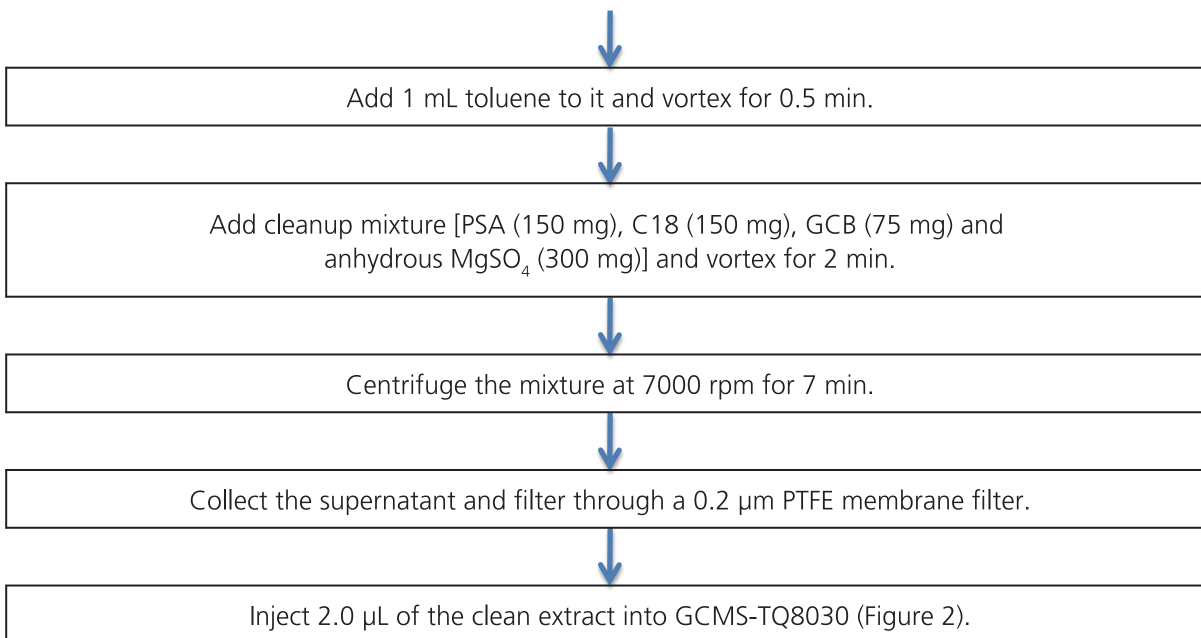


Figure 2. GCMS-TQ8030 Triple quadrupole system by Shimadzu

Key Features of GCMS-TQ8030

- ASSP™ (Advanced Scanning Speed Protocol) enables high-speed scan and data acquisition for accurate quantitation at 20,000 u/sec
- Capable of performing simultaneous Scan/MRM
- UFsweeper® technology efficiently sweeps residual ions from the collision cell for fast, efficient ion transport ensuring no cross-talk
- Two overdrive lenses reduce random noise from helium, high-speed electrons and other factors to improve S/N ratio
- Flexible platform with EI (Electron Ionization), CI (Chemical Ionization), and NCI (Negative Chemical Ionization) techniques
- Full complement of acquisition modes including MRM, Scan/MRM, Precursor Ion, Product Ion and Neutral Loss Scan

