2023 迷你食品應用文集

第一期

SCIEX
The Power of Precision

內容提要

動植物產品中農藥殘留分析

食品組學與營養成分析

食品包材溶出物

動物源性食品中五氯酚殘留量的LC-MS/MS定量分析檢測

Determination of pentachlorophenol residue in animal-derived foods by LC-MS /MS

張小剛[1]，王霞[2]，楊總[1]，劉冰潔[1]，郭立海[1]

Zhang Xiaogang[1], Wang Xia[2], Yang Zong[1], Liu Bingjie[1], Guo Lihai[1]

*SCIEX*應用支援中心，中國[1]，上海市農產品品質安全中心[2]

SCIEX Application Support Center, China[1], Shanghai Center of Agri-products Quality and Safety[2]

Keywords: animal-derived foods , pentachlorophenol

引言

五氯酚（Pentachlorophenol, PCP）及其鈉是一種高毒有機氯農藥，常被當作除草劑、殺蟲劑等使用。五氯酚具有致畸、致癌、致突變等毒副作用，其化學性質穩定難以降解，殘存於水體、土壤中並通過食物鏈進入高等動物體內影響人體安全。目前五氯酚鈉殘留量的分析方法主要有氣相法、氣相色譜-質譜法、高效液相色譜法、液相色譜-串聯質譜法等。氣相色譜法和氣質色譜法需柱前衍生，操作相對繁瑣，液相色譜法易出現假陽性，液相色譜-串聯質譜法具有選擇性好、靈敏度高等特性，目前廣泛應用於五氯酚鈉的測定。目前測定五氯酚的液質聯用標準主要為中國食品安全國家標準GB 23200.92-2016，然而在實驗過程中會碰到諸多問題，例如前處理操作不當會導致回收率偏低、色譜分離不好會有嚴重的基質幹擾等問題。基於此本文採用SCIEX液相色譜串聯三重四極杆質譜建立了動物源性食品中五氯酚的快速定量分析檢測方法以解決五氯酚測定問題。

本實驗方法具有如下特點：

1、詳細優化了色譜條件，有效的避開基質幹擾，定量結果更準確。

2、回收率高、穩定性好：肉空白基質添加1.0 μg/kg 和5.0 μg/kg 兩個濃度，每個添加濃度重複6次，平均回收率分別是80.5%和96.4%（n=6），相對標準差小於3.1%。

3、該方案解決了回收率偏低的問題：氮吹不宜吹幹，定容液用PTFE膜進行過濾。

1 實驗方法

1.1 樣品前處理

稱取試樣2 g（精確到0.01 g）於50 mL離心管中，加入5 mL 5%三乙胺的 乙腈-水溶液均質1 min。10000 r/min離心5 min，收集上清液於一具刻度離心管中。離心後的殘渣再用5 mL 5%三乙胺的乙腈- 水溶液重複提取1次，合併上清液。將上清液轉入經甲醇和水預處理過的MAX固相萃取柱中，棄去流出液。依次用5 mL 5%氨水溶液、5 mL甲醇、 5mL 2 % 甲酸的甲醇-水溶液淋洗，抽幹。以5 mL 8%甲酸甲醇溶液洗脫，收集洗脫液，于40 ℃水浴下吹氮濃縮低於2 mL，用水定容至2 mL，渦旋混勻，過0.22 μm PTFE濾膜，供測定。

1.2 液相色譜條件

液相系統：SCIEX ExionLC™ AD 系統

色譜柱：Phenomenex C18（100×2.1 mm, 1.7 μm）

流動相：A為5 mmol/L乙酸銨溶液，B為甲醇

流速：0.3 mL/min

柱溫：40℃

洗脫程式：梯度洗脫（表1）

表1. 液相梯度洗脫

Time (min)	A%	B%
0.0	85	15
0.5	85	15
4.0	5	95
6.0	5	95
6.1	85	15
8.0	85	15

1.3 質譜條件

掃描模式：多反應監測MRM，負離子模式，MRM離子對見（表2）

離子源：ESI源；

噴霧電壓（IS）：-4500V； 離子源溫度（TEM）：550℃；

氣簾氣（CUR）：35psi； 碰撞氣（CAD）：Medium；

霧化氣（GS1）：35psi； 輔助霧化氣（GS2）：60psi。

表2.離子對資訊

母離子 (m/z)	子離子 (m/z)	保留時間 (min)	化合物名稱	去簇電壓(V)	碰撞能量(eV)
262.8	262.8	4.89	PCP 1	-75	-10
264.8	264.8	4.89	PCP 2	-75	-10
266.8	266.8	4.89	PCP 3	-75	-10
268.8	268.8	4.89	PCP 4	-75	-10

2 實驗結果與討論

2.1 色譜條件優化

實驗詳細優化了色譜條件，比較了不同品牌、不同型號的色譜柱以及流動相，最終選擇的色譜柱是Phenomenex C18，流動相為A為5 mmol/L乙酸銨溶液，B為甲醇，保證五氯酚有較好的保留（圖1），並且有效的避開基質幹擾，定量結果更準確。

