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Spectrométrie de Masse

Volume 7 - Spécial ASMS 2018











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DES COLLABORATEURS EXPERTS ET PASSIONNÉS

Shimadzu a toujours misé sur le talent de ses collaborateurs passionés pour transformer l'industrie, à l'image de Genzo Shimadzu Jr., récompensé comme l'un des dix plus grands inventeurs japonais de tous les temps ou encore de Koichi Tanaka, lauréat du prix Nobel de Chimie en 2002 pour ses travaux de recherche sur la spectrométrie de masse et les macromolécules.

UN PIONNIER DANS L'INNOVATION

Tout au long de son histoire, Shimadzu n'a jamais cessé d'innover et a été à l'origine de nombreuses découvertes et premières mondiales. Aujourd'hui, notre groupe est présent à l'international dans plus de 100 pays avec des centres de Recherche & Développement et de production en Europe, au Japon, en Chine, en Australie et aux Etats-Unis. Plus de 11h000 collaborateurs Shimadzu se mobilisent chaque jour autour d'un seul but : contribuer à l'amélioration de la société à travers la Science et la Technologie.





DES SOLUTIONS ADAPTÉES À VOS BESOINS

Notre effort en Recherche & Développement est constant. Nos nouvelles technologies sont le fruit d'une collaboration avec différentes institutions universitaires et centres de recherche. Cette synergie nous permet de mettre au point des solutions fiables et adaptées aux différentes problématiques analytiques. Nous disposons de plusieurs laboratoires de démonstration ainsi que de centres de formation pour chaque gamme. Avec une présence régionale complète, nous proposons des solutions suivies pour chacun de nos clients à travers tout le pays.

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UN ACTEUR MAJEUR EN SPECTROMÉTRIE...

L'analyse par spectrométrie de masse de type triple quadripôle est la méthode de prédilection pour une quantification précise avec une grande gamme dynamique et une confirmation de traces d'analytes dans des matrices complexes. Des progrès de pointe ont été réalisés par Shimadzu dans les domaines clés de l'acquisition ultra rapide : 555 MRM par seconde, contamination croisée quasi nulle, inversion de polarité ultra rapide...

... À L'ORIGINE DES DERNIÈRES TECHNOLOGIES DE POINTE

Avec nos spectromètres de masse LCMS-8040, LCMS-8045, LCMS-8050 et LCMS-8060, vous valorisez vos analyses quantitatives et bénéficiez d'une productivité élevée ainsi que de performances inégalées. Nos technologies Ultra-Fast MS combinées à notre UHPLC à très grande vitesse, Nexera, vous permettront d'accélérer vos analyses et de réduire vos coûts de consommables (gaz, solvants, flacons etc..) pour un retour sur investissement maximisé.



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Instrumentation Linear Ion Trap with Multipole fields

QTOF

MALDI



Development of the microflow LC solvent delivery unit for stable pumping at µL/min level

ASMS 2018 MP 660

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PO-CON1844E

Development of the microflow LC solvent delivery unit for stable pumping at µL/min level

Overview

The newly designed solvent delivery pump unit, "LC-Mikros" enabled accurate and stable solvent flow at µL/min levels for higher sensitivity on LC-ESI-MS.

Introduction

LC-ESI-MS has been a workhorse to determine a huge variety of compounds due to its high selectivity and sensitivity. However, there is still room to improve the sensitivity on the most popular LC-MS platform, with 2.1 mm i.d. columns and 0.1-1 mL/min of mobile phase flow. A decrease in the mobile phase flow with narrower columns results in higher sensitivity, hence nanoflow LC/MS has been a gold standard in the discovery stage. A drawback of nano-LC/MS is that it requires a highly-skilled chemist to operate the system in the correct manner. Microflow LC/MS balances high sensitivity and simple operation. Here we will present some technical background information to achieve robust and stable microflow with a LC-MS system, focusing on the solvent delivery unit.



Figure 1. Nexera Mikros microflow LC-MS system



Figure 2. Demand for Microflow LC-MS system

Development of the microflow LC solvent delivery unit for stable pumping at µL/min level

Instruments and Method

Configuration with UV detector for an evaluation of pump performance

The microflow UHPLC system consisted of UHPLC units (Shimadzu). Gradient elution was performed by LC-Mikros binary pump. The test compound was injected by autosampler SIL-30AC. The separation column (C18,

1.9 μ m, 150 mmL. \times 0.3 mm i.d.) was installed with 0.05 mm i.d tubing, between the autosampler and UV detector SPD-20A which the microflow cell was mounted on.

Test solution preparation

The commercially available peptide standard mixture (angiotensin II, Gly-Tyr, Leu-enkephalin, Met-enkephalin, Val-Tyr-Val) was dissolved and diluted in 0.1% formic acid aq.

Analytical conditions with UV detector

Mobile phase A Mobile phase B	: 0.1% formic acio : 0.1% formic acio	d in water d in acetonitrile		
Gradient	Time [min]	%B	Time [min]	%B
	0.00	1	0.00	1
	0.50	1	1.00	1
	9.00	40	11.00	40
	9.10	90	11.01	90
	11.10	90	13.00	90
	11.20	1	13.01	1
	18.00	1	25.00	1
Column	: 150 x 0.3 mm M	icro Column C18	1.9 µm (Prototype)	
Flow rate	: 5 µL/min			
Column oven temperature	: 40 °C			
Injection volume	: 0.5 µL			
UV wavelength	: 280 nm			

Configuration for an evaluation of LC-ESI-MS system's robustness

The microflow UHPLC Trap & Elute system consisting of UHPLC units (Nexera Mikros series, Shimadzu) was coupled to a triple quadrupole mass spectrometer LCMS-8060 (Shimadzu) with a Micro ESI unit. The trapping process was performed by two LC-30AD pumps, autosampler SIL-30AC, and switching valve FCV-32AH. Gradient elution for the separation was performed by LC-Mikros binary pump.

Human plasma sample preparation

Human plasma was prepared by the addition of acetonitrile in a ratio of 3 : 1 (acetonitrile : plasma). The plasma sample was then vortex mixed for one minute and subsequently centrifuged at 15,000 relative centrifugal force (RCF) for fifteen minutes. The supernatant was then removed, and pipetted into an LC vial. At regular intervals of fifty injections, a QC standard, consisting of nortriptyline, was monitored to access chromatographic performance over the test period.

Development of the microflow LC solvent delivery unit for stable pumping at μ L/min level

Analytical conditions

LC Conditions	
Mobile phase A	: 0.1% acetic acid in water
Mobile phase B	: 0.1% acetic acid in acetonitrile
Trapping	: 50 μL/min for 1.2 min, 5% B
Gradient	Time [min] %B
	0.00 20
	1.00 20
	2.50 95
	4.30 95
	4.50 20
	7.00 20
Trap column	: 35 x 0.5 mm Shim-pack MCT C8 3 µm
Analytical column	: 50 x 0.175 mm Shim-pack MC C18 1.9 μm
Flow rate	: 4 µL/min
Column oven temperature	: 55 ℃
Injection volume	: 1 µL
MS CONDTIONS	
Ionization mode	: ESI +ve;
MRM (Nortriptyline)	: 264.05 > 233.15 (-16.0 eV)
CID gas pressure	: 210 kPa
Nebulizer gas	: 0.5 L/min
DL temperature	: 300 °C
Heated block temperature	: 300 °C

Results

The principle mechanism of the developed microflow solvent delivery unit

The new microflow solvent delivery unit is a binary pump, which two linear-drive actuators for each plunger reciprocate. Each actuator pair comprises a single reciprocating serial pump. The pressure values monitored on each pressure sensor, the first pump-head, and the second pump-head, are used to compensate the action of two linear-drive actuators and reduce the pressure pulse.

For microflow LC/MS analysis, extremely accurate flow rate of the solvent is essential to obtain good reproducibility on MS chromatograms. Hence, three aspects are highly required;

- 1) A leak-free mechanism.
- 2) Temperature stability on the solvent pump.
- 3) Less influence of expansion and contraction of the fluid under high pressure.

1) Leak-free mechanism

As the flow rate is very small, it's difficult to determine solvent leakage. The sealing structure against high pressure in the check valve was newly designed to prevent solvent leakage.



Development of the microflow LC solvent delivery unit for stable pumping at μ L/min level

2) Temperature stability on the solvent pump

The thermal influence is expressed by the following equation.

$$\Delta f = V_{hp} \times \beta \times \Delta T$$

 Δf : Variation of flow rate V_{hp} : High pressure line volume β : Coefficient of thermal expansion ΔT : Variation of environmental temperature per minute

The newly developed microflow solvent delivery unit is designed to reduce V_{hp} as much as possible. Additionally, by increasing the thermal resistance value around the high-pressured line, ΔT could be reduced.

3) Less influence of expansion and contraction of the fluid under high pressure. The influence of the system pressure to the flow rate variation is expressed by the following equation.

 $\Delta f = V_{hp} \times k \times \Delta P$

k: Compressibility ΔP : Variation of system pressure per minute

Assuming that the LC system behaves as the first-delay lag system due to the pressure damper and fluidics resistance, the pressure change is expressed by following function.

$\Delta P \propto R, V_{hp}$ R: Fluidic resistance

 V_{hp} is affected by the position of the plunger in the reciprocal motion. To obtain good accuracy and reproducibility in solvent delivery, the pressure curve should give identical curves.

The plungers mounted on LC-Mikros are controlled to be put at precisely the same position when the analysis starts.

Performance evaluation of solvent delivery unit

To evaluate the performance of the solvent delivery unit at µL/min levels, the pressure behaviour were monitored first. Stable pressure change following the organic solvent ratio was observed with good reproducibility (Figure. 3).

Development of the microflow LC solvent delivery unit for stable pumping at μ L/min level



Figure 3. System pressure traces for 6 injections.

To evaluate the accuracy of solvent delivery, five small peptides (Gly-Tyr, Val-Tyr-Val, angiotensin II, Leu-enkephalin, and Met-enkephalin) were injected six times, and the UV chromatograms were acquired. Excellent reproducibility of retention time in table 1, which is equal to that in the semi-micro analysis, indicates that the accuracy of flow rate is superior with good repeatability.

	Average Rt [min]	SD [min]	RSD
Gly-Tyr	5.31	0.009	0.16%
Val-Tyr-Val	7.97	0.006	0.07%
Angiotensin II	9.32	0.015	0.16%
Leu-encephalin	9.71	0.011	0.11%
Met-enkephalin	10.5	0.012	0.11%

Table 1. Reproducibility of retention time for peptide

Evaluation of system robustness for biological sample analysis

We also evaluated the robustness of the micro LC-ESI/MS/MS system for biological sample analysis. Figure.4 illustrates that good system robustness was maintained even after 1500 injections of human plasma, prepared by simple protein precipitation with acetonitrile.

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Development of the microflow LC solvent delivery unit for stable pumping at µL/min level





Conclusions

- The newly designed solvent delivery unit "LC-Mikros" with its various features was evaluated, and it was determined that its performance was prominently stable and accurate at µL/min levels.
- The retention time reproducibility (0.07- 0.16% RSD, n=6) for peptides was superior, with microflow analysis.
- Even after 1500 injections of plasma samples, Nexera Mikros with LC-Micros still provided brilliant retention time stability.

Nexera Mikros[™] and LCMS-8060 is intended for Research Use Only (RUO). Not for use in diagnostic procedures.



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Analysis of Perfluorinated Alkyl Acids Specified in EPA M537 and Beyond Using LCMS-8045

ASMS 2018

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PO-CON1827E

Analysis of Perfluorinated Alkyl Acids Specified in EPA M537 and Beyond Using LCMS-8045

Novel Aspects

The scope of EPA Method 537 has been expanded to include 7 additional perfluorinated alkyl acids.

Introduction

There has been an increasing awareness of the presence of perfluoroalkyl sulfonates (PFOS) and perfluoroalkyl carboxylic acids (PFCAs) in water. Although perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the most studied polyfluoroalkyl substances (PFASs), perfluoroalkyl and polyfluoroalkyl substances with chain lengths varying from C2-C14 have also been detected in various sample matrices. The continued use of these compounds presents a long-term challenge to scientist, industry leaders, and public health officials worldwide. This presentation describes a highly sensitive Solid Phase Extraction (SPE) – Liquid Chromatography -Tandem Mass Spectrometry (LC-MS/MS) Method for the determination of twenty-four specific polyfluorinated chemical (PFCs), fourteen of which are listed in EPA M537 in drinking waters.

Methods

MRM transitions were optimized using Flow Injection Analysis (FiA) for all compounds. Compounds were separated, including PFHxS and PFOS isomers (Figures 1 through 3), using a Restek Raptor ARC-18 150 x 2.1 mm (Part No. 9314A62) using 20 mM ammonium acetate for mobile phase A and methanol for Mobile Phase B. Standards were purchased from Wellington Laboratories.

SPE Method

Extractions were performed using Biotage[®] ISOLUTE[®] 101 polystyrene-divinylbenze (SDVB) cartridges (Part No. 101-0050-C) as outlined in EPA 537. A vacuum manifold with a high-volume sampling kit outfitted with PEEK tubing was used to reduce potential contamination. Each cartridge was conditioned first with methanol, followed by LCMS grade water as outlined in EPA 537. Each water sample (250 mL) was fortified with surrogates and passed through the cartridge. Compounds were eluted from the solid phase with 8 mL of methanol and evaporated to dryness using nitrogen. Extracted samples were reconstituted to a final volume of 1 mL in 96:4 Methanol: H_2O with internal standards added.

Calibration

A series of 10 calibration levels ranging from 1.25 ppb to 100 ppb were injected four times over the course of two weeks. The initial calibration curve was used to quantitate the subsequent injections. Table 1 lists the calculated concentration as well as the %RSD. All calibration curves met the criteria listed in EPA 537. Compounds added to improve method performance are italicized.



Analysis of Perfluorinated Alkyl Acids Specified in EPA M537 and Beyond Using LCMS-8045



Figure 1: TIC from a low-level calibrator (20 ppb)



Figure 2: TIC from a method blank

Compound	Retention	D2	Low (2	20 ppb)	Mid (5	0 ppb)	High (1	00 ppb)
Compound	Time	n	Conc	%RSD	Conc	%RSD	Conc	%RSD
PFBS	8.046	0.9977	20.68	1.97	45.85	2.53	102.91	1.87
4-2FTS	8.558	0.9928	22.21	2.48	45.16	6.96	94.19	1.09
PFHxA	8.614	0.9968	21.02	3.54	48.19	6.48	102.05	3.45
PFPeS	8.666	0.9985	20.89	2.00	46.17	1.87	99.64	1.11
PFHpA	9.512	0.9974	20.96	4.92	46.44	4.67	101.33	2.33
PFHxS	9.558	0.9968	20.64	2.68	46.04	4.84	104.38	2.70
6-2 FTS	10.77	0.9968	20.96	4.15	43.81	4.34	94.52	2.28
PFOA	10.84	0.9967	21.04	4.63	47.23	7.39	103.01	2.63
PFHpS	10.859	0.9982	20.61	4.29	44.98	7.56	103.75	5.86
PFOS	12.55	0.9986	19.99	6.13	43.74	7.42	102.64	12.29
PFNA	12.545	0.9975	21.11	10.35	46.75	1.60	100.12	3.36
8-2 FTS	14.436	0.994	22.67	13.73	45.39	12.80	94.29	12.62
PFNS	14.469	0.9978	21.07	2.38	45.84	5.58	100.05	4.74
PFDA	14.486	0.9969	20.83	2.62	47.20	3.04	98.24	1.67
N-MeFOSAA	15.423	0.9979	21.04	3.28	46.68	1.09	100.38	2.68
N-EtFOSAA	16.411	0.998	21.66	3.98	47.79	2.17	101.97	4.93
PFDS	16.397	0.997	20.89	3.57	45.39	11.22	102.82	5.33
PFUnA	16.449	0.9973	20.87	4.15	47.57	4.22	100.21	5.99
PFDoA	18.339	0.9975	20.60	3.45	47.91	3.40	103.32	5.65
PFTriA	20.035	0.9967	20.37	5.03	45.30	5.08	100.55	4.82
PFTreA	21.549	0.9966	21.05	5.39	47.36	4.05	102.69	2.92

Analysis of Perfluorinated Alkyl Acids Specified in EPA M537 and Beyond Using LCMS-8045