Table 1. List of pesticides

Sr. No.	Pesticide	Sr. No.	Pesticide	Sr. No.	Pesticide	Sr. No.	Pesticide
1	2,6-Dichlorobenzamide	52	Cyfluthrin-3	103	Fipronil sulphone	154	Permethrin-1
2	2-Phenylphenol	53	Cyfluthrin-4	104	Flucythrinate-1	155	Permethrin-2
3	3,4-Dichloraniline	54	Cyhalofop-butyl	105	Flucythrinate-2	156	Pethoxamid
4	3-Chloroaniline	55	Cypermethrin-2	106	Flufenacet	157	Phosalone
5	4-Bromo 2-Chloro phenol	56	Cypermethrin-3	107	Flumoixazine	158	Phosmet
6	4,4-Dichlorobenzophenone	57	Cypermethrin-4	108	Fluquinconazole	159	Pirimicarb
7	Acetochlor	58	Cyprodinil	109	Flurochloridone-1	160	Pretilachlor
8	Acrinathrin	59	Delta-HCH	110	Flurochloridone-2	161	Procymidone
9	Alachlor	60	Demeton-s-methyl	111	Flutolanil	162	Profenofos
10	Aldrin	61	Demeton-S-methyl sulphone	112	Flutriafol	163	Propanil
11	Azinphos-ethyl	62	Dialifos	113	Fluxapyoxad	164	Propaquizafop
12	Azinphos-methyl	63	Diazinon	114	Folpet	165	Propazine
13	Azoxystrobin	64	Dichlobenil	115	Fuberidazole	166	Propham
14	Barban	65	Dichlofluanid	116	Heptachlor	167	Propiconazole-1
15	Beflubutamid	66	Diclofop	117	Hexaconazole	168	Propisoclor
16	Benfluralin	67	Dicloran	118	Iprobenfos	169	Propyzamide
17	Benoxacor	68	Dieldrin	119	Isoprocarb	170	Proquinazid
18	Beta-endosulfan	69	Diethofencarb	120	Isoprothiolane	171	Pyraflufen-ethyl
19	Bifenox	70	Difenoconazole-1	121	Isopyrazam	172	Pyrazophos
20	Bifenthrin	71	Difenoconazole-2	122	Isoxaben	173	Pyrimethanil
21	Bitertanol	72	Diflubenzuron	123	Lactofen	174	Pyriprooxyfen
22	Boscalid	73	Diflufenican	124	Lambda-cyhalothrin	175	Pyroquilon
23	Bromacil	74	Dimethipin	125	Malaoxon	176	Quinoxyfen
24	Bromophos-ethyl	75	Dimethomorph-1	126	Malathion	177	Simazine
25	Bromopropylate	76	Dimethomorph-2	127	Mepanipyrim	178	Spirodiclofen
26	Bromuconazole-1	77	Dimoxystrobin	128	Mepronil	179	Sulfotep
27	Bromuconazole-2	78	Diniconazole	129	Metalaxyl	180	Swep
28	Butralin	79	Dinoseb	130	Metalaxyl M	181	Tebufenpyrad
29	Butylate	80	Dinoterb	131	Metazachlor	182	Tebupirimfos
30	Carbaryl	81	Dioxathion	132	Metconazole	183	Tebuthiuron
31	Carbofuran	82	Edifenfos	133	Methabenzthiazuron	184	Tefluthrin
32	Carfentrazone	83	Endosulfan sulphate	134	Methacrifos	185	Terbacil
33	Chlordane-trans	84	Endrin	135	Methidathion	186	Tetraconazole
34	Chlordecone	85	Epoxiconazole	136	Methiocarb	187	Tetradifon
35	Chlorfenvinphos	86	Ethalfluralin	137	Metholachlor-s	188	Thiobencarb
36	Chlormephos	87	Ethoprophos	138	Methoxychlor	189	Tolylfluanid
37	Chlorobenzilate	88	Etoxazole	139	Metribuzin	190	Tralkoxydim
38	Chloroneb	89	Etridiazole	140	Mevinphos	191	Triadimefon
39	Chlorothalonil	90	Etrimfos	141	Monolinuron	192	Tri-allate
40	Chlorpyriphos-ethyl	91	Famoxadone	142	Myclobutanyl	193	Triazophos
41	Chlorpyriphos-methyl	92	Fenamidone	143	Napropamide	194	Tricyclazole
42	Chlorpyriphos-oxon	93	Fenarimol	144	Nitrapyrin	195	Trifloxystrobin
43	Chlorthal-dimethyl	94	Fenbuconazole	145	Oxadiargyl	196	Triflumizole
44	Cinidon-ethyl	95	Fenchlorphos	146	Oxadiazon	197	Triflumuron
45	Cis-1,2,3,6 tetrahydrophthalimide	96	Fenchlorphos oxon	147	Oxycarboxin	198	Trifluralin
46	Clodinafop propargyl	97	Fenhexamid	148	p,p-DDE	199	Triflusulfuron
47	Clomazone	98	Fenobucarb	149	Parathion-ethyl	200	Triticonazole
48	Crimidine	99	Fenoxycarb	150	Parathion-methyl	201	Valifenalate
49	Cyanophos	100	Fenthion sulphoxide	151	Penconazole	202	Vinclozolin
50	Cyfluthrin-1	101	Fenvalerate	152	Pencycuron (Deg.)	203	Zoxamide (Deg.)
51	Cyfluthrin-2	102	Fipronil	153	Pendimethalin		

GCMS/MS Analytical Conditions

The analysis was carried out on Shimadzu GCMS-TQ8030 as per the conditions given below.