Compound	Spiked Conc (ppt)	Calculated Conc (ppt)	Accuracy	%RSD	MDL
PFBS	5	4.17	83.31	12.19	1.47
4-2FTS	5	5.22	104.45	14.09	2.13
PFHxA	5	4.07	81.44	9.95	1.17
PFPeS	5	4.06	81.16	12.84	1.51
PFHpA	5	4.18	83.64	8.72	1.06
PFHxS	5	4.25	84.95	5.61	0.69
6-2 FTS	5	4.59	91.88	17.06	2.27
PFOA	5	4.59	91.83	11.94	1.59
PFHpS	5	3.99	79.74	8.92	1.03
PFOS	5	4.03	80.65	14.94	1.74
PFNA	5	3.99	79.73	7.13	0.82
8-2 FTS	5	5.02	100.41	22.38	3.25
PFNS	5	4.04	80.78	9.05	2.06
PFDA	5	4.13	82.61	8.11	0.97
N-MeFOSAA	5	3.87	77.50	15.14	1.70
N-EtFOSAA	5	3.82	76.49	10.75	1.19
PFDS	5	4.12	82.34	18.04	2.15
PFUnA	5	4.10	81.98	12.22	1.45
PFDoA	5	3.97	79.41	13.98	1.61
PFTriA	5	3.92	78.41	12.55	1.43
PFTreA	5	3.97	79.38	15.30	1.76

Table 2: Method Detection Limit (MDL) results

Analysis of Perfluorinated Alkyl Acids Specified in EPA M537 and Beyond Using LCMS-8045

Compound	Extract 1	Extract 2	Extract 3	Extract 4	Extract 5	Extract 6	Extract 7	Average	Percent Recovery	%RSD
PFBS	56.51	53.06	54.00	62.97	47.78	42.11	50.85	52.47	87.4	12.6
4-2FTS	57.18	64.03	53.27	58.52	46.92	43.49	55.36	54.11	90.2	12.9
PFHxA	56.22	52.76	52.34	61.03	46.62	42.27	52.80	52.01	86.7	11.8
PFPeS	64.33	53.80	56.44	63.02	47.02	43.18	52.42	54.31	90.5	14.3
PFHpA	62.71	57.86	51.39	59.75	42.22	41.87	52.44	52.61	87.7	15.6
PFHxS	64.20	54.20	52.08	60.50	47.16	43.88	52.84	53.55	89.3	13.2
6-2 FTS	69.30	58.81	57.66	56.75	44.84	47.45	49.37	54.88	91.5	15.3
PFOA	61.39	49.06	49.13	62.86	46.19	43.49	50.81	51.85	86.4	14.3
PFHpS	63.59	56.78	52.94	59.57	49.45	42.77	51.64	53.82	89.7	12.8
PFOS	63.93	55.54	56.96	59.89	42.73	40.21	52.39	53.09	88.5	16.5
PFNA	62.92	52.69	48.53	58.59	44.04	39.20	53.49	51.35	85.6	15.9
8-2 FTS	61.41	58.87	57.68	56.63	36.93	43.16	44.83	51.36	85.6	18.5
PFNS	67.17	54.84	53.66	58.26	46.52	42.96	51.95	53.62	89.4	14.7
PFDA	59.59	51.56	50.29	60.47	45.73	42.59	53.15	51.91	86.5	12.7
N-MeFOSAA	60.48	56.23	59.16	56.22	52.97	38.88	44.63	52.65	87.8	15.2
N-EtFOSAA	63.56	59.29	57.93	59.58	50.20	40.35	47.10	54.00	90.0	15.4
PFDS	58.38	59.38	50.25	54.26	42.70	37.82	58.55	51.62	86.0	16.5
PFUnA	55.83	50.89	51.21	56.52	50.66	40.11	52.99	51.17	85.3	10.6
PFDoA	57.20	53.99	54.28	57.77	47.48	36.54	52.75	51.43	85.7	14.4
PFTriA	55.42	50.69	48.72	56.65	45.61	36.63	49.87	49.08	81.8	13.6
PFTreA	54.28	51.17	49.84	54.57	49.46	34.95	50.11	49.20	82.0	13.5

Table 3: Precision and Accuracy Study Results

Table 4: Surrogate Recovery Results

Compound	Extract 1	Extract 2	Extract 3	Extract 4	Extract 5	Extract 6	Extract 7	Average	Percent Recovery	%RSD
MPFxA	44.16	42.51	40.33	52.78	38.95	33.90	45.57	42.60	106.5	13.9
MPFDA	47.88	44.26	44.68	51.56	41.80	35.31	40.65	43.73	109.3	11.9
MNEt-FOSAA	191.14	188.34	182.79	206.44	157.42	138.83	157.02	174.57	109.1	13.7

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Analysis of Perfluorinated Alkyl Acids Specified in EPA M537 and Beyond Using LCMS-8045



Figure 3: Overlaid Chromatograms for PFOS from the Method Detection Limit Study



Figure 4: Calibration Curves for PFOA and PFOS ranging from 1.25 ppb to 100 ppb



Analysis of Perfluorinated Alkyl Acids Specified in EPA M537 and Beyond Using LCMS-8045

Results and Discussion

A Method Detection Limit (MDL) study was conducted by spiking 250 mL samples at 5 ng/L (5 ppt). These samples were then extracted and concentrated to a final volume of 1 mL in 96:4 MeOH:H₂O. Nine samples were extracted over the course of three days, as described in 40 CFR Part 136 Appendix B. The results from this study are outlined in Table 2. Compounds added to improve method performance are italicized. The precision and accuracy study was carried out by spiking LCMS grade water at 60 ppt and extracted seven times each. Table 3 lists the results of this study. All recoveries were within 20 percent of the true value, exceeding the criteria listed in EPA 537. Compounds that were added to improve method performance are italicized.

All extracted saples were spiked with 10 ng of MPFxA, 10 ng of MPFDA, and 40 ng of MNEt-FOSAA giving a sample concentration of 40 ppt for MPFxA and MPFDA and 160 ppt for MNEt-FOSAA. The calculated recoveries are shown in Table 4 using a Mean Response Factor. All recoveries were within +/-10 percent, well exceeding there requirements of section 9.3.5 of EPA 537.

Conclusion

The Shimadzu LCMS-8045 and Biotage[®] ISOLUTE 101 cartridges exceed the performance criteria specified by EPA 537. Method Detection limits ranging from 0.69 to 3.25 ppt were obtained with recoveries of at least 80% for all compounds.

Acknowledgements

Shimadzu Scientific Instruments would like to thank Biotage for providing the cartridges used to generate this data

References

- EPA Method 537 rev1.1, Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) (U.S. Environmental Protection Agency, Washington, D.C., Sept. 2009).
- (2) ASTM D7979-16, Standard Test Method for Determination of Perfluorinated Compounds

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A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry

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A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry

Overview

Development of a rapid simple screening method by LC/MS/MS for mycotoxins containing fumonisins.

Introduction

Mycotoxin is a chemical substance produced by mold. In terms of harmful substances to health of human and livestock, regulatory limitation is defined in each country. In recent years, the risk management of mycotoxins has been gaining wider acceptance all over the world. In this report, we demonstrate a simultaneous screening analysis for 18 kinds of mycotoxins in grains by LC/MS/MS. For the purification of grain extract, Multitoxin Spin Column (Romer Labs) was utilized in order to perform a simple and rapid clean-up treatment for mycotoxins, which has individual chemical properties, such as aflatoxins, ochratoxin A, trichothecenes, and fumonisins.

Methods and Materials

Analytical conditions

Analysis was performed by a LCMS-8050 which was equipped with a Nexera[™] X2 UHPLC. Pentafluorophenyl (PFP) bounded column was used to separate the regioisomeric pair (3-AcDON / 15-AcDON, FB2 / FB3) by gradient elution with a series of mobile phases containing ammonium acetate, acetic acid and methanol. Quantitative limits had been deemed to be less than or equivalent to the minimum values specified in EC/1886/2006. The developed method achieved the simultaneous determination of mycotoxins such as aflatoxins (B1, B2, G1, G2), fumonisins (B1, B2, B3), ochratoxin A (OTA), trichothecenes [(3-acetyldeoxynivalenol(3-AcDON), 15-acetyldeoxynivalenol (15- AcDON), deoxynivalenol (DON), HT-2, nivalenol (NIV), T-2, zearalenone (ZEN)), Fusarenon-X (FUX), Diacetoxy- scirpenol (DAS)] and patulin (PAT) in 15 minutes analytical cycle. This analytical method was developed by the modified LC/MS/MS method package for mycotoxin (Shimadzu Corporation, Japan).



Inject method

Improving peak shape of NIV solved in more than 50% of acetonitrile aqueous solution, the sample solutions should be injected with additional water. SIL-30AC autosampler has this useful function shown as above.



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High Speed Mass Spectrometer Ultra Fast Polarity Switching -5 msec Ultra Fast MRM -Max.555 transition/sec



Column : Mastro PFP 2 (150 mm×2.1 mm, 3 µm) Mobile phase A : 10 mmo/L Ammonium acetate-water
Mobile phase A : 10 mmo/L Ammonium acetate-water
Mobile phase B : Methanol including 2% acetic acid
Flow rate : 0.4 mL/min
Time program : B conc.15%(0 min) -35%(1.51 min) –
45%(5.50 min) - 60%(5.51 min) –
95%(9.50-12.00 min) - 15%(12.01-15.00 min)
Column temp. : 40 °C
Injection vol. : 2.5 µL with 50 µL Water
Rince R0 : Mobile phase A
Rince R1 : 10 mmol/L Sodium citrate aqueous solution
Rince R2 : Water/ Methanol / Acetonitrile/ IPA = 1/1/1/1 including 1% formic acid
Needle rinse program : inside: $R1 \rightarrow R0 \rightarrow R2 \rightarrow R0$, outside: $R3(1 \text{ sec}) \rightarrow R0$
MS conditions (LCMS-8050)
Ionization : ESI, Positive/Negative MRM mode
DL temp. : 150 °C

DL temp.	. 150 °C
Interface temp.	: 200 °C
Heat block temp.	: 400 °C
Nebulizer gas	: 2.5 L/min
Heating gas	: 15 L/min
Drving gas	: 5 L/min

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No.	Mycotoxin	Retention Time (min)	Polarity	Precursor Ion (<i>m/z</i>)	Product Ion (<i>m/z</i>)	CE (V)
1	Nivalenol (NIV)	2.261	-	371.10	281.10	15
2	Patulin (PAT)	2.569	-	153.00	109.10	13
3	Deoxynivalenol (DON)	2.998	-	355.10	295.10	10
4	Fusarenon-X (FUX)	3.827	-	413.10	353.10	9
5	15-AcetylDeoxynivarenol (15-AcDON)	5.582	+	339.10	261.10	-12
6	3-AcetylDeoxynivarenol (3-AcDON)	5.751	+	339.10	231.10	-14
7	Aflatoxin G2 (AF G2)	7.197	+	331.10	245.10	-30
8	Diacetoxy- scirpenol (DAS)	7.480	+	384.20	307.10	-13
9	Aflatoxin G1 (AF G1)	7.433	+	329.10	243.10	-27
10	Aflatoxin B2 (AF B2)	7.669	+	315.10	259.10	-30
11	Fumonisin B1 (FB1)	7.804	+	722.40	334.10	-42
12	Aflatoxin B1 (AF B1)	7.904	+	313.10	241.10	-39
13	HT-2 toxin (HT-2)	8.060	+	442.20	263.10	-13
14	Fumonisin B3 (FB3)	8.107	+	706.40	336.10	-35
15	Fumonisin B2 (FB2)	8.475	+	706.40	336.10	-39
16	T2-toxin (T-2)	8.705	+	484.30	185.10	-23
17	Ochratoxin A (OTA)	8.987	+	404.10	239.10	-24
18	Zearalenone (ZEN)	9.532	-	317.10	131.10	30

Table 1 MRM transitions of Mycotoxins

Sample preparation

Analytical samples were prepared through the extraction protocol of MycoSpin[™]400 (Romer Labs), which is a very convenient method without evaporator nor nitrogen purge procedures. The operation of MycoSpin[™]400 was completed within 5 minutes.



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2.5 g Wheat / Corn (add 100 ng each standard)

Add 10 mL 50% Acetonitrile Solution (or 85% Acetonitrile Solution)

Shake for 90 minutes

Centrifuge at 3200 rpm., 10 minutes

1 mL of the supernatant, add 50 µL acetic acid

Transfer 750 µL of the solution to the MycoSpin[™] column

Cap MycoSpin[™] column and vortex for 1 minute

Break bottom tip off of MycoSpin[™]

Centrifuge at 10000 rpm., 30 seconds

Obtain 350 µL of purified extract

Each 45 μL of purified extract with 5 μL standard solution (0, 100 ,200, 500 ng/mL)

Inject onto the LC/MS/MS system

Figure 2 Protocol of sample preparation



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Result

Analysis of Standard Solution

Figure 3 shows MRM chromatograms of the 18 mycotoxin standards (each 10 ng/mL).

At first, we evaluated the solvent for better recovery of the mycotoxin from MycoSpin[™] column.

In comparison, with 85% acetonitrile aqueous solution, better recovery of fumonisins was obtained with 50% acetonitrile aqueous solution (Fig.4). Thus, we decided to use 50% acetonitrile aqueous solution for the extraction solvent.



Figure 3 MRM chromatograms of the 18 mycotoxin standards (each 10 ng/mL).



Figure 4 Recovery (%) of the mycotoxin standard from MycoSpin[™] column. (Each 10 ng/mL standard mixture was applied on the column.)

Evaluation of the matrix effect

Figure 5 shows recovery (%) of the mycotoxin standards in the four kinds of extraction as wheat, corn powder, peanut powder, and almond powder. MycoSpin[™] protocol was convenient in short timescale. However, even after the clean-up, many matrix compounds remained and was affected.(Fig. 5). Although under this situation, it usually requires each labeled internal standard for target compound, we tried to investigate the quantify by the standard additive method.

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Quantitative analysis

The results of quantitative analysis from wheat and corn powder using the sample preparation protocol (2.2) is summarized in table 2 through using the standard additive method instead of the internal standard method. The results indicates that the standard addition calibration method could help correct and improve the recovery rate even under the influence of the matrix effect. Using this method, only a small amount of mycotoxins were detected in corn powder below the regulation value.

	Wheat			Corn			
	Result (mg/Kg)	Recovery (%)	%RSD (n=2)	Result (mg/Kg)	Recovery (%)	%RSD (n=2)	
NIV	N.D.	93	0.86	N.D.	134	2.00	
PAT	0.0350	94	5.36	N.D.	79	15.60	
DON	0.0148	128	6.34	0.1376	114	10.66	
FUX	N.D.	101	0.30	N.D.	149	0.02	
15-AcDON	N.D.	119	10.73	0.0213	114	14.30	
3-AcDON	N.D.	106	7.64	N.D.	117	8.26	
AF G2	N.D.	104	4.27	N.D.	115	1.80	
DAS	N.D.	118	4.24	N.D.	137	2.25	
AF G1	N.D.	101	3.45	N.D.	119	1.11	
AF B2	N.D.	110	0.07	N.D.	125	5.95	
FB1	N.D.	60	18.33	0.0407	94	15.16	
AF B1	N.D.	104	2.06	0.0011	124	2.32	
HT-2	N.D.	107	9.21	0.0008	132	3.91	
FB3	N.D.	70	19.66	0.0078	83	7.19	
FB2	N.D.	66	6.23	0.0094	79	5.80	
T-2	N.D.	105	4.79	0.0005	119	1.80	
ΟΤΑ	N.D.	97	12.29	N.D.	109	4.38	
ZEN	N.D.	97	0.90	0.0120	103	3.37	

Table 2	The results	of wheat	and	corn	nowder
TUDIC Z	THE TESUILS	or writeat	anu	COIII	powuci

A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry



Figure 6 Recovery (%) of the mycotoxin standards from wheat and corn powder (Each 100 ng standard was spiked)



<u>Corn</u>

Figure 7 Chromatograms and calibration curves of the corn extraction (DON and FB3).



A rapid screening method of mycotoxins in grains by liquid chromatograph tandem mass spectrometry

Conclusions

- A rapid screening method for mycotoxins had been established.
- The LC/MS/MS method package for mycotoxin (Shimadzu Corporation, Japan) is useful tools for this type of analysis.
- The extraction solvent to improve the recovery rate of fumonisins was optimized.
- We investigated the standard addition method in order to compensate for the effect of the matrix.
- We plan to continue to evaluate multi-function columns or ion exchange columns for further improvement of the clean-up.





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ASMS 2018

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Introduction

There has been an increasing awareness of the presence of polyfluorinated alkyl substances (PFAS) in water. A simple and robust method with quick turn-around time to determine these compounds is essential to providing accurate responses in a timely manner. In the method presented here, direct injection without solid-phase extraction (SPE) allows us to maximize throughput and to minimize background caused by the sample preparation step. We used liquid chromatography with triple-quadrupole mass spectrometry (LC–MS/MS) to analyze the fluorotelomer and unsaturated fluorotelomer acids included in ASTM International method 7968/7979. Fluorotelomer acids are observed as [M-H]- and [M-HF-H]-. Since the loss of HF in the fluorotelomer acids results in an ion with the same formula as the unsaturated fluorotelomer acids, and these two classes of compounds showed very similar retention times, we reduced the electrospray ionization (ESI) heater temperature to reduce HF loss and minimize false identifications.

Experimental

The method was developed to study 30 PFAS compounds and 19 surrogates, for a total of 49 compounds. The target list includes new PFAS of concern, including

- Perfluoroalkane sulfonic acids (PFASs),
- Perfluoroalkyl carboxylic acids (PFCAs),
- Perfluorooctanesulfonic acids (PFOSs),
- Perfluorooctane sulfonamide acetic acids (FOSAAs),
- Fluorotelomer alcohols (FTOHs),
- Fluorotelomer acids (FTAs),
- Unsaturated Fluorotelomer acids (FTUAs)
- Fluorotelomer sulfonic acids (FTSs)

Parameter	Value					
LCMS Analytical Column Solvent Delay Column Column Oven Temperature Injection Volume Mobile Phase Gradient Flow rate	 Shimadzu LCMS-80 Shim-pack GIST Phe Shim-pack XR-ODS 40 °C 10 μL Reagent A = 20 mn Reagent B = Acetor 0.4 mL/minute 	Shimadzu LCMS-8060 Shim-pack GIST Phenyl-Hexyl (2.1 mm ID. x 100 mm L., 3 μm) Shim-pack XR-ODS (3 mm ID. x 50 mm L., 2,2 μm) 40 °C 10 μL Reagent A = 20 mmol Ammonium Acetate in LCMS grade water Reagent B = Acetonitrile 0.4 mL/ minute				
Gradient	Time (minutes)	% Reagent B				
	0	10				
	1	10				
	3	30				
	14	65				
	14.1	98				
	17.1	10				
	20	10				
Run time Nebulizing gas flow Heating gas flow Interface temperature Desolvation Line temperature Heat Block temperature Drying gas flow Acquisition cycle time Total MRMs	 20 minutes 3 L / minute 15 L /minute 300 °C 100 °C 200 °C 5 L / minute 20 minutes 74 					

Table 1: Instrument conditions

Instrument Operating Conditions

The analysis of PFAS was performed using a Shimadzu Nexera X2 SIL-30AC autosampler and a LCMS-8060 triple quadrupole mass spectrometer. Table II lists the compounds tested, the MRM transitions, and retention times.

Chromatography was adjusted to obtain maximum resolution between peaks in the shortest time possible. This approach minimizes the coelution of isomers. The total run time of 20 minutes includes a final wash out with concentrated acetonitrile to remove contamination. Fluorotelomer acids, observed as [M-H]- and [M-HF-H]-, can result in an ion with the same formula as the unsaturated fluorotelomer acid. Even under optimized chromatography, these compounds have near identical retention times. The lower desolvation line temperature reduces HF loss and minimizes false identification. The modified mobile phase reagents compared to the ASTM method do not use reagent C containing 400 mM ammonium acetate in 95:5 acetonitrile–water. The new reagents are easier to prepare, and obtain equivalent, if not better, peak shape and sensitivity. Reagents commonly contain PFAS contamination. Mobile phase passes through the delay column positioned between the pump and the injection valve shifting the retention time of contamination peaks and ensuring that they are not coeluted with the analyte.

		Retention Time		Calibration Range % Recovery % RSD (
Component	Transition (m/z)	(minutes)	MDL (ng/L)	(ng/L)	(20 ng/L)	ng/L)
PFBA	212.90 > 169.00	3.092	4.1	5 - 200	112	6.6
MPFBA	217.00 > 172.10	3.095	5.0	5 - 200	86	10.2
PFPeA	263.00 > 219.00	4.753	0.9	5 - 200	101	2.9
M5PFPeA	268.00 > 223.00	4.754	0.6	5 - 200	100	1.4
4-2 FTS	327.00 > 307.00	5.347	1.7	5 - 200	102	3.2
M4-2 FTS	329.00 > 309.00	5.347	1.2	5 - 200	92	3.0
PFHxA	312.90 > 269.00	5.652	1.3	5 - 200	101	3.9
M4PFHxA	317.90 > 273.00	5.653	1.1	5 - 200	101	2.3
PFBS	298.90 > 80.10	5.824	1.5	5 - 200	101	10.4
M3PFBS	301.90 > 80.10	5.825	1.1	5 - 200	98	4.1
FHUEA	357.00 > 293.00	6.210	2.6	5 - 200	108	5.6
FHEA	376.90 > 293.00	6.225	32.5	100 - 4000	99*	5.3*
PFHpA	362.90 > 319.00	6.642	1.4	5 - 200	103	4.2
M4PFHpA	366.90 > 322.00	6.643	0.7	5 - 200	99	2.2
PFPeS	348.90 > 79.90	6.992	1.1	5 - 200	100	4.7
6-2 FTS	427.00 > 406.90	7.194	2.5	5 - 200	113	7.3
M6-2 FTS	429.00 > 408.90	7.195	1.8	5 - 200	101	3.8
PFOA	412.90 > 369.00	7.635	5.1	5 - 200	96	5.7
M8PFOA	420.90 > 376.00	7.636	0.7	5 - 200	99	2.0
FhpPA	440.90 > 337.00	7.965	9.4	5 - 200	84	28
FOEA	476.90 > 393.00	8.066	48.3	100 - 4000	103*	5.5*
FOUEA	456.90 > 392.90	8.076	1.6	5 - 200	104	3.6
PFHxS	398.90 > 80.10	8.094	1.5	5 - 200	96	9.8
M3PFHxS	401.90 > 80.10	8.102	1.7	5 - 200	100	3.4
PFNA	462.90 > 418.90	8.588	1.7	5 - 200	104	6.3
M9PFNA	471.90 > 426.90	8.589	1.6	5 - 200	103	4.2
8-2 FTS	526.90 > 506.90	9.011	3.2	5 - 200	90	25.2
M8-2 FTS	528.90 > 508.90	9.012	1.8	5 - 200	89	12.3
PFHpS	448.90 > 79.90	9.131	1.6	5 - 200	99	8.2
N-MeFOSAA	569.90 > 419.00	9.410	3.6	5 - 200	101	15.0
d3M N-MeFOSAA	572.90 > 419.00	9.420	5.4	5 - 200	102	9.6
PFDA	512.90 > 468.90	9.486	2.3	5 - 200	108	5.7
M6PFDA	518.90 > 473.90	9.487	1.1	5 - 200	98	4.7
FDEA	576.90 > 493.00	9.762	35.5	100 - 4000	89*	7.0*
N-EtFOSAA	583.90 > 419.00	9.767	5.3	5 - 200	118	16.3
M N-EtFOSAA	588.90 > 419.00	9.768	4.2	5 - 200	130	13.0
PFOS	498.90 > 80.10	10.076	3.0	5 - 200	105	7.8
M8PHOS	506.90 > 80.10	10.077	1.5	5 - 200	107	5.0
PFUdA	562.90 > 519.00	10.330	2.9	5 - 200	100	11.6
M7PFUdA	569.90 > 525.00	10.331	1.5	5 - 200	103	4.6
PFNS	548.90 > 79.90	10.946	1.3	5 - 200	112	7.3
PFDoA	612.90 > 568.90	11.122	2.2	5 - 200	98	6.5
MPFDoA	614.90 > 569.90	11.123	0.8	5 - 200	100	4.1
FOSA	497.90 > 77.90	11.586	0.6	5 - 200	88	6.8
M8FOSA	505.90 > 77.90	11.588	1.6	5 - 200	94	5.4
PFDS	598.90 > 79.90	11.760	2.1	5 - 200	108	5.4
PFTrDA	662.90 > 618.90	11.877	1.1	5 - 200	99	4.6
PFTeDA	712.90 > 668.90	12.586	1.1	5 - 200	92	3.5
M2PFTeDA	714.90 > 669.90	12.587	0.7	5 - 200	92	4.3

Table 2: MRM transitions, retention times, calibration range, MDL, and accuracy and precision (n=8).

* FHEA, FOEA, FDEA (spike :mdl at100 ng/L; p&a at 400 ng/L)

All compound parameters, including precursor ion, product ion, and collision energies, were optimized. There are at least two multiple reaction monitoring (MRM) transitions for most of the analytes. The plots shown in Figure 1 demonstrate that the 50% methanol in water solution used in the ASTM methods sufficiently dissolves the PFAS and keeps them in solution. Standard were purchased from Wellington Laboratories.



Figure 1: Shelf life study showing recovery for the 50% methanol solution in (a) glass vials and (b) polypropylene vials.

Calibration and Standardization

Nine calibration standards containing the PFAS compounds were prepared from purchased stock standard solutions. Target analytes and surrogate spike solutions were combined to ensure consistency. Each standard was diluted with a 50:50 methanol–water mixture containing 0.1% acetic acid.

Next, 5 mL aliquots of each calibration standard were diluted with 50:50 methanol–water containing 0.1% acetic acid. Solutions were transferred to 2-mL amber glass LC vials. Calibration standards may be used multiple times since glass vials with removable caps are being used.

Calibration standards are not filtered.

Inject each standard and obtain its chromatogram. Figure 2 shows a chromatogram of 49 PFAS, which includes PFOA, PFOS, and related compounds. The concentration of each compound was determined using the external calibration technique with the nine point calibration curves developed earlier. Concentrations were calculated using LabSolutions software to generate a linear regression. The point of origin is excluded and a fit weighting of 1/x is used to give more emphasis to the lower concentrations.



Figure 2: Direct Inject Method ASTM D7968/D7979 - Standard Test Method for Determination of PFAS Compounds by LCMS–8060 : Chromatogram at 100 ng/L Using Optimized Conditions

Sample Extraction and Analysis

A surrogate spiking solution containing each isotopically labeled PFAS was added to all samples, including method blanks, duplicates, laboratory control samples, matrix spikes, and reporting limit checks. The results obtained for the surrogate recoveries fell within the 70–130 % method criteria.

Next, 5 mL of sample was collected in a 15-mL centrifuge vial. Also, 5 mL of reagent water for a blank was prepared

as well as an LCS containing known amounts of PFAS and a reporting limit check containing each PFAS at the lowest calibration concentration. Then 40 μ L of the surrogate spiking solution and 5 mL of methanol were added to each sample, blank, and control sample. Next, the sample was shaken and mixed on a vortex mixer for 2 minutes. 10 μ L of acetic acid was added, and it was transferred to an autosampler vial for analysis.

Instrument and Method Performance

Experiments were made to verify linearity, accuracy, precision, and method detection limits (MDL). A series of nine initial calibration standards ranging from 5 to 200 ng/L (100ng/L – 4000ng/L for FTA mix) were prepared. The

linearity of the curves were evaluated using 1/x weighting and ignoring the origin. Figure 3 shows the calibration curves for the calibration range. MDLs and accuracy & precision data are shown in Table 2.
Excellence in Science Improved Analysis of Polyfluorinated Alkyl Substances (PFAS) in Environmental Samples Using Optimized ASTM Method 7968/7979



Figure 3: Calibration Curves of select PFAS compounds obtained by direct injection 10 uL of PFAS standard mixtures using LCMS-8060

Conclusion

This poster evaluated ASTM D7968/D7979 for the "direct" analysis of 30 PFAS and 19 mass-labeled surrogates. Instead of solid-phase extraction, as is used by the EPA drinking water Method 537, the ASTM methods specify solubilizing the PFAS in a methanol–water mix, filtering if necessary, and injecting the sample into an LC–MS/MS system. 19 isotopically labeled surrogates were used in this study. The results obtained for the surrogate recoveries were well within the QC limits. The results obtained for labeled surrogates were corrections for the native compounds.

The method simplifies extraction and analysis, eliminating sources of contamination and potential loss of analyte. With the exception of a few method-allowed modifications, we ran the method exactly as written. The method appears to be rugged and of sufficient accuracy and precision to analyze the matrices listed in the scope. The new methods takes the advantage of new technology that minimizes labor while still obtaining data suitable for its intended purpose.

Improved Analysis of Polyfluorinated Alkyl Substances (PFAS) in Environmental Samples Using Optimized ASTM Method 7968/7979

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Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

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PO-CON1807E

Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

Introduction

There are various substances that can threaten the food safety, such as pesticides, mycotoxins. LC-MS/MS analysis is a prevailing technique for the detection of these substances in food. Mycotoxins are especially frequent contaminants of agricultural products, and brewers are concerned that they can give serious damages to consumers, for example liver cancer, nephritis, pulmonary edema and so on. This is the reason why most countries have adopted regulations to limit exposure to mycotoxins, while the regulated mycotoxins and value differ with countries. The toxicity and potential health hazards induced by mycotoxins demand the need for sensitive, robust analytical methodologies. This research provides a LC-MS/MS system for quantitative screening of mycotoxins and includes a multi-mycotoxin sample preparation column to cover worldwide regulations. Although LC-MS/MS is a highly sensitive analytical technique, the problem of carryover occurs frequently. Metal-free column and multi-rinse mode were performed for reduction of carryover.



Fig 1. LC-MS/MS system (Nexera X2+LCMS-8060, Shimadzu Corporation.)

Methods and Pretreatment

19 mycotoxins (Nivalenol, Patulin,

Doxynivalenol-3-Glucoside, Deoxynivalenol, Fusarenon-X, Neosoraniol, 3-Acetyl-Deoxynivalenol,

15-Acetyl-Deoxynivalenol, Aflatoxin B1, B2, G1, G2, Diacetoxyscirpenol, Fumonisin B1, B2, B3, T-2 toxin, Ochratoxin A, Zearalenone) were used for evaluation of matrix effect and recovery rates in wheat. These mycotoxins were diluted with ACN at 5 ng/mL. Ground wheat flour samples were mixed with water/acetonitrile. After filteration, extracts were diluted with aqueous acetic acid solution and mixed with mycotoxins at 5 ng/mL. The solution were loaded to into the spin purification column (MycoSpin[™]400, Romer Lab) and analyzed using a triple quadrupole mass spectrometer (LCMS-8060, Shimadzu Corp.).