Chromatographic parameters

Parameter	Value		
• Column	: Rxi-5Sil MS (30 m L x 0.25 mm I.D.; 0.25 µm)		
• Injection Mode	: Splitless		
• Sampling Time	: 2.0 min		
• Split Ratio	: 5.0		
• Carrier Gas	: Helium		
• Flow Control Mode	: Linear Velocity		
• Linear Velocity	: 40.2 cm/sec		
• Column Flow	: 1.2 mL/min		
• Injection Volume	: 2.0 µL		
• Injection Type	: High Pressure Injection		
• Total Program Time	: 41.87 min		
• Column Temp. Program	: Rate (°C /min)	Temperature (°C)	Hold time (min)
		70.0	2.00
	25.00	150.0	0.00
	3.00	200.0	0.00
	8.00	280.0	10.00

Mass Spectrometry parameters

Parameter	Value
• Ion Source Temp.	: 230.0 °C
• Interface Temp.	: 280.0 °C
• Ionization Mode	: EI
• Acquisition Mode	: MRM

Results

For MRM optimisation, well resolved pesticides were grouped together. Standard solution mixture of approximately 1 ppm concentration was prepared and analyzed in Q3 scan mode to determine the precursor ion for individual pesticides. Selected precursor ions were allowed to pass through Q1 & enter Q2, also called as Collision cell. In Collision cell, each precursor ion was bombarded with collision gas (Argon) at different energies (called as Collision Energy-CE) to produce fragments (product ions). These product ions were further scanned in Q3 to obtain their mass to charge ratio. For each precursor ion, product ion with highest intensity and its corresponding CE value was selected, thereby assigning a characteristic MRM transition to every pesticide. Based on MRM transitions, the mixture of 203 pesticides was analyzed in a single run (Figure 3).

Method was partly validated for each pesticide with respect to linearity (0.5 to 25 ppb), reproducibility, LOQ and recovery. The validation summary for two pesticides namely Mevinphos and Parathion-ethyl (Sr. Nos.140 and 149 in Table 1) is shown in Figures 4 and 5. The summary data of linearity and LOQ for 203 pesticides is given in Table 2 and 3 respectively.