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Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

[LC] Nexera[™] X2 System : Mastro[™] PFP2 (Shimadzu GLC Ltd) 2.1 mml.D.×150 mmL., 3 µm Analytical Column Solvent A : 10 mmol/L ammonium acetate Solvent B : 2% acetic acid in methanol Gradient Program Time (min) %В 1.00 40 1.50 40 1.51 50 5.50 50 5.51 65 9.50 70 95 9.51 13.00 95 13.01 20 15.00 STOP Flow Rate : 0.4 mL/min : 40 °C Column Temp [MS] LCMS-8060 Ionization : ESI (Positive/Negative) Nebulizer Gas : 2 L/min : 300 °C Interface temperature : 250 °C Desolvation Line Heat Block temperature : 500 °C Heating Gas : 10 L/min Drying Gas : 10 L/min



< Rinse Program >

R0	10 mmol/L ammonium acetate
R1	10 mmol/L sodium citrate
R2, R3	1% Formic acid + water/MeOH/ACN/IPA= 1/1/1/1 (v/v)

Table 1. LC and MS conditions

Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

		Table 2. MRM transi	itions for mycotoxins	:Pos	itive itive :Negative
No.	Mycotoxins	RT (min)	Polarity	transition	CE
1	NIV	1.88	-	371.10>281.10	16
2	PAT	2.22	-	153.00>109.00	11
3	D3G	2.30	-	517.20>427.20	21
4	DON	2.40	-	355.10>265.10	15
5	FUX	2.94	-	413.10>353.10	9
6	NEO	3.05	+	400.20>305.10	-12
7	15-ADON	3.74	+	339.10>261.10	-11
8	3-ADON	3.86	+	339.10>231.10	-14
9	AF G2	4.87	+	331.10>245.10	-31
10	AF G1	5.55	+	329.10>243.10	-30
11	DAS	5.78	+	384.20>307.10	-13
12	AF B2	6.22	+	315.10>259.10	-30
13	AF B1	6.96	+	313.10>241.10	-39
14	FB1	7.37	+	722.40>334.10	-43
15	FB3	8.08	+	706.40>336.10	-38
16	T-2	8.71	+	484.30>185.10	-20
17	FB2	8.97	+	706.40>336.10	-39
18	OTA	9.73	+	404.10>239.10	-24
19	ZEN	10.8	-	317.10>130.10	35

Details of sample preparation

1. Mix a ground wheat flour sample (50.0 g) with 100.0 mL of water/acetonitrile (15/85), and shake for

30 minutes



4. Load 1.0 mL of Solution A into the spin purification column and mix using vortex mixer for 1 minute while capped



2. Filter the supernatant using glass-fiber filter paper (pore size < 0.7 um)



5. Remove the bottom tip of the column and centrifugation for 2 minutes at 10,000 rpm



3. Add 500.0 µL of acetic acid to the filtrate (10.0 mL): Solution A



6. Transfer the supernatant into a vial then serve to the sample



Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

Results

Typical MS chromatogram for mycotoxins in ACN are shown in Fig. 2. An LC-MS/MS method was developed that achieved good separation and sensitivity for the detection of all mycotoxins without separating method for its polarity. Autosampler rinsing capabilities and metal free column were used to minimize the carryover of the fumonisins. Matrix effect was calculated by the peak area of mycotoxins (5 ng/mL) in ACN and post spiked samples. Recovery rate was calculated by the peak area of post spiked samples (5 ng/mL) and pre spiked samples (5 ng/mL) which is shown in Table 3. NIV, DON, AF B1, T-2, ZEN were influenced wheat extractions which dramatically decrease the ionization efficiency of the mycotoxins. Recovery rate of the NIV, D3G, DON, T-2, ZEN were also insufficient. Therefore, internal standards are required for achieving accurate quantitative results.



Fig 2. Typical MS chromatogram for mycotoxins mixture (50 ppb)

No.	Mycotoxins	Matrix Effect (%)	Recovery Rate (%)
1	NIV	35.0	156.6
2	PAT	71.6	115.8
3	D3G	34.4	166.8
4	DON	47.3	143.9
5	FUX	81.8	99.3
6	NEO	74.2	95.1
7	15-ADON	72.6	87.7
8	3-ADON	87.6	78.6
9	AF G2	78.3	70.7
10	AF G1	85.6	65.5

Table 3. Matrix effect and recovery rate of the mycotoxins in wheat matrix (5 ppb)

No.	Mycotoxins	Matrix Effect (%)	Recovery Rate (%)
11	DAS	84.6	76.4
12	AF B2	80.6	75.0
13	AF B1	33.8	65.3
14	FB1	73.6	128.6
15	FB3	71.5	120.1
16	T-2	51.8	52.0
17	FB2	68.6	122.2
18	ΟΤΑ	42.3	111.5
19	ZEN	40.4	28.0

Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS



Fig 3. MS chromatograms of NIV, D3G, DON, T-2, ZEN which are pre-spiked in and post-spiked in wheat extraction at 5 ng/mL

Sensitive multi-mycotoxins analysis with a single sample preparation by LC-MS/MS

Conclusion

- This LC-MS/MS method and one step sample preparation measured various types of mycotoxins which spiked in wheat matrix.
- Sensitivity of some mycotoxins were decreased because of matrix effect.
- For accurate quantitative measurement, internal standard is necessary

Reference

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Glyphosate and Aminomethylphosphonic acid (AMPA) analysis in plants using LC-MS/MS

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PO-CON1817E



Glyphosate and Aminomethylphosphonic acid (AMPA) analysis in plants using LC-MS/MS

Novel Aspects

A Glyphosate and AMPA quantitative analysis method was developed on a triple quadrupole mass spectrometer for plant analysis without derivatization.

Introduction

Glyphosate (Gly) is one of the most widely used herbicides in the world. It acts as an enzyme inhibitor to stunt the growth and vitality of plants. The potential health effects on humans exposed to glyphosate have been a concern. Therefore, methods that analyze glyphosate and

aminomethylphosphonic acid (AMPA), the main degradant of glyphosate, are highly desired. An LCMS glyphosate and AMPA quantitation method was therefore developed using a Shimadzu triple quadrupole mass spectrometer for research use.

Method – Mass Spectrometry

A LCMS-8060 triple quadrupole mass spectrometer was used to analyze glyphosate and AMPA in ESI negative mode. The MRM transitions (Table 1) were determined and optimized using LabSolutions software. Instrument conditions are listed in Table 2.

Analyte	MRM	Q1 Bias (V)	Collision Energy (V)	Q3 Bias (V)
Churchasata	168.10>62.90	17	23	15
Giyphosate	168.1>78.95	17	37	19
	110.00>79.00	21	27	27
AIVIPA	110.00>63.00	21	23	13

Table 1	MRM	transitions	for	Glv	and $\Delta MP\Delta$
Table L.	1011/101	LI ALISILIO IIS	101	UIY	anu Aivii A

Table 2. MRM transitions for Gly and AMPA

Heating gas	:	20 L/min
Drying gas	:	3 L/min
Nebulizing gas	:	3 L/min
Interface temperature	:	350 °C
Heat block temperature	:	400 °C
DL temperature	:	250 °C

Glyphosate and Aminomethylphosphonic acid (AMPA) analysis in plants using LC-MS/MS

Method- Reversed Phase Liquid Chromatography

A Bio-Rad Micro-Guard Cation H+ Cartridge (30 x 4.6 mm) was used for the separation. Mobile phase was composed of A: 0.1% FA in H2O, B: ACN, and C: 0.2% phosphoric acid in H2O. A divert valve was used for mobile phase C to wash the column without contacting

the mass spectrometer. Chromatography details can be found in Table 3. In effort to minimize glyphosate adsorption, post autosampler injection port tubing was changed to peek.

Time (min)	Command	Value
0	Valve	To Waste
0	В%	20%
0	A+B flow	0.5 mL/min
0.5	Valve	To MS
1	В%	20%
2.5	В%	0%
4	A+B flow	1 mL/min
6.9	Valve	To waste
7	A+B flow	0.5 mL/min
7	C flow	0.5 mL/min
10	В%	0%
10.09	C flow	0.5 mL/min
10.1	В%	20%
10.1	C flow	0 mL/min
12.6	Controller	Stop

Table	3.	Chromatography	method

Solid standards of glyphosate and AMPA were acquired from Sigma-Aldrich. Dilution and extraction solvent was 0.1% formic acid. Six point calibration curves were created for both AMPA and glyphosate to ensure instrument sensitivity from 0.1 ppb to 10 ppb.

Barley and lemongrass were chosen as sample matrices. They were ground to increase extraction surface area. 1 gram of plant samples were put in to each extraction vial along with 10 mL of 0.1% FA. Glyphosate and AMPA in various concentrations were spiked in the sample to create matrix matched external calibration curve for lemongrass and standard addition for barley. Samples were filtered and directly inject (20 μ L) onto the system for analysis.

Results- Standards in neat

Decent chromatographic peaks were obtained in both LLOQ and ULOQ for both Gly and AMPA. Chromatograms of blank, LLOQ (0.1 ppb), and 10 ppb were shown in Figure 1.



Figure 1. Chromatograms of Gly and AMPA in blank, at 0.1 ppb, and 10 ppb

Glyphosate and Aminomethylphosphonic acid (AMPA) analysis in plants using LC-MS/MS

Calibration curves of Gly and AMPA were obtained (triplicates) in neat standard from 0.1 ppb to 10 ppb. (Figure 2). Acceptable linearity was obtained with R^2 =0.9999 for Gly and R^2 =0.9997 for AMPA.



Figure 2. Calibration curves of Gly and AMPA in neat standards

Results- Glyphosate and AMPA in plant matrix

USDA approved organic lemongrass was used as a low glyphosate matrix to generate matrix matched calibration curve. Due to the complexity of matrix and the lack of sample cleaning steps for this initial study, source saturation was observed at 10 ppb. A linear curve was still maintained from 0.1 ppb to 5 ppb. Linearity of R^2 = 0.993 for glyphosate and R^2 =0.994 for AMPA were obtained. This result shows the LCMS-8060 has the ability to perform matrix matched calibration even at low level (0.1 ppb).



Figure 3. Lemongrass matrix matched calibration for Gly and AMPA

Glyphosate and Aminomethylphosphonic acid (AMPA) analysis in plants using LC-MS/MS

Barley was chosen to represents matrices already containing glyphosate. For sample matrices containing Gly and AMPA, standard addition can be used to evaluate the levels. Increments of 2 ppb were added to the barley samples. Linear calibration curves were observed with R^2 =0.997 for Gly and R^2 =0.999 for AMPA. Gly in Barley was found to be 1.084 ppb and AMPA as 0.235 ppb.



Figure 4. Barley standard addition analysis for Gly and AMPA

Conclusion

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MRM transitions for glyphosate and AMPA were identified and optimized using the LCMS-8060 in ESI negative mode. A Bio-Rad Micro-Guard Cation H+ Cartridge was used to obtain separation. Good linearities were obtained from 0.1 ppb to 10 ppb for both glyphosate and AMPA in neat standard. Glyphosate and AMPA were spiked into plant matrices to show feasibility of future research and quality control applications. This analysis demonstrated the capability of LCMS-8060 in quantifying glyphosate and AMPA in various matrices. Matrix matched calibration curves can be used to quantify Gly and AMPA for samples that do not contain (or with trace amount of) glyphosate. For matrices that contain glyphosate at low levels, standard addition can be used to assess the amount of Gly and AMPA present. Furthermore, Shimadzu LCMS-8060 has a high scanning speed that allows additional herbicides or other compounds of interested to be added to this method for simultaneous analysis.



Glyphosate and Aminomethylphosphonic acid (AMPA) analysis in plants using LC-MS/MS

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PO-CON1801E

Overview

- nSMOL proteolysis (Ref. 1-7) has been reported as the novel technology to standardize the sample preparation workflow and to improve the sensitivity of mass spectrometric assay of therapeutic monoclonal antibodies in human serum or plasma.
- We developed highly sensitive bioanalysis method to achieve low ng/ml sensitivity of <u>Trastuzumab in human plasma</u> with the combination of the nSMOL proteolysis and the newly developed Nexera Mikros system.

Introduction

Mass spectrometric (LC-MS/MS) determination of therapeutic monoclonal antibodies in serum or plasma is increasingly used for pharmacokinetic studies in the preclinical, clinical, and therapeutic phases. One major advantage of this approach over conventional ligand binding assay (LBA) is high specificity for the target antibodies that can be achieved by selecting tryptic peptides derived from the complementarity-determining region (CDR) as the antibody signature peptide and subjecting it to LC-MS/MS quantitation. Moreover, LC-MS/MS approach requires much less assay developmental work than LBA, which completes within days rather than several months (Table 1). Our recent advancement of sample preparation strategy, namely **nano-surface and molecular-orientation limited (nSMOL) proteolysis** (Fig 1), have further simplified the method development process. nSMOL proteolysis yields extremely clean CDR peptide mixture thereby alleviating the need to address interference from biological matrix.



Figure 1. The working principle of Fab-selective reaction of IgG by nSMOL proteolysis.



Despite various advantages, one drawback of LC-MS/MS assays is that the level of sensitivity depends on the mass spectrometric response (efficiency of ionization and fragmentation) of the signature peptide, which is essentially unpredictable and uncontrollable. For example, recently reported bioanalyses of therapeutic mAb ^[ref.1-8] showed varying LLOQ levels ranging from 0.06-0.58 µg/mL in plasma. Currently there is risk that a newly-developed

assay might not fulfil the sensitivity requirement for pre-clinical trials.

Here we aim to overcome this issue by implementation of a robust microflow LC-MS/MS system to measure signature peptides at increased sensitivity than conventional semi-microflow systems, while maintaining the same level of robustness, analysis turnaround time and ease of system configuration.

	nSMOL+LCMS	LBA
Ab for Collection/Detection	Not needed	6++ months to develop
Cross-reactivity test	Not needed	Mandatory and tricky
Pre-validation	1 - 3 days	2 - 3 weeks
Full validation	3 - 4 w	3 - 4 w
Sample prep.	3 - 5 h	2 - 4 h
Data features	Highly selective and reliable, wide dynamic range, easy to multiplex, independent of antibodies	Highly dependent on quality of detection Ab.

		c		
Table 1.	Comparison	of nSMOL-	⊦LCMS a	nd LBA

Development of LCMS bioanalysis in combination with nSMOL proteolysis is much faster, and can dramatically accelerate the total R&D workflow period of biologics by alleviating the bottleneck that typically occur when entering the preclinical and clinical phase.

Methods

Sample and Pretreatment

Pooled <u>human plasma</u> sample was purchased from Kohjin Bio (Saitama, Japan). Trastuzumab was spiked at various concentrations (0, 0.00763, 0.0153, 0.0305, 0.0610, 0.122, 0.0244, 0.488, 0.977, 1.95, 3.91, 7.81, 15.6, 31.3, 62.5 µg/mL) for calibration curve and independently at four concentration set for QC samples. QC set 1 and 2 were prepared and ran on two separate days. Spiked and blank plasma samples were pretreated after keeping at -80°C for 24 h or longer using the nSMOL[™] Antibody BA Kit (Shimadzu Corporation, Japan) in accordance with the instruction manual.



Figure 2 Standard protocols of nSMOL workflow.

System Configuration

The newly developed micro-LC system by Shimadzu Corporation, Nexera Mikros (Fig. 2), consists of

- (1) LC-Mikros, the 'micro to semi-micro flow' pump with 1-500 µL/min range and 800 bar pressure tolerance,
- (2) CTO-Mikros, the new-design column oven that couples any analytical column directly to the ion source by the UF-Link[™] technology (Fig. 3) to minimize post-column void volume,
- (1) Micro ESI-8060, the camera-equipped and X-Y adjustable ESI ion source for maximum ionization efficiency and usability.



Figure 3. Nexera Mikros system, equipped with additional modular pumps for Trap & Elute

[LC] Nexera Mikros	
Analytical Column	: Shim-Pack MC C18 (0.175 mm I.D. x 50 mm L.)
Trap column	: CERI L-column2 Micro (0.3 mm I.D. x 50 mm L.)
Oven Temp.	: (Analytical) 50 deg.C, (Trap) 40 deg.C
Solvent A	: 0.1% Formic Acid in water
Solvent B	: 0.1% Formic Acid in Acetonitrile
Gradient	: 0.00-0.50 min 5%B \rightarrow 4.50 min 22%B \rightarrow 4.51 min 95%B \rightarrow 5.50 min 95%B
	\rightarrow 5.60 min 5%B \rightarrow 11.00 min STOP
Analytical flow Rate	: 4 µL/min
Inj. Volume	: 10 µL
[MS] LCMS-8060 wi	th Micro ESI-8060
[MS] LCMS-8060 wi	th Micro ESI-8060 : ESI Positive
[MS] LCMS-8060 wi lonization DL Temp.	th Micro ESI-8060 : ESI Positive : 250 deg.C
[MS] LCMS-8060 wi lonization DL Temp. Heat Block Temp.	th Micro ESI-8060 : ESI Positive : 250 deg.C : 400 deg.C
 [MS] LCMS-8060 wi lonization DL Temp. Heat Block Temp. ESI Temp.	th Micro ESI-8060 : ESI Positive : 250 deg.C : 400 deg.C : 100 deg.C
[MS] LCMS-8060 wi lonization DL Temp. Heat Block Temp. ESI Temp. Nebulizer Gas	th Micro ESI-8060 : ESI Positive : 250 deg.C : 400 deg.C : 100 deg.C : 2 L/min.
[MS] LCMS-8060 wi lonization DL Temp. Heat Block Temp. ESI Temp. Nebulizer Gas Drying Gas	th Micro ESI-8060 : ESI Positive : 250 deg.C : 400 deg.C : 100 deg.C : 2 L/min. : OFF
[MS] LCMS-8060 wi lonization DL Temp. Heat Block Temp. ESI Temp. Nebulizer Gas Drying Gas Heating Gas	th Micro ESI-8060 : ESI Positive : 250 deg.C : 400 deg.C : 100 deg.C : 2 L/min. : OFF : 3 L/min.