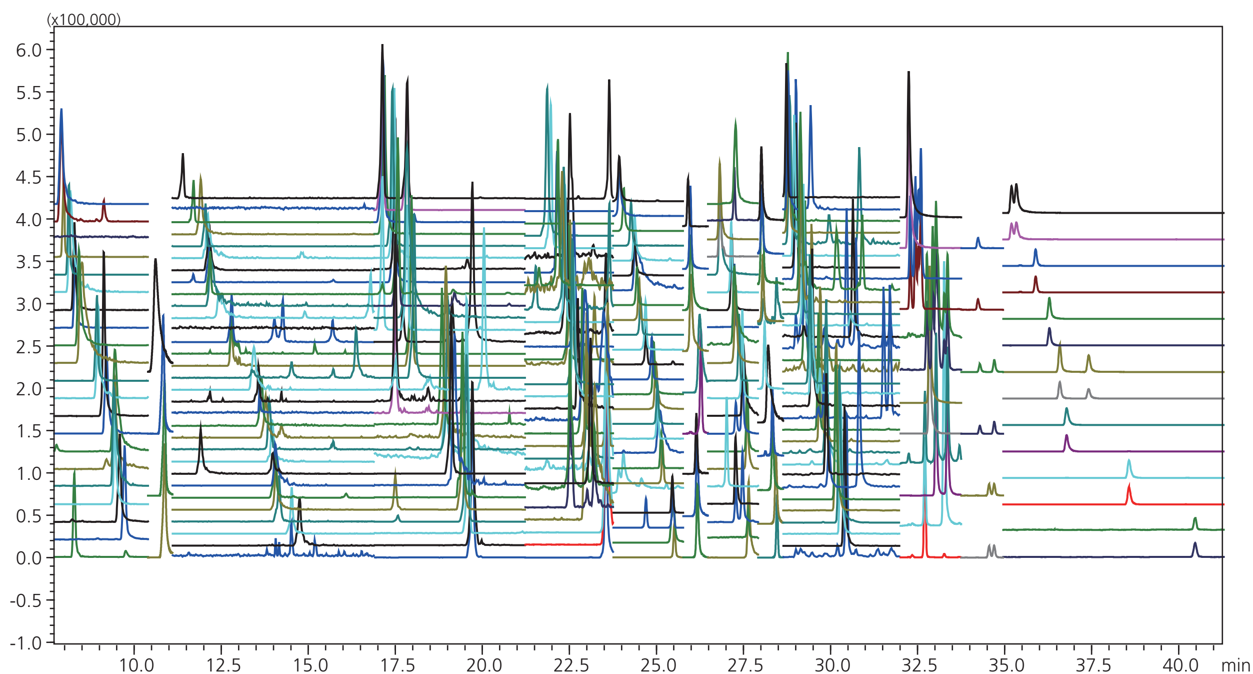
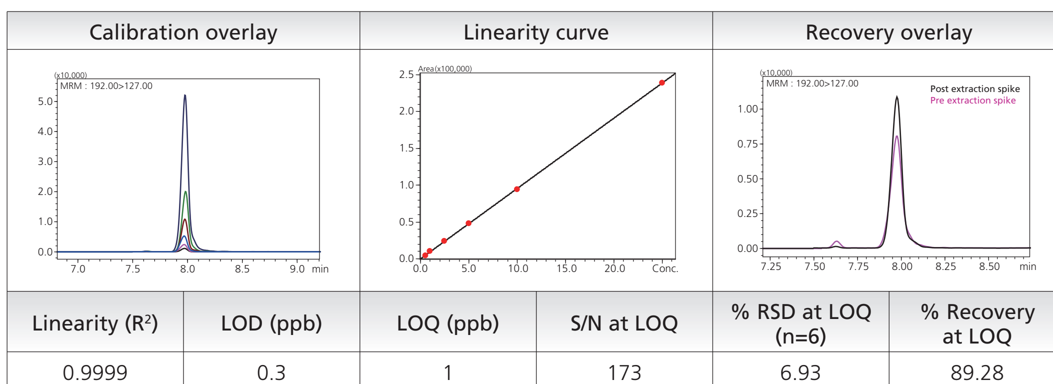
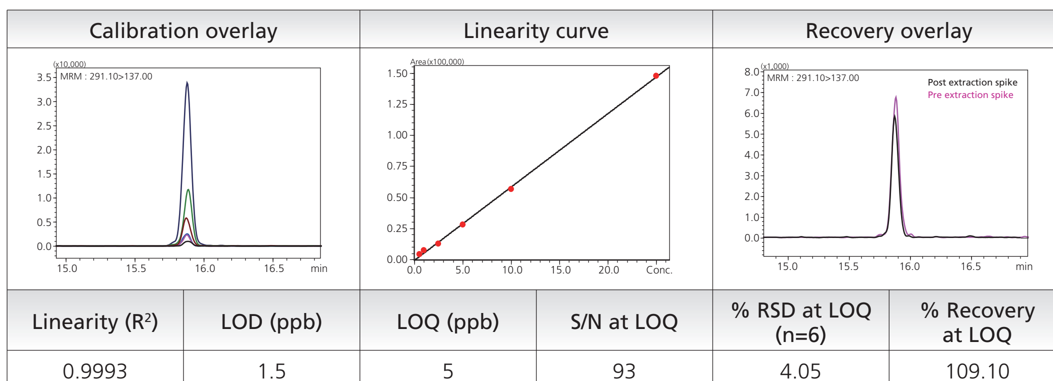


Figure 3. MRM Chromatogram for 203 pesticides mixture



Calibration overlay		Linearity curve		Recovery overlay	
Linearity (R^2)	LOD (ppb)	LOQ (ppb)	S/N at LOQ	% RSD at LOQ (n=6)	% Recovery at LOQ
0.9999	0.3	1	173	6.93	89.28

Figure 4. Summary data for mevinphos



Calibration overlay		Linearity curve		Recovery overlay	
Linearity (R^2)	LOD (ppb)	LOQ (ppb)	S/N at LOQ	% RSD at LOQ (n=6)	% Recovery at LOQ
0.9993	1.5	5	93	4.05	109.10

Figure 5. Summary data for parathion-ethyl

Multi pesticide residue analysis in tobacco
by GCMS/MS using QuEChERS as an extraction method

Table 2. Linearity Summary

Sr. No.	Linearity (R^2)	Number of pesticides
1	0.9950 - 1.0000	193
2	0.9880 - 0.9950	10

Table 3. LOQ Summary

Sr. No.	LOQ (ppb)	Number of pesticides	% RSD range (n=6)	S/N Ratio range	% Recovery range
1	1	15	6 – 15	16 – 181	70 – 130
2	5	18	3 – 15	19 – 502	
3	10	158	0.95 – 15	10 – 14255	
4	25	12	1 – 10	19 – 660	

Conclusion

- A highly sensitive method was developed for quantitation of 203 pesticides in complex tobacco matrix by using Shimadzu GCMS-TQ8030.
- The MRM method developed for 203 pesticides can be used for screening of pesticides in various food commodities. For 90 % of the pesticides, the LOQ of 10 ppb or below was achieved.
- Ultra Fast scanning, UFsweeper® and ASSP™ features enabled sensitive, selective, fast, reproducible, linear and accurate method of analysis.