Results

Calibration curve in plasma matrix showed good linear response in the range 7.6 ng/mL to 62.5 μ g/mL (Fig 4). Compared to the LLOQ of 0.06 μ g/mL as previously reported for Trastuzumab (also using nSMOL proteolysis and LCMS-8060), switching to the Nexera Mikros system contributed to sensitivity improvement by nearly one order

of magnitude. Notably, the chromatographic peak shape and elution band was equivalent to UHPLC system with average W0.5h of 3.7 seconds, most likely due to near-zero post-column dead volume achieved by the UF-Link.

Quantitation range in human plasma : 0.00763 to 62.5 μ g/mL, Averaged accuracy : 101 %



Peptide	MRM transition	Objectives
	542.8>404.7 (y7++)	Quantifier
IYPTNGYTR	542.8>808.4 (y7+)	Qualifier
	542.8>610.3 (y5+)	Qualifier
Peptide	MRM transition	Objectives
	512.1>292.3 (b3+)	Quantifier
P ₁₄ R (IS)	512.1>389.3 (b4+)	Qualifier
	512.1>660.4 (b6+)	Qualifier
L		·

Figure 5. Calibration curve for Trastuzumab bioanalysis.



Figure 6. Representative MRM chromatograms

As part of assay validation, intra-day repeatability (%RSD) was evaluated using two sets of QC samples. The results are shown in Table 2. Good repeatability was observed (<20% for LLOQ and otherwise well under 15%) and

accuracies fell under 85-115% range, which are the commonly accepted criteria for quantitative adequacy from FDA Bioanalytical Method Validation.

Set conc.	QC s	et 1 (N=5 for each l	evel)	QC set 2 (N=5 for each level)		
(µg/mL)	Determined	Accuracy	Repeatability	Determined	Accuracy	Repeatability
0.00763	0.00741	97.1%	5.69%	0.00762	100%	11.3%
0.0229	0.0234	102%	6.68%	0.0232	101%	2.84%
5.86	6.19	106%	2.67%	5.83	99.4%	3.12%
50.0	46.9	94%	6.36%	45.8	91.7%	7.23%

Table 2. Results of assay repeatability evaluation using QC samples.

Conclusion

- Combination of Nexera Mikos[™]r and nSMOL[™] Antibody BA Kit achieved single digit ng/mL LLOQ in the bioassay of Trastuzumab in 11 minutes of analysis runtime.
- Enhancement in sensitivity may be attributed to increased ionization efficiency at lower flow rate, while peak shape was maintained by the UF-Link column connection at ion source. The system is also suitable for routine analysis without the use of specialized tubings that typically suffer from clogging.
- Assuming same level of sensitivity enhancement for other signature peptides of therapeutic mAbs, it now became highly probably that a developed LC-MS/MS assay will satisfy the sensitivity required for both preclinical and clinical studies.

Acknowledgement

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ASMS 2018 TP 338

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Overview

- The purpose of this study is to quickly look the many measurement results obtained and to create new knowledge and hypotheses.
- We developed a pipeline for automated visualization of the multiomics data (metabolomics, proteomics, fluxomics and transcriptomics) on the Garuda platform¹.
- By utilizing the Garuda platform, we succeeded in an easy visualization the four omics data on the metabolic map.

Introduction

Objective

In order to understand biological systems, it has become common to analyze over 100 metabolites. In particular, multiomics analysis which attempts to understand biological systems from multiomics data, has been utilized. With the increase in the number of metabolites and the number of proteins to be analyzed, there is now a big need for a tool to quickly look the many measurement results obtained and to create new knowledge and hypotheses. We previously reported that we developed a pipeline for automated visualization of the multiomics data combining protein, metabolite and metabolic flux on the Garuda platform that provides the framework to connect, discover, and navigate through different software called "gadgets". This study has made it possible to handle transcriptome data.



Figure 1 Automated visualization of multiomics data on Garuda

Garuda platform

Garuda is an open, community-driven, and common platform for systems biology, healthcare and beyond.Garuda provides a framework to connect, discover, and navigate through different analytics applications, databases and services (called "gadgets" available on a dashboard).





Garuda dashboard

Figure 2 Garuda platform

Methods

Synechocystis sp. PCC 6803

The Synechocystis sp. PCC 6803 strain was cultured under three conditions: 1) the autotorphic condition, 2) the mixotorphic condition and 3) the photoheterotrophic condition (Figure 3). For each condition, the transcriptome, proteome, metabolome and metabolic flux data have been acquired by the Shimizu et al. Group at Osaka University^{2,3,4}.

Photosynthesis



Nutritional conditions	photosynthesis	Glucose assimilation
Autotorphic (Auto)	+	-
Mixotorphic (MIxo)	+	+
Photoheterotrophic (Hetero)	-	+

Autotorphic condition



Mixotorphic condition



Photoheterotrophic condition



Figure 3 Estimated metabolic flux distribution (Red arrow : photosynthesis, Blue arrow : glucose assimilation)



Shimadzu multiomics analysis gadgets

Data import and analytic tools were specifically developed as gadgets on the Garuda platform, namely, the "Shimadzu MS Data Import" and the "Multiomics Data Mapper". Furthermore, these gadgets were connected with downstream gadgets for analysis and visualization by VANTED⁵, available freely on the Garuda platform. Similarly, other analysis workflows are realized by connecting iPATH2⁶, Cytoscape and Shimadzu multiomics analysis gadgets (Figure 4).

Shimadzu multiomics analysis gadget pack (free version) are now available! Installation guide is available on http://www.garuda-alliance.org/gadgetpack/shimadzu



Figure 4 Analysis workflows

Results

We attempted a visualization of four omics layers using four pieces of data. In addition to the three sets of data (proteome, metabolome and metabolic flux) that could already be visualized, this study has made it possible to handle transcriptome data (Figure 5).





The data was visualized on the metabolic map of the Calvin Benson cycle including the RuBisCO, which is an enzyme involved in the first major step of carbon fixation in terms of photosynthesis (Figure 6).

The carbon fixation catalyzed by RuBisCO (RbcL / RbcS) showed that the metabolic flux (Flux_rbc1) decreased in the order of an autotorphic condition, a mixotorphic

condition and a photoheterotrophic condition. The RuBP of the substrate metabolite, the 3PG content of the product and the expression level of the rbcL / rbcS gene encoding the RuBisCO protein did not clearly correlate with the metabolic flux. On the other hand, the change in the expression level of rbcL / rbcS, which is a RuBisCO protein, was similar to the change in metabolic flux.



Figure 6 Multiomics changes between conditions

Conclusions

• By utilizing the Garuda platform, we succeeded in an easy visualization the four omics data on the metabolic map.

• This research can be expected to interpret the data by connecting others gadgets on Garuda platform.

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Determination of pyrrolizidine alkaloids in plant material using on-line SPE coupled to UHPLC-MS/MS



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Determination of pyrrolizidine alkaloids in plant material using on-line SPE coupled to UHPLC-MS/MS

Introduction

Pyrrolizidine alkaloids (PAs) are secondary plant metabolites that are supposed to be carcinogenic and genotoxic. They occur mainly in plants of the Boraginaceae, Asteraceae and Fabaceae families. They contain a pyrrolizidine core and make up a large group of heterocyclic alkaloids mainly derived from the 4 Necin bases platynecine, retronecine, heliotridin and ontonecin. PAs are hepatotoxic if they carry a 1,2-double bond as well as an esterified side chain which is a structural prerequisite for their hepatic activation. Plant food and beverage, phytopharmaceuticals or even animal feed can easily be contaminated with PAs and enter the food chain. Currently there are discussions on possible regulatory measures caused by the presence of PAs in honey, tea, herbal infusions and food supplements. Existing methods include laborious sample preparation, e.g. solid-liquid extraction followed by solid phase extraction for clean-up. Here we report an on-line SPE UHPLC-MS/MS method, which overcomes the difficulties of combining low pressure online SPE with high pressure analytical UHPLC.



Figure 1 General structure of pyrrolizidine alkaloids

Methods and Materials

Sample Preparation

Tea samples were extracted twice with 0.05M sulfuric acid by sonication. Before centrifugation the pH of the combined extracts was adjusted with ammonium hydroxide.

UHPLC method

Instrument	: Nexera UHPLC, Shimadzu
Column	: Shim-pack XR-ODS III, 150 mm x 2.0 mm, 2.2 µm, Shimadzu
Mobile phase A	: 5 mM ammonium formate + 0.1% formic acid
В	: methanol + 5 mM ammonium formate + 0.1% formic acid
Flow rate	: 0.4 mL/min
Time program	: B conc. 1% (0-1.6 min) -50% (14.6 min) – 71.5% (18.1 min)
	– 95% (18.2 min – 20.2 min) -1% (20.3 min – 25 min)
Column temperature	: 30 °C

Determination of pyrrolizidine alkaloids in plant material using on-line SPE coupled to UHPLC-MS/MS

Online SPE method

Column Mobile phase	 EVOLUTE® EXPRESS ABN, 30 x 2.1 mm, Biotage 5 mM ammonium formate + 0.1% formic acid for sample loading methanol / H2O + 5 mM ammonium formate + 0.1% formic acid methanol isopropagal for washing of SPE column.
Flow rate	: 0.2 / 2 mL/min
Injection vol.	: 50 μL
Column temperature	: RT

MS conditions

Instrument	: LCMS-8060, Shimadzu
Ionization	: pos ESI
Nebulizing gas	: 3 L/min
Heating gas	: 15 L/min
Drying gas	: 5 L/min
Interface temperature	: 400 °C
DL temperature	: 300 °C
Heat block temperature	: 400 °C
CID gas	: 270 kPa
Interface voltage	: 1 kV

Result

Method development of the online SPE

The neutralized and centrifuged tea extract samples were put into the autosampler and transferred to the on-line SPE column using an aqueous solution. After washing the sample was eluted with only 10 μ L solvent and trapped into a loop. By switching the loop the eluted sample was transferred to the analytical column. A binary gradient

separated the PAs for quantification. Due to this hardware set-up UHPLC with high backpressure and on-line SPE which is pressure limited were successfully combined. By careful fine-tuning of the SPE elution and the chromatographic conditions the separation of critical peak pairs could be maintained.



Figure 2 Typical chromatogram of pyrrolizidine alkaloids in tea matrix including the separated pressure curves of the analytical column (Pump A and B pressure) and the online SPE column (Pump C pressure)



Determination of pyrrolizidine alkaloids in plant material using on-line SPE coupled to UHPLC-MS/MS



Figure 3 Setup of the on-line SPE analytical system

Quantitative Analysis of tea samples

By using the reported instrument set-up, analysis and thus the quantification of 16 PAs and 14 of their related N-Oxides could be performed. Calibration curves in different tea matrices (black tea, green tea and herbal tea) determined in duplicate showed good precision and accuracy and even in a complex matrix like tea we were able to easily quantify the PAs in at least the range of 10 to 400 µg/kg. This is comparable to the established methods using manual sample preparation. For all analytes, weighted regression resulting in r^2 0.99 could be achieved, with S/N >10 for LLOQ levels.

Exemplary calibration curves obtained for the 30 compounds are shown in Figure 4, Chromatograms of exemplary LLOQs are shown in Figure 5, the LLOQs which could be achieved in the different tea matrices are shown in Table 1.

Determination of pyrrolizidine alkaloids in plant material using on-line SPE coupled to UHPLC-MS/MS



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Figure 4 Exemplary calibration curves in different tea matrices

0.2 - 20 ng/mL (4 - 400 µg/kg)

Calibration Curve Senecyphilline in green tea

20

Conc (ng/ml)

15

2 0000

1.000e5

0.000e



8.0 8.1 8.2 8.3 8.4 8.5 8.6 8.7 8.8









LLOQ of Retrosine-N-oxide in black tea / 10µg/kg

LLOQ of Senecyphilline in green tea / 4.0 $\mu\text{g/kg}$ Figure 5 Exemplary chromatograms of LLOQs different tea matrices
Determination of pyrrolizidine alkaloids in plant material using on-line SPE coupled to UHPLC-MS/MS

	Blac	k tea OQ	Gree	n tea OQ	Herbal tea LLOQ		
	ng/mL	µg/kg	ng/mL	µg/kg	ng/mL	µg/kg	
Echimidine	0.05	1.0	0.05	1.0	0.05	1.0	
Echimidine-N-oxide	0.05	1.0	0.05	1.0	0.05	1.0	
Erucifoline	0.20	4.0	0.20	4.0	0.50	10.0	
Erucifoline-N-Oxide	0.10	2.0	0.20	4.0	0.10	2.0	
Europine	0.05	1.0	0.05	1.0	0.05	1.0	
Europine -N-Oxide	0.05	1.0	0.05	1.0	0.05	1.0	
Heliotrine	0.05	1.0	0.05	1.0	0.05	1.0	
Heliotrine N-oxide	0.05	1.0	0.05	1.0	0.05	1.0	
Intermedine	0.05	1.0	0.05	1.0	0.05	1.0	
Intermedine N-oxide / Indicine N-oxide	N.A	N.A	0.05	1.0	0.05	1.0	
Jacobine	0.20	4.0	0.50	10.0	0.50	10.0	
Jacobine N-oxide	0.05	1.0	0.10	2.0	0.10	2.0	
Lasiocarpine	0.05	1.0	0.10	2.0	0.05	1.0	
Lasiocarpine N-oxide	0.10	2.0	0.10	2.0	0.05	1.0	
Lycopsamine / Indicine	N.A	N.A	0.05	1.0	0.05	1.0	
Lycopsamine N-oxid	0.05	1.0	0.05	1.0	0.05	1.0	
Monocrotaline	0.50	10.0	0.20	4.0	0.50	10.0	
Monocrotaline-N-oxide	0.10	2.0	0.10	2.0	0.10	2.0	
Retrosine	0.10	2.0	0.05	1.0	0.10	2.0	
Retrosine N-oxide	0.20	4.0	0.50	10.0	0.50	10.0	
Senecionine	0.05	1.0	0.50	10.0	0.50	10.0	
Senecionine N-oxide	0.05	1.0	0.20	4.0	0.50	10.0	
Senecyphilline	0.10	2.0	0.20	4.0	0.50	10.0	
Senecyphilline N-oxide	0.05	1.0	0.20	4.0	0.10	2.0	
Senecivernine	0.20	4.0	0.50	10.0	0.20	4.0	
Senecivernine N-oxide	0.05	1.0	0.20	4.0	0.10	2.0	
Senkirkine	0.05	1.0	0.05	1.0	0.05	1.0	
Trichodesmine	0.05	1.0	0.10	2.0	0.20	4.0	

Table 1 LLOQs of the pyrrolizidine alkaloids in different tea matices

A total of 29 commercially available tea samples were analyzed. Among these samples there were 6 samples of green tea, 10 samples of black tea and 13 samples of herbal tea. In 59% of all analyzed tea samples one or more of the pyrrolizidine alkaloids could be detected above their LLOQ. 3 out of 6 green tea samples, 5 out of 10 black tea samples and 9 out of 13 herbal tea samples where contaminated with pyrollizidine alkaloids.



Determination of pyrrolizidine alkaloids in plant material using on-line SPE coupled to UHPLC-MS/MS

Conclusions

An on-line SPE method for high-sensitivity analysis was successfully developed for PA analysis in plant material. The manual sample preparation could be reduced to a minimum as the set up of on-line SPE followed by UHPLC-MS/MS saves additional clean-up steps without compromising the performance of the assay.

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Introduction

The majority of clinical decisions are based on laboratory test results. For many laboratories. Triple quadrupole MRM methods are used to deliver highly sensitive, selective and robust for precise quantification and identification verification. To help transition towards a more effective data review and higher confidence in reporting results. we have been rethinking the capability of MRM in compound identification and verification. In this workflow, 6-10 fragment ion transitions were monitored for each target compound as opposed to a conventional approach using 2-3 fragment ions. By acquiring a high number of fragment ion transitions, each target compound had a corresponding fragmentation spectra which could be used in routine library searching and compound verification using reference library match to clinical and forensic toxicology.

Methods and Pretreatment

Whole blood was spiked with 33 drugs (Hypnotics). Calibration samples were prepared by modified QuEChERS method. In this study, MRM spectrum mode acquired a library of typically 7 or more MRM's per compound using certified reference materials. The library included retention time, CAS number, formula and MRM transitions for each target compound. As a comparison, MRM triggered product ion scan for three collision energies corresponding to CE: 15, 30, 45V as well as a fourth merged CE spectrum was performed for qualitative.