Reference

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PO-CON1453E

Simultaneous quantitative analysis of 20 amino acids in food samples without derivatization using LC-MS/MS

ASMS 2014 TP 510

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Simultaneous quantitative analysis of 20 amino acids in food samples without derivatization using LC-MS/MS

Introduction

In order to detect many kinds of amino acids with high selectivity in food samples, the LC/MS analysis have been used widely. Amino acids are high polar compound, so they are hard to be retained to reverse-phased column such as ODS (typical method in LC/MS analysis). It needs their derivartization or addition of ion pair reagent in mobile phase to retain them. For easier analysis of amino acids, it is expected to develop the method without using reagents mentioned above.
This time, we tried to develop a simultaneous high sensitive analysis method of 20 amino acids by LC/MS/MS with mix-mode column (ion exchange, normal-phase) and the typical volatile mobile phase suitable for LC/MS analysis.

Methods and Materials

Amino acid standard regents and food samples were purchased from the market. Standards of 20 kinds of amino acids were optimized on each compound-dependent parameter and MRM transition. As an LC-MS/MS system, HPLC was coupled to triple quadrupole mass spectrometer (Nexera with LCMS-8050, Shimadzu Corporation, Kyoto, Japan). Sample was eluted with a binary gradient system and LC-MS/MS with electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode.

High Speed Mass Spectrometer

UF-MRM
High-Speed MRM at 555ch/sec

UFswitching
High-Speed Polarity Switching 5msec

Figure 1 LCMS-8050 triple quadrupole mass spectrometer

Result

Method development

First, MRM method of 20 amino acids was optimized. As a result, all compounds were able to be detected high sensitively and were detected in positive MRM transitions. As the setting temperature of ESI heating gas was found to affected on the sensitivity of amino acids, it was also optimized. Even though amino acids were not derivartized and ion-pairing reagent wasn’t used, 20 amino acids were retained by using a mixed-mode stationary phase structure and separated excellently on the below-mentioned condition.

Simultaneous quantitative analysis of 20 amino acids in food samples without derivatization using LC-MS/MS

HPLC conditions (Nexera system)

Column	: Intrada Amino Acid (3.0mmI.D. x 50mm, 3um, Imtakt Corporation, Kyoto, Japan)
Mobile phase	
Case1	
A	: Acetonitrile / Formic acid = 100 / 0.1
B	: 100mM Ammonium formate
Time program	: B conc.14%(0-3 min) -100%(10min) - 14%(10.01-15min)
Case2 (High Resolution condition)	
A	: Acetonitrile / Tetrahydrofuran / 25mM Ammonium formate / formic acid = 9 / 75 / 16 / 0.3
B	: 100mM Ammonium formate / Acetonitrile = 80 / 20
Time program	: B conc.0%(0-2 min) - 5%(3min) - 30%(6.5min) - 100%(12min) - 0%(12.01-17min)
Flow rate	: 0.6 mL/min
Injection volume	: 2 uL
Column temperature	: 40 °C

MS conditions (LCMS-8050)

Ionization	: ESI, Positive MRM mode

MRM transition are shown in Table 1.