Fig.1 Comparison of conventional MRM and MRM spectrum mode with flunitrazepam



Table 1. LC and MS conditions

[LC] Nexera [™] X2 Syste	em					
Analytical Column Guard Column Solvent A Solvent B	: Phenomenex Kinetex [®] XB-C18 (2.1 mml.D.×100 mmL., 2.6 μm) : Phenomenex Security Guard Ultra C18 2.1 mmlD : Water + 10 mmol/L ammonium formate + 0.1% formic acid : Methanol + 10 mmol/L ammonium formate + 0.1% formic acid					
Gradient Program	: Time (min)	%B				
	0	5				
	7.5	95	_			
	10	95	_			
	10.01	5				
	15	STOP				
Flow Rate	: 0.3 mL/min					
Column Temp	: 40 °C					
[MS] LCMS-8060						
Ionization	: ESI (Positive)					
Nebulizer Gas	: 3 L/min					
Interface temperature	: 300 °C					
Desolvation Line	: 250 °C					
Heat Block temperatur	re : 400 °C					
Heating Gas	: 15 L/min					
Drying Gas	: 10 L/min					



MRM spectrum mode and Library Searching for Enhanced Reporting Confidence in Forensic Toxicology

Results

Quantitation

Conventional qualitative data acquisition by triple quadrupole LC-MS/MS which typically uses 2 MRM per compound. MRM product mode acquires a higher number of precursor-fragment ion transitions to generate a library searchable product ion spectrum. For quantitative analysis, these compounds and QuEChERS extraction were diluted at 0.05, 0.1, 0.5, 1.0 ng/mL for calibration points. For accurate quantitative, internal standard was required. Low similarity score was obtained for some compounds, because some drugs have almost same mass which can't separate by nominal mass. Good LC separation was required for the good similarity score.



Fig.2 MS chromatogram of flunitrazepam and estazolam post-spiked in whole blood at 1 ng/mL



Fig.3 Calibration curve with MRM spectrum mode post-spiked sample (0.05, 0.1, 0.5, 1.0 ng/mL).

Compound	Similarity Score	RT (min)	Accuracy (%)	R ²
7-aminoflunitrazepam	97	4.67	107	0.9960
7-aminoclonazepam	88	4.25	106	0.9969
Estazolam	93	6.18	85	0.9996
Zolpidem	96	4.68	78	0.9998
Flunitrazepam	98	6.16	77	0.9998
Bromazepam	65	5.78	115	0.9960
Clotiazepam	93	7.14	79	0.9913
Midazolam	98	5.82	111	0.9950
4-hydroxymidazolam	87	5.51	92	0.9992
Triazolam	76	6.32	87	0.9862
Ethyl loflazepate	77	6.95	89	0.9931
Flurazepam	100	5.46	89	0.9998
Nordiazepam	90	6.71	82	0.9833

 Table 2
 Similarity score, retention time, recovery rate, accuracy of typical compounds

 pre-spiked (2.0 ng/mL) and R² for post-spiked calibration curve with MRM spectrum mode.

Library Identification

To minimize the possibility of false reporting without compromising the accuracy, precision and limits of detection, methods were developed to combine the sensitivity of MRM detection with the identification power of a product ion spectrum. The methods have the capability of simultaneously using both precursor and product ion information enabling precise, accurate quantification and library searchable compound identification. To assess the impact of methods designed to increase reporting confidence by library searching on quantification both product ion spectrum methods were compared to a data generated using conventional 2 MRM methods; MRM triggered product ion spectrum and MRM spectrum mode.



Fig.4 MS chromatogram of 7-aminonitrazepam, 7-aminoclonazepam, 7-aminoflunitrazepam, and flurazepam.

Compound	7-amino nitrazepam	7-amino clonazepam		7-amino flunitrazepam	Flurazepam
MS/MS	acquisition mode			MRM Spectrum N	Node
Precursor	252.1	28	6.1	284.1	388.2
Product 1	121.1	22	2.1	135.1	315.0
Product 2	208.1	19	4.1	148.1	225.0
Product 3	119.1	20	9.1	236.2	288.0
Product 4	207.5	20	5.1	264.1	134.1
Product 5	180.1	16	7.1	163.2	317.0
Product 6	94.1	12	1.1	227.1	287.0
Product 7	77.1	25	0.1	226.1	271.0
Product 8	224.1	94	l.1	93.1	109.0
Product 9	146.1	77.1		240.1	130.1
Product 10	104.1	19	5.1 256.1		224.0
MS/MS	acquisition mode			MRM Triggered Produ	ct lon Scan
Precursor	252.1	28	6.1	284.1	388.2
Product 1	121.1	22	2.1	135.1	315.0
Product 2	94.1	12	1.2	226.2	317.0
CE: 15	20 : 262.1	20:2	296.1	20 : 294.1	20 : 398.2
CE: 30	20 : 262.1	20 : 2	296.1	20 : 294.1	20 : 398.2
CE: 45	20:262.1	20:2	296.1	20 : 294.1	20 : 398.2

Table 3 Acquisition parameters of MRM Spectrum Mode and MRMTriggered Product Ion Scan (Threshold was an intensity of 10,000 counts).

MRM Spectrum Mode



MRM Triggered Product Ion Spectrum (Merged Spectrum CE: 15/30/45)



Fig.5 Compared to a conventional MRM triggered product ion scan and MRM spectrum mode, MRM spectrum mode has almost same qualitative ability for these compounds.



Conclusion

MRM spectrum mode results in high data densities and a high data sampling rate across a peak. This approach generates a consistent loop time and sampling rate producing reliable guantification and peak integration without threshold triggering and creates new opportunities in toxicological screening.

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ASMS 2018

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PO-CON1823E



Introduction

Pesticides and other chemicals are used in the production of many agricultural products, including botanicals for use as dietary supplements. Supplements are widely used but their raw materials, often sourced from remote locations, are subjected to fewer regulatory controls than staple foods. To ensure quality the US FDA requires identity and quality testing, but most botanicals do not have specific regulations. To analyze complex botanicals for residual chemicals such as pesticides, LC-MS-MS is needed for high sensitivity, high confidence results. We developed an LCMS method with improved detection sensitivity for chemical residues in botanicals.

Photo credits: Echinacea, Giancarlo Dessi; Cayenne, H. Zell; Valerian, Lairich Rig; Ginseng, National Institute of Korean Language; Tumeric, Simon A. Eugster; Passionflower, Bob Peterson; St Johns Wort, Glyn Baker. All photos obtained through wikimedia commons under creative commons attribution-share alike 2.0 or higher



Echinacea E. purpurea



Passionflower (tea) Passiflora sp.





Cayenne Capsicum annuum



Korean Ginseng Panax ginseng



Valerian Valeriana officinalis



St. Johns Wort Hypericum perforatum

Figure 1 A selection of popular dietary supplements tested. Various parts of the above-pictured plants may be used in actual dietary supplement formulations.



Method

Representative samples of powdered botanicals were removed from their gelatin capsules, homogenized and extracted with acetonitrile accompanied by shaking and sonication. Samples were additionally cleaned up using dispersive solid phase extraction to remove unwanted matrix components. Analysis was carried out by LC-MS-MS using a triple quadrupole mass spectrometer. The mass spectrometer interface parameters were carefully adjusted to improve the signal for the majority of the analytes. Spiking experiments were used to determine recovery and matrix-matched standards were used to prepare calibration curves.

LC Column	: Raptor ARC18 (2.1×150 mm, 2.7 μm)
Mobile Phase A	: 0.1% Formic Acid with 5 mM Am. Formate
Mobile Phase B	: Methanol
Flow Rate	: 0.5 mL/min
Probe Voltage	: +0.5 kV or -0.5kV
Interface Temp	: 100 °C
Nebulizing Gas	: 3 L/min
Drying Gas	: 10 L/min
DL Temp	: 100 °C
Heat Block Temp	: 100 ℃

Table 1 Instrument parameters used for analysis



Figure 2 Sample preparation of botanicals for LC-MS analysis



Results and Discussion

Eight popular botanical supplements were selected for testing, including Cayenne, Valerian, Passionflower tea, Korean Ginseng, St. John's Wort, Tumeric and two varieties of Echinacea. The Tea and Echinacea variety 1 were labeled as organic, while the other supplements were not labeled as organic. For each sample, a single-point standard addition sample at 500 ng/g dried material was prepared in addition to check matrix-specific effects. Compared with a conventional method, we found significant improvement in instrument response for many analytes by careful adjustment of interface temperature and spray voltage. For quantitation, matrix matched calibration curves were linear within the quantitation limits established for each compound, which was compound dependent. Detection limits and quantitation limits were required to have 3:1 and 10:1 signal to noise respectively, and quantitation limits were required to have less than 20% RSD in triplicate injections. Using our newly developed method, we are able to characterize the extent of residual pesticides t in popular botanicals.



Figure 3 Representative chromatogram of pesticides spiked into a sample of Korean Ginseng at the 500 ng/g of dried material level.



Figure 4 Acetonitrile extracts (before dSPE cleanup) of various botanicals. From left: Valerian, Cayenne, Echinacea-1, Tumeric, Echinacea-2, St. John's Wort, Korean Ginseng, and Passionflower Tea.

	Cayenne	Echinacea-1	Echinacea-2	Korean Ginseng	St. John's Wort	Passionflower tea	Tumeric	Valerian root
Azoxystrobin	15	ND	ND	7	ND	ND	ND	ND
Carbaryl	ND	ND	ND	ND	ND	35	ND	ND
Carbofuran	3.7	ND	ND	ND	ND	ND	ND	ND
Chlorpyrifos	ND	ND	ND	ND	ND	ND	9.4	ND
Cypermethrin	140	ND	ND	ND	ND	ND	ND	ND
Dimethomorph	ND	ND	ND	36	ND	ND	ND	ND
Imidacloprid	11	ND	ND	ND	ND	ND	ND	ND
Metalaxyl	ND	ND	ND	ND	ND	ND	8.3	ND
Methoprene	196	ND	ND	ND	ND	ND	ND	ND
Novaluron	24	ND	ND	ND	ND	ND	ND	ND
Propiconazole	ND	ND	ND	24	ND	ND	ND	ND
Pyraclastrobin	6.5	ND	ND	ND	ND	ND	ND	ND
Tebuconazole	59	ND	ND	4.8	ND	ND	ND	ND
Trifloxystrobin	10	ND	ND	ND	ND	ND	ND	ND

Table 2 Chemical residues detected in botanical extracts. Values reported in ng/g dry material.

Table 3 List of compounds measured and limits of quantitation in ng/g.

Abamectin	30	Daminozide	15	Hexythiazox	15	Propiconazole	60
Acephate	5	Deltamethrin	30	Imazalil	10	Propoxur	2
Acequinocyl	60	Diazinon	<2	Imidacloprid	4	Pyraclostrobin	10
Acetamiprid	<2	Dichlorvos	15	Kresoxim-methyl	4	Pyrethrin I	100
Aldicarb	<2	Dimethoate	<2	Malathion	2	Pyridaben	2
Allethrin	50	Dimethomorph	5	Metalaxyl	2	Resmethrin	35
Azoxystrobin	4	Dinotefuran	2	Methiocarb	4	Spinetoram	2
Bifenazate	2	Dodemorph	4	Methomyl	<2	Spinosad	<2
Bifenthrin	4	Endosulfan-sulfate	4	Methoprene	50	Spirodiclofen	10
Boscalid	4	Ethoprophos	2	Mevinphos	4	Spiromesifen	20
Buprofezin	<2	Etofenprox	4	MGK-264	500	Spirotetramat	2
Carbaryl	10	Etoxazole	<2	Myclobutanil	10	Spiroxamine	2
Carbofuran	<2	Fenhexamid	20	Naled	2	Tebuconazole	2
Chlorantraniliprole	2	Fenoxycarb	2	Novaluron	15	Tebufenozide	5
Chlorpyrifos	10	Fenpyroximate	10	Oxamyl	2	Teflubenzuron	15
Clofentazine	4	Fensulfothion	5	Paclobutrazol	2	Tetrachlorvinphos	4
Clothianidin	4	Fenthion	10	Permethrin	10	Tetramethrin	4
Coumaphos	4	Fenvalerate	100	Phenothrin	10	Thiacloprid	<2
Cyantraniliprole	2	Fipronil	2	Phosmet	10	Thiamethoxam	<2
Cyfluthrin	500	Flonicamid	25	Piperonyl butoxide	5	Thiophanate-methyl	5
Cypermethrin	60	Fludioxonil	2	Pirimicarb	2	Trifloxystrobin	<2
Cyprodinil	10	Fluopyram	2	Prallethrin	10		





Figure 5 Recovery for each analyte in a 500 ng/g spike of each sample. Tumeric and Cayenne had the greatest number of analytes with low recovery, due to signal suppression.



Figure 6 (Left) Cypermethrin (four isomers) detected in Cayenne sample. (Right) Carbaryl detected in organic passionflower tea sample.



Conclusion

We developed a high performance method for sensitive detection of pesticides in popular botanical supplements with a simple sample preparation and applied the method to measure pesticides in selected botanical products offered for retail sale. We found matrix effects to be minimal with the exception of Tumeric and Cayenne. For these matrices, additional sample cleanup may be useful to minimize signal suppression by the matrix. Several pesticides were detected in some of the dietary supplements. Cayenne had the greatest number of detections and with the highest levels approaching 200 ng/g. Significantly, the Passionflower tea, which was labeled as organic, was found to contain 35 ng/g carbaryl. Our rapid, sensitive, and selective method is well-suited to high throughput detection of pesticide residues in popular botanical products.





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PO-CON1816E

Novel Aspects

A single LC-MS/MS method was developed and optimized to identify and determine a limit of detection (LOD) for several synthetic opioids. including fentanyl, and several fentanyl analogues as well as a few of their major metabolites in blood and urine matrices.

Introduction

The potency of these compounds result in small concentrations being detected in real-world samples, thus requiring methods utilized by a forensic or clinical laboratory to be very sensitive. Hyphenated techniques such liquid chromatography-tandem mass spectrometry (LC-MS/MS) can achieve this needed sensitivity through multiple reaction monitoring (MRM). The Shimadzu LCMS-8060 features a heated dual ionization source (DUIS) coupled with a along with ultrafast MRM acquisition software and polarity switching for increased accuracy, sensitivity and robustness. DUIS combines electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI) by using the ESI source and the corona needle used in APCI. The development process involved MRM optimization, MS source optimization and finally column selection for a method that successfully separated and identified a mixture of synthetic opioids. This analytical method when used in combination with a validated solid phase extraction procedure, achieved sub-ng/mL detection limits in blood and urine samples.



Chemical Structure of Fentanyl (top) and Carfentanyl (bottom)

Method

Method development began with optimizing the compounds using flow injection analysis and LabSolutions MRM Optimization Wizard. The MRM transitions were fully optimized to enhance sensitivity of all of the compounds in the mixture.

The LC flow rate was set at 0.5 mL/min with a 50:50 mixture of mobile phase A and B. The mass spectrometer

source parameters and LC conditions are listed below. The optimized MRM transitions for each compound are listed in results section.

Following the MRM optimization, four LC columns were analyzed using a five minute LC gradient starting at 20% mobile phase B increasing to 80%.

Chromatography Pa	Chromatography Parameters				
Column	: Restek Raptor Biphenyl				
Column Temp	: 40 °C				
Autosampler Temp	: 15 °C				
Injection Volume	: 30 µl				
Mobile Phase A	: H2O: MeOH w/ Formic Acid				
Mobile Phase B	: MeOH w/ Formic Acid				
Injection Solvent	: 80 MPA: 20 MPB				
Flow Rate	: 0.5 mL/min				
LCMS-8060 Paramet	ers				
Nebulizing Gas	: 1.5 L/min				
Interface Temp	: 400 °C				
DL Temp	: 200 °C				
Heat Block Temp	: 250 °C				
Drying Gas Flow	: 5 L/min				
Heating Gas Flow	: 15 L/min				
Interface Temp	: 400 °C				
Ion Source	: DUIS				

Serial dilutions (1:5) of a 1000 pg/mL solution were used to create a 6 point calibration curve in certified blank urine and certified blank whole blood. Fentanyl-d5 was used as the internal standard.