Case1

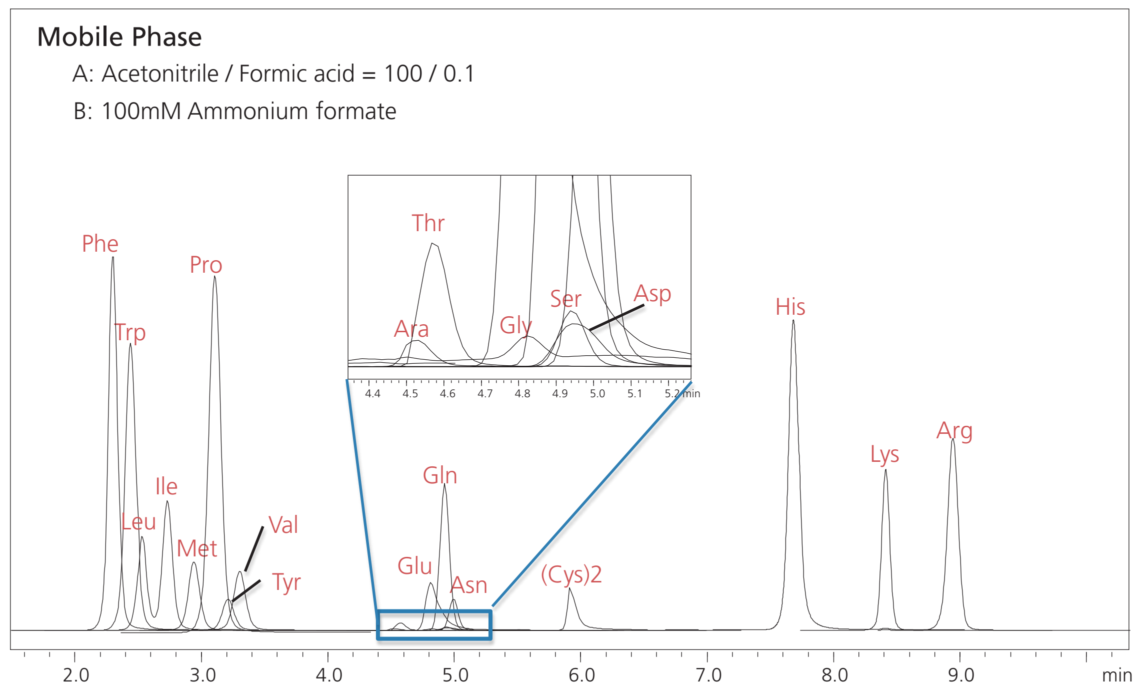

Figure 2 Mass Chromatograms of 20 Amino acids (concentration of each compound : 10nmol/mL)

Simultaneous quantitative analysis of 20 amino acids in food samples without derivatization using LC-MS/MS

Case2 (High Resolution condition)

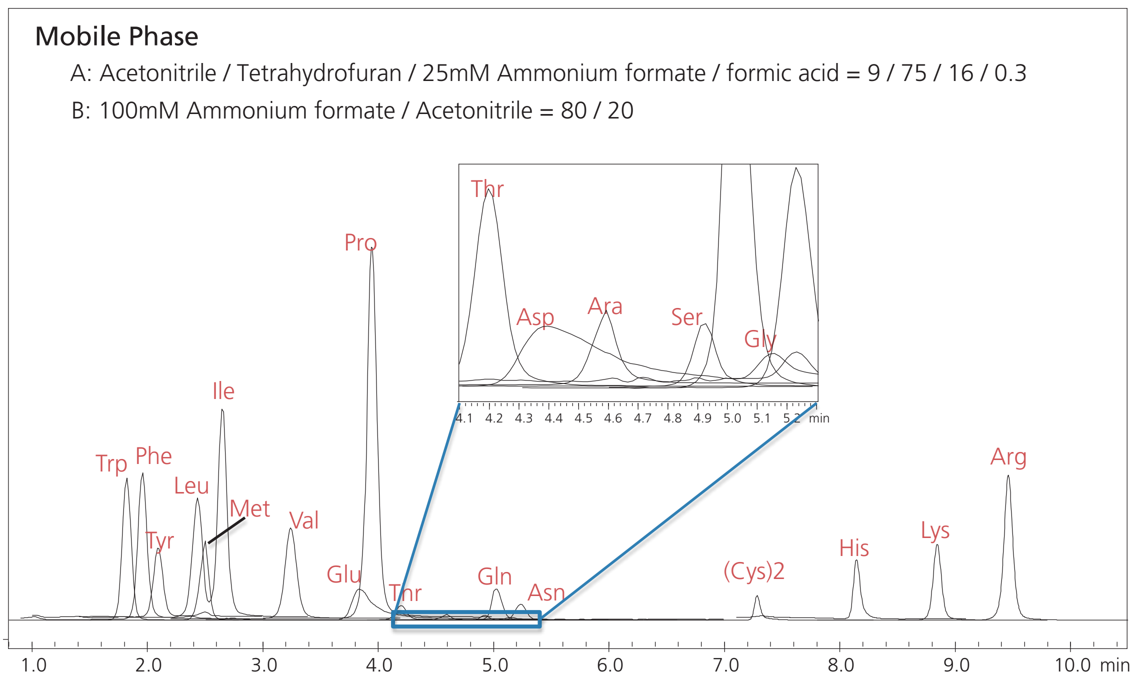

Figure 3 Mass Chromatograms of 20 Amino acids (concentration of each compound : 10nmol/mL)

In this study, two conditions of mobile phase were investigated. It was found that 20 amino acids were separated with higher resolution in case2.
As the mobile phase condition of case1 is more simple and the result of case1 was sufficiently well, case1 analytical condition was used for quantitative analysis. The dilution series of these compounds were analyzed. All amino acids were detected with good linearity and repeatability (Table1).