The samples were extracted for analysis using a simple solid phase extraction. The sample was added to the cartridge after the SPE cartridge was conditioned. The wash solutions were added to the cartridge and the compounds were eluted off using a mixture of DCM/IPA/NH₄OH. Once eluted the samples were dried down and reconstituted in a 80:20 Water:MeOH mixture. The calibration curve was prepared in triplicate to along with two blank samples; a certified matrix blank spiked with internal standard and a solvent blank, and a single unknown blood sample. The case sample was run against both matrix curves.

Results- Columns and LOD





The Restek Raptor Biphenyl was chosen for the study after a comparison between peak shape, peak separation, and sensitivity on column was made. The overall chromatograms for the four columns are represented above.

Compound	Quant Ion	Qual Ion	Blood LOD (pg/mL)	Urine LOD (pg/mL)
Methylfentanyl	351.10>105.10	351.10>202.25	1.6	40
6-Acetylmorphine	328.00>181.10	328.00>165.10	1.6	40
Naloxone	328.00>310.20	328.00>212.10	200	8
Fentanyl	337.30>188.20	337.30>105.10	8	8
Acetyl Fentanyl	323.10>188.20	323.10>105.15	1.6	40
Butyryl Fentanyl	351.10>105.25	351.10>188.20	8	40
Norfentanyl	233.00>84.05	233.00>55.20	0.32	8
4-ANPP	281.10>188.20	281.10>105.10	8	8
Valeryl Fentanyl	365.10>188.20	365.10>105.05	8	8
Ocfentanyl	371.10>188.20	371.10>355.15	1.6	1.6
MT-45	349.30>181.15	349.30>166.10	1.6	40
Furanyl Fentanyl	375.10>188.20	375.10>105.15	1.6	8
Carfentanyl	395.10>335.30	395.10>113.25	1.6	8
Norcarfentanyl	291.10>231.30	291.10>146.25	0.32	40
Fentanyl D5	342.10>188.20	342.10>105.25		

MRM optimization resulted in the above transitions. These transitions were used for both the urine and blood testing. The limit of detection (LOD) for each compound was determined by the lowest injection level that had a signal to noise calculation of greater than 3. The LOD of the

compounds ranged from 0.32 pg/mL to 8 pg/mL in blood with the exception of Naloxone which was 200 pg/mL. The LODs in urine were slightly higher than in blood and ranged from 1.6 to 40 pg/mL.

Results- Calibration Curve

The below chromatogram is of the highest calibration point (1000 pg/mL) in the extracted matrix curve. Below are the chromatogram are four out of fourteen calibration curves in urine. All of the compounds were linear over the range of 0.32 to 1000 pg/mL with an R2 value greater than 0.998.





Conclusion

The analytical conditions shown in this application note have demonstrated the ability of the LCMS-8060 to detect Fentanyl, Fentanyl analogs and metabolites at picogram per milliliter levels. The continuation of this study will include linear ranges, limit of quantitation of the compounds as well as precision and accuracy of the method and extraction.

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Overview

Purpose

The aim of this study is to develop an online SFE-LC/MS system that provides completely automated analytical process for metabolites analysis.

Methods

- SFE (supercritical fluid extraction) was used for multi-step extraction.
- Newly developed polymer-based column was used as the trap column for displacing SFE extractant with LC mobile phase.

Results

We successfully developed

Multi-step extraction method that afforded automatic extraction of typical metabolites from microbial cells and low background noise in MS detection due to clean-up step.

An online SFE-LC/MS system using newly developed polymer-based trap column for analysis of metabolites in microbial cells.

Introduction

In bio-based fine chemical production, a short breeding cycle time of microbes is a key issue for improving productivity. Metabolomics has been widely used as a quick and comprehensive analytical procedure due to its excellent features of dynamic monitoring and quick evaluation for bio-production. On the other hand, sample pretreatment procedure is still generally tedious and time consuming while high-throughput analytical methods using LC/MS and GC/MS.

We present the development of online SFE-LC/MS system that provides completely automated analytical process resulting labor-saving.

Methods and Materials

Instrumentation

SFE conditions	
Modifier	: 0.1% ammonium formate-methanol
Flow rate	: 1.0 mL/min
Extraction	: Static extraction : 3 min.
	Dynamic extraction : 2 min.
BPR	: 15 MPa
Vessel	: 0.2 mL
LC/MS conditions	
Column	: SUPELCO Discovery HS F5-3 (4.6 x150 mm, 3 μm)
Mobile Phase	: 0.1% formic acid-water / 0.1% formic acid acetonitrile
Gradient program	: 0%B (0-2min) => 25%B (5min) => 35% B (11min) => 95%B (15-20min)
	=> 0%B (20.01-25min)
Flow rate	: 0.8 mL/min
Oven temperature	: 40 °C
Ionization	: ESI positive, negative
Mode	: MRM



Figure 1 Nexera UC SFE system





Samples & target compounds

- E.coli. and yeast cells were used as tested samples.
- Typical metabolic precursors for secondary metabolites on the shikimate, mevalonate, and MEP pathways were selected.

Mevalonate
Mevalonate-5-phosphate
Mevalonate-5-diphosphate
DXP
MEP
DOXP
Malonyl CoA





Results

Component technologies for the online SFE-LC/MS

Multi-step extraction with auto-clean up using SFE

- Most of tested metabolites were extracted when the modifier concentration was more than 20% (Fig. 4). Therefore, multi-step extraction method was employed (Fig. 5).
- Some phosphoric compounds were not extracted due to metal ion liganding property.





Figure 5 Single / multi-step extraction in SFE



Multi-step extraction method afforded

- Automatic extraction of typical metabolites from microbial cells without any additional pretreatment.
- Low back ground noise in MS detection due to clean-up step (i.e. 1st extraction with 0% of modifier).



Figure 6 Chromatograms of sample extracts (E.coli.) by single / multi-step extraction

Trapping technique using newly developed polymer-based trap column

- The extract from SFE must be trapped in the trap column before introduction into the LC column for displacing SFE extractant with appropriate solvent due to poor miscibility of SFE and LC mobile phases.
- Newly developed polymer-based column that showed large retention under SFE condition whereas quick elution under LC condition (Fig. 7 and 8) was employed.



Retention behaviours of the metabolites in new polymer-based column

Under SFE condition

(a) Effect of modifier concentration



(b) Effect of additive in the modifier (modifier conc. 20%)



Figure 7 Chromatograms of typical metabolites under SFE condition

Under LC condition



Figure 8 Chromatograms of typical metabolites under LC condition

Component technologies for the online SFE-LC/MS



Figure 9 System configuration of newly developed online SFE-LC/MS



1. 1st step extraction



2. 2nd step extraction



3. Releasing back pressure



4. Conditioning



5. LC/MS analysis



Figure 10 Schematic sequence of the online SFE-LC/MS

Metabolites analysis in real samples using SFE-LC/MS

- E.coli. and yeast cells collected from their culture mediums by centrifugation were used.
- Some metabolites were successfully extracted from E.coli. and yeast cells without any pretreatment.



Figure 11 Chromatograms of the metabolites in E.coli. and yeast cells

Conclusions

- An online SFE-LC/MS system using newly developed polymer-based trap column for analysis of metabolites in microbial cells has been successfully developed.
- This system provides completely automated analytical process resulting labor-saving.

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PO-CON1798E

Overview

We have developed a novel cell culture media analysis platform, C2MAP, that can carry out automated deproteinization of cell-free culture supernatant samples and simultaneous analysis of up to 95 compounds found in basal media components and secreted metabolites using triple quadrupole LC-MS/MS.

Introduction

Optimization and control of cell culture processes are essential to increase production efficiency of biopharmaceuticals. In the field of cell therapy, enhanced control of the culture process is also becoming important to reduce cell variability and improve consistency of mass production of the cells. Comprehensive monitoring of culture supernatant components gives researchers useful information for these purposes. However, current technologies for process monitoring are limited to measurement of pH, dissolving gases, and some small compounds such as glucose, glutamine, lactate, and ammonia. We have developed a "Cell Culture Media Analysis Platform, C2MAP system" that can perform automated sample pretreatment and simultaneous analysis of up to 95 compounds listed below. We also developed a viewer software that can easily visualize temporal change in each measured compounds through the cell culture. In this poster, we present features of C2MAP system and its applications.

_	and at an up a minute							
No.	Compound Name	Class.	No.	Compound Name	Class.	No.	Compound Name	Class.
1	2-Isopropylmalic acid	IS	33	N-Acetylaspartic acid	Amino acid	65	Cytidine	Nucleic acid
2	Gluconic acid	Carbohydrate	34	N-Acetylcysteine	Amino acid	66	Cytidine monophosphate	Nucleic acid
3	Glucosamine	Carbohydrate	35	Ornithine	Amino acid	67	Deoxycytidine	Nucleic acid
4	Hexose (Glucose)	Carbohydrate	36	Oxidized glutathione	Amino acid	68	Guanine	Nucleic acid
5	Sucrose	Carbohydrate	37	Phenylalanine	Amino acid	69	Guanosine	Nucleic acid
6	Threonic acid	Carbohydrate	38	Pipecolic acid	Amino acid	70	Guanosine monophosphate	Nucleic acid
7	2-Aminoadipic acid	Amino acid	39	Proline	Amino acid	71	Hypoxanthine	Nucleic acid
8	4-Aminobutyric acid	Amino acid	40	Serine	Amino acid	72	Inosine	Nucleic acid
9	4-Hydroxyproline	Amino acid	41	Threonine	Amino acid	73	Thymidine	Nucleic acid
10	5-Glutamylcysteine	Amino acid	42	Tryptophan	Amino acid	74	Thymine	Nucleic acid
11	5-Oxoproline	Amino acid	43	Tyrosine	Amino acid	75	Uracil	Nucleic acid
12	Alanine	Amino acid	44	Valine	Amino acid	76	Uric acid	Nucleic acid
13	Alanyl-glutamine	Amino acid	45	4-Aminobenzoic acid	Vitamin	77	Uridine	Nucleic acid
14	Arginine	Amino acid	46	Ascorbic acid	Vitamin	78	Xanthine	Nucleic acid
15	Asparagine	Amino acid	47	Ascorbic acid 2-phosphate	Vitamin	79	Xanthosine	Nucleic acid
16	Aspartic acid	Amino acid	48	Biotin	Vitamin	80	Penicillin G	Antibiotics
17	Citrulline	Amino acid	49	Choline	Vitamin	81	2-Aminoethanol	Other
18	Cystathionine	Amino acid	50	Cyanocobalamin	Vitamin	82	2-Ketoisovaleric acid	Other
19	Cysteine	Amino acid	51	Ergocalciferol	Vitamin	83	3-Methyl-2-oxovaleric acid	Other
20	Cystine	Amino acid	52	Folic acid	Vitamin	84	4-Hydroxyphenyllactic acid	Other
21	Glutamic acid	Amino acid	53	Folinic acid	Vitamin	85	Citric acid	Other
22	Glutamine	Amino acid	54	Lipoic acid	Vitamin	86	Ethylenediamine	Other
23	Glutathione	Amino acid	55	Niacinamide	Vitamin	87	Fumaric acid	Other
24	Glycine	Amino acid	56	Nicotinic acid	Vitamin	88	Glyceric acid	Other
25	Glycyl-glutamine	Amino acid	57	Pantothenic acid	Vitamin	89	Histamine	Other
26	Histidine	Amino acid	58	Pyridoxal	Vitamin	90	Isocitric acid	Other
27	Isoleucine	Amino acid	59	Pyridoxine	Vitamin	91	Lactic acid	Other
28	Kynurenine	Amino acid	60	Riboflavin	Vitamin	92	Malic acid	Other
29	Leucine	Amino acid	61	Tocopherol acetate	Vitamin	93	O-Phosphoethanolamine	Other
30	Lysine	Amino acid	62	Adenine	Nucleic acid	94	Putrescine	Other
31	Methionine	Amino acid	63	Adenosine	Nucleic acid	95	Pyruvic acid	Other
32	Methionine sulfoxide	Amino acid	64	Adenosine monophosphate	Nucleic acid	96	Succinic acid	Other

List of Compounds





Figure 1 Cell Culture Media Analysis Platform



Fig.2 Pretreatment and measurement flow





Fig.3 C2MAP software





A dedicated software, C2MAP software, can control both pretreatment module and LC/MS/MS system, making it possible to carry out seamless analysis and to associate the treated sample and the measurement results easily because pretreatment and analysis are carried out with the common sample ID. Tempral changes in each component can be graphed with the dedicated viewer software, C2MAP TRENDS, using LC/MS/MS data set. Users can monitor variations in basal media components and secreted metabolites during cultivation.

Materials and Methods

Various media analysis using C2MAP

Various kinds of mammalian cell culture media showed in Table I were analysed with C2MAP. IgG was added to the CHO cells media (final conc. at 10 mg/ mL) prior to analysis. Fetal bovine serum (FBS) often affects cell growth. Detection of component amount variation among the product lots was also tested using three different lots of FBS.

Analysis for culture supernatant of human iPS cells

A spent medium of human iPS cells was collected every 24 hrs. The temporal changes in the components in the culture supernatant were monitored using the C2MAP system. Maintaining undifferentiated state is one of important characters of iPS cells. In this experiment,

C2MAP system was used to compare the temporal changes in the culture supernatant components in undifferentiated human iPS cells and its differentiated counterparts.

Results

Applicability of C2MAP for culture media analysis

The following cell culture media showed in Table I were analysed (six replicates each). Coefficient of variations for all detected compounds from tested media were less than 10% (data not shown).

Cell Type	CHO cells	iPS/ES cells	T cells	Mesenchymal stem cells
Culture Media	BalanCD® CHO	AK03N	X-VIVO [™] 10	MSCBM
	1×CD CHO	Essential-8™	X-VIVO [™] 15	MesenPRO™
	EX-CELL [®] CHO	mTeSR [™] 1 / TeSR [™] -E8 [™]	TexMACS™	Stempro®

Table I. Tested cell culture media

Fifty-six compounds were detected from FBS. Overall pattern of mass chromatogram from each lot was similar, whereas significant differences were detected in some compounds.



Fig.5 Evaluation of lot to lot variation of FBS
Spent media analysis of human iPS cells

Spent media analysis showed which medium components were favoured and consumed by cells, and which metabolites were secreted by cells. This information provides useful insights into optimization of the culture media composition and the culture process.



Fig.6 Time course of components in culture supernatant

C2MAP system was used to find biomarker candidates that can evaluate cell differentiation state using cell culture supernatant as the sample. Three germ layers differentiation was induced by the addition of appropriate cytokines and chemicals. Spent media was collected every 24 hours and analyzed with C2MAP. As a result, significant difference could be found in the time course of some compounds.





A novel cell culture media analysis platform for culture process development

Conclusion

- C2MAP is applicable for analysis of wide range of mammalian cell culture media.
- C2MAP can give useful insights into optimization of the culture media composition and the culture process.
- Multi components analysis using C2MAP is also useful for finding for potential critical process parameters.

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Improved performance of linear ion trap mass spectrometer with added octopole and dodecapole fields



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Improved performance of linear ion trap mass spectrometer with added octopole and dodecapole fields

Overview

- The linear ion trap mass spectrometer with added octopole and dodecapole fields was evaluated.
- The simulation program was developed by LabVIEW.
- The electrode geometry was optimized. The experimental instrument was constructed and it was confirmed that the mass isolation performance was improved.

Introduction

The geometry of commercial ion trap mass spectrometer has been intentionally distorted. The distortion generates additional higher order multipole components. The non-linear resonance caused by the multipole components results in rapid ejection of ions and mass resolution and mass accuracy was improved⁽¹⁾. The non-linearity of the ion trap also increases the trap efficiency and improve sensitivity and dynamic range. But excess amount of multipole components degrade the mass isolation performance. For example, with the sole presence of positive octopole field in addition to quadrupole field, the resonance curve has sharp edge on high frequency side but has gentle slope on low frequency side and that degrades the mass isolation resolution. We have reported that the mass isolation performance was improved by optimizing 3D ion trap geometry⁽²⁾. Here we report that the same improvement can be achieved for linear ion trap mass spectrometer.

Methods

To evaluate the characteristics of ion trap, it is crucial to accurately calculate the electric field. This can be done by surface charge method (SCM). But SCM is time consuming because it requires to integrate all the surface charge density on discretized electrode elements. In this work, multipole coefficients were primary calculated from surface charge⁽³⁾ and secondary, electric field was calculated from multipole coefficients. This method enabled fast calculation of the electric field with high accuracy.