Simultaneous quantitative analysis of 20 amino acids in food samples without derivatization using LC-MS/MS

Table1 Linearity and Repeatability of 20 amino acids

	MRM Transition	Linearity		Repeatability*
		Range (nmol/mL)	Coefficient (r2)	%RSD
Trp	205.10>188.10	0.01-100	0.9950	1.4
Phe	166.10>120.10	0.01-100	0.9971	1.2
Tyr	182.10>136.00	0.05-100	0.9900	1.7
Met	150.10>56.10	0.05-200	0.9963	0.1
Lue, Lle	132.10>86.15	0.01-100	0.9955	0.7
Val	118.10>72.05	0.05-100	0.9991	1.9
Glu	148.10>84.10	0.05-10	0.9965	4.5
Pro	116.10>70.10	0.01-50	0.9933	1.5
Asp	134.20>74.10	0.5-500	0.9953	1.4
Thr	120.10>74.00	0.1-50	0.9923	4.5
Ala	90.10>44.10	0.5-500	0.9989	16.2
Ser	106.10>60.20	0.5-500	0.9988	6.5
Gln	147.10>84.10	0.05-1	0.9959	3.9
Gly	76.20>29.90	5-200	0.9974	11.0
Asn	133.10>74.05	0.05-20	0.9939	6.1
(Cys)2	241.00>151.95	0.05-20	0.9909	2.3
His	156.10>110.10	0.05-200	0.9983	1.7
Lys	147.10>84.10	0.05-5	0.9908	0.9
Arg	175.10>70.10	0.01-100	0.9956	0.5

*@ 0.5nmol/mL : except for Gly, 5nmol/mL : for Gly

The analysis of 20amino acids in food samples

The analysis of the amino acids contained in sports beverage on the market was carried out. In the case of sports beverage, all amino acids written in the package were detected.

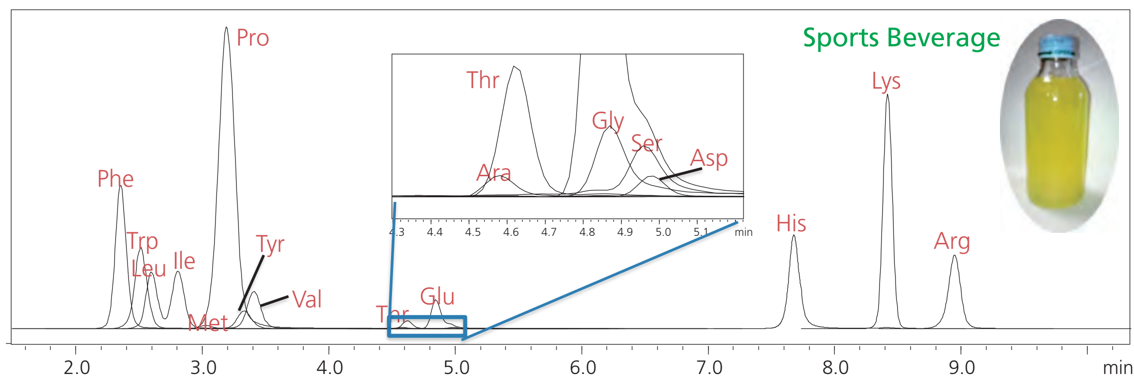

Figure 4 Mass Chromatograms of Sports Beverage (100 fold dilution with 0.1N HCl)

Simultaneous quantitative analysis of 20 amino acids in food samples without derivatization using LC-MS/MS

Furthermore, Japanese Sake, Beer and sweet cooking rice wine (Mirin) were analyzed using this method. Japanese Sake and Beer were diluted with 0.1N HCl. Sweet cooking rice wine was diluted in the same way after a deproteinizing preparation. These were filtered through a 0.2um filter and then analyzed. MRM chromatograms of each food samples are shown in Figure 5,6,7. Amino acids of each sample were detected with high sensitivity.