The electric potential of linear ion trap shown in Fig.1 can be expressed as

$$\Phi(\rho,\theta) = V \sum_{n=0}^{\infty} \left(\frac{\rho}{x_0}\right)^n A_n \cos(n\theta), \qquad (1)$$

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where V is RF trapping voltage. A_n is n-th order multipole coefficients. x_0 is the normalizing constant which is half distance between X-electrodes. Equation (1) is differentiated to acquire the electric field E_x and E_y ;

$$-E_{x} = \frac{\partial \phi}{\partial x} = V \sum_{0}^{\infty} A_{n} \left(\frac{1}{x_{0}}\right)^{n} \left\{ n\rho^{n-2}x\cos(n\theta) + \frac{\partial}{\partial x}\cos(n\theta) \right\}$$
(2)
$$-E_{y} = \frac{\partial \phi}{\partial y} = V \sum_{0}^{\infty} A_{n} \left(\frac{1}{x_{0}}\right)^{n} \left\{ n\rho^{n-2}y\cos(n\theta) + \frac{\partial}{\partial y}\cos(n\theta) \right\}$$
(3)

To enhance the accuracy, multipole coefficients up to 100^{th} order (A_{100}) were calculated by following recursive definition of cosine function.

$$\cos((n+2)\theta) = 2\cos\theta\cos((n+1)\theta) - \cos(n\theta)$$
(4)

A 2D ion trajectory calculation program using SCM was developed by LabVIEW. The electric field components of quadrupole (A_2), octopole (A_4), dodecapole (A_6) and higher multipole up to 100th order was directly calculated from electrode surface charge⁽³⁾. The ion trajectory was

calculated by 4th order Runge - Kutta method using electric field derived from multipole component for the purpose of fast computation. The resonance curve was obtained by scanning the frequency of dipole voltage which is applied between X-electrodes.



Fig.1. Coordinate system

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3D simulation program using GPU (NVIDIA Quadro GP100) was also developed for more realistic simulation as shown in Fig.2. The surface integration was performed in parallel. Finally, the experimental instrument was constructed to confirm the validity of the simulation results.



Fig.2. 3D trajectory simulation by LabVIEW

Results

The inscribed radius of hyperbolic surface of original LIT electrodes in Fig.1 was $x_0 = y_0 = 3.924$ mm. The geometry modification of LIT was done by shifting the pair of X-electrodes and Y-electrodes to inner direction as shown in Fig.3. In this study, dx was fixed to 0.40mm and dy was varied as (A) 0.60mm, (B) 0.65mm, (C) 0.7mm, (D) 0.75mm, (E) 0.80mm. The multipole components of A₂, A₄, A₆ were calculated. Fig.4 shows the results of A₄/A₂, A₆/A₂, A₄/A₆ for these models. It is shown that A₄/A₂ is positive and A₆/A₂ is negative. Model A is dodecapole(A₆) dominant and Model E is octopole(A₄) dominant. Model D has the same absolute value and opposite sign of octopole and dodecapole. It is known that with presence of appropriate higher multipole field, the resonance curve has sharp edge which is caused by non-linear resonance⁽¹⁾. The resonance curve was calculated for all models as shown in Fig.5. Model A shows the sharp edge on low frequency side by negative dodecapole. Model E shows the sharp edge on high frequency side by positive octopole. Model B, C, D shows sharp edge on both side because of balanced amount of octopole and dodecapole. The sharp resonance edge on both frequency side of resonance curve expected to improve the mass isolation performance.

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Fig.3. Geometry modification

dx = 0.4mm					
MODEL	А	В	С	D	E
dy(mm)	0.60	0.65	0.70	0.75	0.80
A4/A2(%)	0.61	0.80	1.00	1.22	1.46
A6/A2(%)	-0.92	-1.01	-1.11	-1.22	-1.33
A4/A6	-0.66	-0.79	-0.90	-1.00	-1.10







Fig.5. Resonance curve of models

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To confirm the validity of the simulation, experimental instrument was constructed based on LCMS-8050 (Shimadzu). A set of electrodes of model D was manufactured and installed where the collision cell was located. Argon cooling gas was introduced and the vacuum of LIT was maintained approximately 0.1 Pa. Isolation q value was 0.81 and CID q value was 0.25. Dipole excitation voltage between X-electrodes was

applied by an isolation transformer which was connected to one of the secondary wiring of resonance circuit. Polyethylene-glycol 1000 in 20:80 methanol/water solution was infused and the isolation profile of m/z 921, 922, 923 were recorded by scanning the trap RF voltage amplitude while applying the isolation FNF waveform to X-electrodes. The result was shown in Fig. 6. Three peaks were more clearly resolved for modified LIT (model D) than original LIT.



Fig.6. Mass isolation profile

The trap efficiency was evaluated by CID performance. Precursor ion m/z 555 was fragmented to produce product ion m/z 547. Fig.7 shows the profile of precursor and product signal intensity by CID excitation voltage. The minimum excitation voltage to fragment precursor ion was 0.004V for original LIT and 0.012V for modified LIT. The voltage increased by a factor of three. Thus the trap efficiency was also improved.



Fig.7. CID profile



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Conclusions

The calculation program of multipole components and ion trajectory of linear ion trap was constructed and ion trap geometry was optimized to produce balanced amount of octopole and dodecapole component with opposite sign. The experimental results shows the improved mass isolation performance and trap efficiency.

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Production and evaluation of micron-sized fine grating having high aspect ratio suitable for time-of-flight mass spectrometer

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Overview

Low-cost grating suitable for orthogonal accelerator of TOF-MS is described (Table 1).

Feature of grating	Effectiveness	Advantageous for
Fine	Suppress ion divergence	Sensitivity
High transmission	Suppress ion loss	Sensitivity
Thick	Withstand high extraction field	Resolving power/sensitivity/mass accuracy
THICK	Suppress field penetration	Resolving power/low background

Table 1. Summary of advantages using present grating for TOF-MS.

Introduction

Ion optical grids are widely used to accelerate or decelerate ions in time-of-flight mass spectrometer (TOF-MS). This "real" grid has the following disadvantages: (a) ion loss on the grids, (b) divergence of ion trajectories due to lens action, and (c) low mechanical strength (Figure 1). To suppress the divergence, it is effective to reduce a pitch of grid [1]. Parallel wire grids (Figure 2a) with 20-um wires at 250-um pitch are available, giving a transmission of 92 %. This kind of grids is especially suitable for ion reflectors. On the other hand, fine electroformed meshes (Figure 2b) are used for orthogonal accelerators; however, thickness of the mesh is limited to typically 10 um by manufacturing reasons. If the extraction field inside the accelerator is increased to improve mass resolving power, mechanical strength is insufficient and these grids would be loosen or broken. It is therefore necessary to develop fine grids with high mechanical strength.



Figure 1 Disadvantages of "real" ion optical grids.



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Figure 2 Conventional ion optical grids used for mass spectrometer.

Methods

We produced a grid with pitches and a transmission similar to those of the conventional electroformed fine meshes, but the thickness was much increased up to 2 mm. This micron-sized fine "grating" having high aspect ratio was made as follows (Figure 3). First, a plurality of thin plates made of stainless steel were stacked with keeping spaces between them by sandwiching a large number of spacers

made of stainless steel, and bonded together to form an integrated block by diffusion bonding (Figure 3a). Secondly, the integrated block was cut in round slices at predetermined intervals by wire-cut discharge machining (Figure 3b). A number of gratings were obtained from one block. By this production method, machining cost per one grating was much reduced.



Figure 3 Production of micron-sized fine grating with high aspect ratio.

Production and evaluation of micron-sized fine grating having high aspect ratio suitable for time-of-flight mass spectrometer

Result

Production of micron-sized fine grating

We produced a fine grating having pitches of 100x1000 um, transmission of 70 %, and thickness of 2 mm (Figure 4). This grating has two advantages. The first is increment of the mechanical strength. To improve resolving power in orthogonal acceleration TOF-MS, it is effective to reduce turn-around time of ions by increasing the extraction field; this will cause the deformation of grids. This adverse effect can be avoided by increasing the strength by using the grating. The second is suppression of field penetration inside the accelerator. When ions are introduced between the push-out electrode and the extraction grid, if the thickness of the grid is thin, a high voltage applied to the flight tube penetrates into the acceleration region. As a result, ions introduced are bent (Figure 5a), and the resolving power and/or the signal intensity will be lowered. Also, ions continue to flow into the flight tube, causing a background noise. To suppress this penetration in the prior art, a potential barrier is formed by using a plurality of grids at some interval apart. However, this has the following problems. (1) These grids are needed to align with high accuracy to keep high transmission. (2) The voltages applied to them need to be timing-controlled independently, thus the cost increases. (3) The jitter of these voltages can result in adverse effect for mass accuracy. Contrary to the conventional grid, our grating can completely avoid the field penetration as confirmed by simulation study (Figure 5b).



Figure 4 Photograph of micron-sized fine grating with high aspect ratio.



Figure 5 Field penetration through grid/grating. Green lines indicates contours.



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Application to orthogonal-acceleration TOF-MS

To evaluate the effectiveness experimentally, the grid was introduced into an orthogonal acceleration TOF-MS (Figure 6). Briefly, high-efficiency ion guide, guadrupole, and the UFsweeper III Collision Cell are all part of the UFMS technologies established for the LCMS-8060. The speed and sensitivity gained by the UFMS architecture are in complete synergy with the following technologies for maximizing mass accuracy and resolving power. The present grating (UFgrating) makes it possible to improve both resolving power and sensitivity by increasing extraction field. Since deformation of the grid electrode during ion ejection can be suppressed, the reproducibility of each ion ejection is improved, contributing to high mass accuracy. Furthermore, the penetration of electric field from the flight tube is completely suppressed, so background ions during data collection can be removed.

An "ideal" reflectron (iRefTOF) is another key technology of the present TOF-MS enabling to obtain both high resolving power and high sensitivity at the same time. When high extraction field is used for the accelerator, energy spread of ejected ions becomes large. Since the energy-focusing ability is low in a reflectron consisting of uniform electric fields, which is widely used, it is necessary to reduce ion spatial spread inside the accelerator to obtain high resolving power at the sacrifice of the sensitivity. We adopted the reflectron consisting of the non-uniform field; this "ideal" electric field enhances the energy-focusing ability while minimizing both the divergence of the reflected ion trajectories and the time-aberration caused for the ions on a path dislocated from the central axis of the reflectron.



Figure 6 Experimental setup of a new orthogonal-acceleration TOF-MS.

As a test of the system, mass spectrum of Na(Nal)_n was obtained (Figure 7a). Although the pulsed voltages were applied to both the push-out electrode and the extraction grating, the observed TOFs plotted against the square root

of the theoretical masses were completely straight within 1 ppm over the whole mass range (Figure 7b). The background due to stray ions were completely suppressed.



Production and evaluation of micron-sized fine grating having high aspect ratio suitable for time-of-flight mass spectrometer



Figure 7 (a) Mass spectrum of Na(Nal)_n. (b) Observed mass error plotted against m/z. Different colours indicate independent measurements.

Conclusions

Low-cost grating which contribute to high resolution, high sensitivity, and high mass accuracy of TOF-MS was produced and experimentally verified.

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Introduction

Extra virgin olive oil (EVOO) is known for its nutraceutical properties, which associate it with several health benefits and a high economic value. For these reasons, EVOO is often a target of adulteration with cheaper, lower-grade vegetable oils, typically, sunflower, corn and soybean. Within the quality control process of EVOO products, it is fundamental to develop rapid, simple and robust analytical methods to detect any fraud. Here, we present a simple approach based on the profiling of triacylglycerols (TAGs) using MALDI-TOF mass spectrometry and an evolved neural network based on a logistic regression machine learning algorithm to reveal the adulteration of extra virgin olive oils by seed oils.



Figure 1. Left: MALDI-8020 Benchtop Linear MALDI-TOF mass spectrometer. Right: sample analysis workflow. (Picture taken from: https://www.iobenessereblog.it/olio-extravergine-di-oliva-benefici/26429).

Methods and Materials

EVOO and sunflower oils were purchased from local stores. Sample preparation involved dissolution of oil aliquots in chloroform. To simulate the adulteration, mixtures of EVOOs containing 5%, 10% and 20% of sunflower oil were prepared. Tricaprin was used as internal standard for mass alignment and the semi-quantitative analyses. LDI (matrix-free) analyses were conducted on a MALDI-8020 benchtop linear MALDI-TOF mass spectrometer (Shimadzu, Manchester, UK; Figure 1), by spotting the oil sample solutions directly onto the MALDI target which was previously pre-coated with NaTFA. Data were acquired in quadruplicates for each scenario and processed using Clover MS software (Clover Bioanalytical Software, Granada, Spain) for peak area calculation and classification with neural networks.

Results and Discussion

MALDI analyses

Figure 2a shows a comparison between the TAG profiles of an EVOO and a sunflower oil. It can be seen how, in EVOO (red trace), naturally rich in palmitic (P) and oleic (O) acids, the TAGs at *m*/*z* 881 and 907, i.e. most likely OPO and OOO, are predominant. In sunflower oil (blue trace), highly rich in linoleic acid (L), the most representative TAGs are those at m/*z* 901, 903 and 905, i.e. most likely LLL, OLL and OLO, respectively. In the oil mixture scenario (Figure 2b), the alteration of the natural TAG ratios in EVOO, e.g. *m/z* 877/907, 881/907, 903/907 and 905/907, can be observed. Interestingly, the TAG at *m/z* 901 (LLL), characteristic of sunflower oil but not normally present in EVOO, is revealed in the EVOO/sunflower mixtures even at the smallest adulteration level.



Figure 2. a) LDI MS spectra of a pure EVOO (red trace) and sunflower oil (blue trace). Right panel: expansion of the mass spectra showing the region of representative TAGs of EVOO (red trace) and sunflower oil (blue trace). b) Expansions of the overlaid mass spectra of EVOO (red trace), EVOO + 5% sunflower oil (blue trace), EVOO + 10% sunflower oil (green trace), EVOO + 20% sunflower oil (orange trace), sunflower oil (purple trace), showing the variation of TAGs and their ratios.
P = palmitic acid; O = oleic acid; L = linoleic acid.
I.S. = internal standard.

Semi-quantitative analyses

Figure 3 shows the plots of the ratios of EVOO's TAG markers and the TAG at *m/z* 907 (the most abundant and representative in EVOO), versus the different levels of adulterant oil (from 0%, i.e. pure EVOO, to 20% sunflower



Figure 3. Plots of *m/z* 877/907, 901/907, 903/907 and 905/907 ratios (*x*-axis) versus percentage of EVOO adulteration (from 0%, i.e. pure EVOO, to 20% sunflower oil; *y*-axis).

Neural Network Training and Classification

Artificial Neural Networks (ANNs) are one of the well-known cutting edge technologies used for classification problems given the huge amount of data available nowadays. They are able to learn specific features from a given dataset. On the other hand, logistic regression models have been typically used for binary classification on linearly separable datasets. We show that the use of ANNs with a logistic regression model seems to be a fast and efficient combination to detect different types of oil samples including the adulterated ones. We have created a three layers neural network able to classify between the EVOO, adulterated EVOO and sunflower oil categories (Figure 4). Prior to the classification, all spectra were aligned and normalised by the 903 Da mass. A total of 267 spectra were used to train and validate the neural network. Thirty single-blinded spectra were used to test the model accuracy (Figure 5).

oil). All TAGs were normalised against the internal standard

using the area of the peaks from quadruplicate analyses. A

good linearity has been achieved along with good

coefficients of determination (R²).



NN Parameters Definition:

- Input Data: (1188, 222)
- Validation Data: (1188, 45)
- Categories: 3
- Epochs: 75
- Batch Size: 10
- Nodes Hidden I: 50
- Nodes Hidden II: 25
- Learning Rate: 0.00025

NN Accuracy and Testing:

- Validation Accuracy: 97.78%
- 30 single-blinded spectra: 100%



Figure 4. Representation of the neural network defined.



Figure 5. The linearity shown in the spectrum (left) after alignment and normalization of the input data using the Clover MS Software. The trained model classification results (left) over the 30 single-blinded samples.

Conclusions

The combination of MALDI-TOF MS and the use of a cutting edge machine learning technique has been proven to be suitable for the detection of adulterated EVOO. The efficiency and simplicity of the methodology proposed is

the key point of this research. The promising results achieved, and the expansion of the dataset and categories to be detected will determine the future viability of the system and its introduction into the oil industry.

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