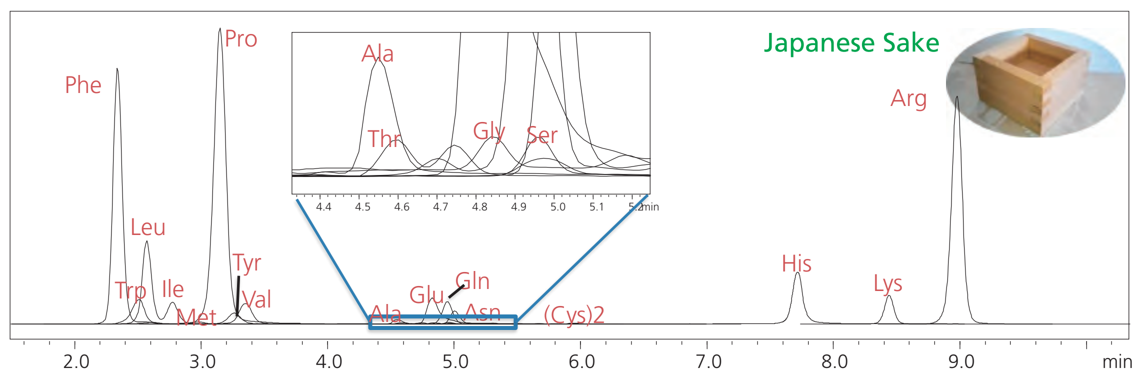

Figure 5 Mass Chromatograms of Japanese Sake (100 fold dilution with 0.1N HCl)

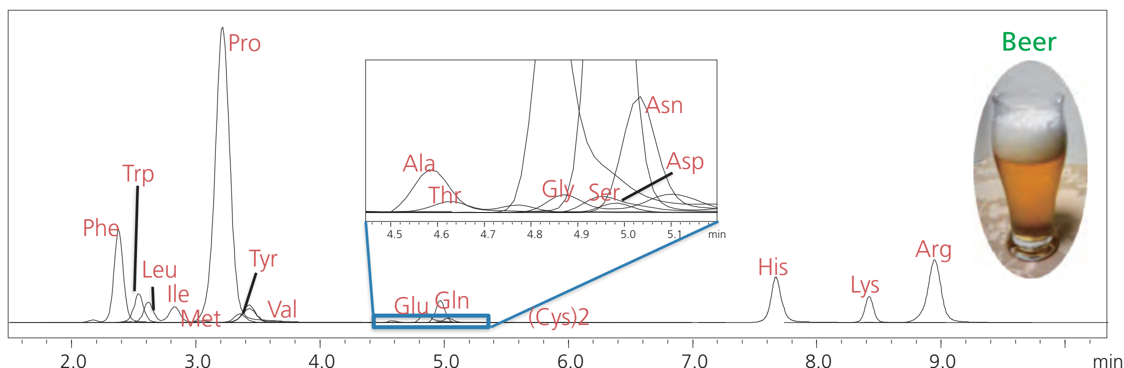

Figure 6 Mass Chromatograms of Beer (10 fold dilution with 0.1N HCl)

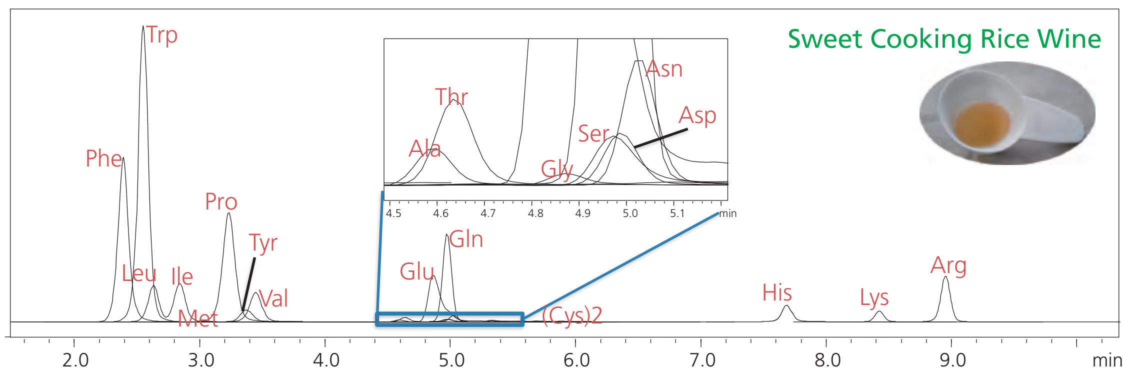

Figure7 Mass Chromatograms of Sweet Cooking Rice Wine (100 fold dilution with 0.1N HCl)

Conclusions

- 20 amino acids could be separated without derivatization using a typical volatile mobile phase suitable for LC/MS analysis and detected with high sensitivity.
- This methods was able to be applied to the analysis of amino acids in various food samples.

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