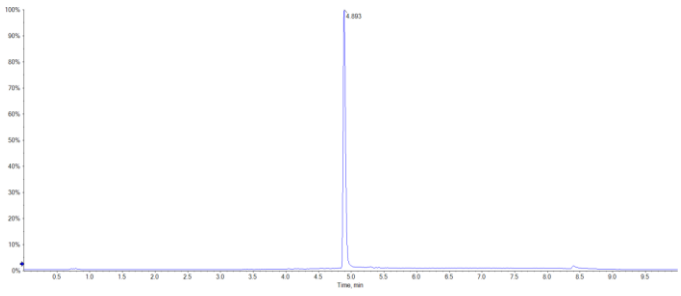


圖1. 五氯酚色譜圖

2.2 方法考察了回收率、重複性、線性等

肉空白基質添加1.0 μg/kg 和5.0 μg/kg 兩個濃度，每個濃度重複6次，平均回收率分別是80.5%和96.4%（n=6） ，相對標準差小於3.1%（表3），實驗結果表明該方法具有較好的回收率以及良好的穩定性。基質加標曲線相關係數r> 0.9996（圖2），表明線性良好。該實驗方法完全滿足標準GB 23200.92-2016定量檢測的要求。

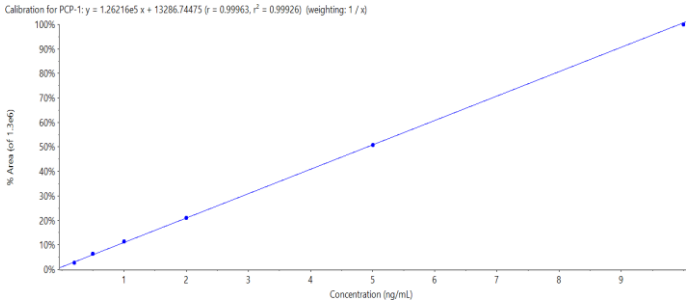


圖2. 五氯酚基質匹配曲線

表3.五氯酚回收率及重複性實驗（n=6）

化合物名稱	添加濃度 (μg/kg)	平均回收率 (%)	相對標準差(%)
五氯酚	1.0	80.5	2.0
	5.0	96.4	3.1

2.3 真實樣本的測試

測定了5個樣本，每個平行測定6次，4#樣本中檢出五氯酚，測定值為3.5 μg/kg ，相對標準差為2.7%（表4），其餘四個均未檢出（ND）。

化合物名稱	樣品編號	測定值 (μg/kg)	相對標準差(%)
五氯酚	1#	ND	/
	2#	ND	/
	3#	ND	/
	4#	3.5	2.7
	5#	ND	/

3 小結

本文建立了高效液相色譜-串聯三重四極杆質譜快速定量分析檢測動物源性食品中五氯酚鈉的檢測方法。實驗詳細優化了前處理過程，保證了該方法有較高的回收率；詳細優化了色譜條件，有效的避開基質幹擾，定量結果更準確。該方法足以滿足GB 23200.92-2016的定量要求，在動物源食品中對污染物五氯酚的分析檢測具有重要的參考意義。

Determination of pyrethroids and macrocyclic lactone insecticides in spices and tea

Using the SCIEX 7500 system

Cathy Lane,[1] Sara Cheikh Ibrahim,[2] Tino Schroeder,[2] Susanne Hergett,[2] Jack Steed,[1] Jianru Stahl-Zeng,[3] Roy Sperling[2]
[1]SCIEX, UK; [2]Bilacon, Germany; [3]SCIEX, Germany

Pyrethroids and macrocyclic lactones are groups of commonly used insecticides in the agriculture and horticulture industries. Macrocyclic lactones are naturally occurring, or semisynthetic, compounds produced as fermentation products in soil-dwelling *Streptomyces avermitilis.*[1] Pyrethroids, on the other hand, are synthetic, and were designed based on the naturally occurring family of pyrethrins, which were originally derived from chrysanthemum flowers.[2]

Due to the widespread use of these compounds in the environment, a comprehensive quantitative method is necessary to monitor and control their concentration in final food products destined for human consumption.

Here, a method has been developed using the SCIEX 7500 system for the simultaneous identification and quantification of pyrethroid and macrocyclic lactone insecticides at detection levels below the maximum residue level defined by the European Commission under regulation 2018/1514.[3]

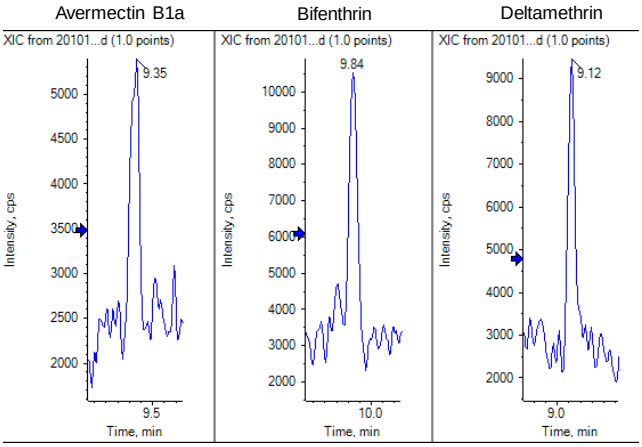


Figure 1. Signals obtained for avermectin B1a, bifenthrin and deltamethrin at their respective LLOQs. A good signal is obtained at a concentration level of 0.02 ng/mL, highlighting the sensitivity that can be achieved when using the SCIEX 7500 system.

Key features of the SCIEX 7500 system for the quantification of pesticides in spices and green tea

- Highly sensitive detection and quantification of avermectin (containing 96% avermectin B1a and 4% avermectin B1b), bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerat, λ-cyhalothrin, milbemectin A3 and A4 and permethrin from QuEChERS extracts of spices and green tea
- Improved sensitivity over previous assays with lower limits of quantification (LLOQs) down to 0.02 ng/mL in solvent (Figure 1)
- Optimization of new parameter Q0D for milbemectin A3 in spices resulted in greatly reduced background and increased signal to noise
- Increased sensitivity allows for the use of lower sample injection volumes, increasing assay robustness and significantly reducing ion suppression in matrix

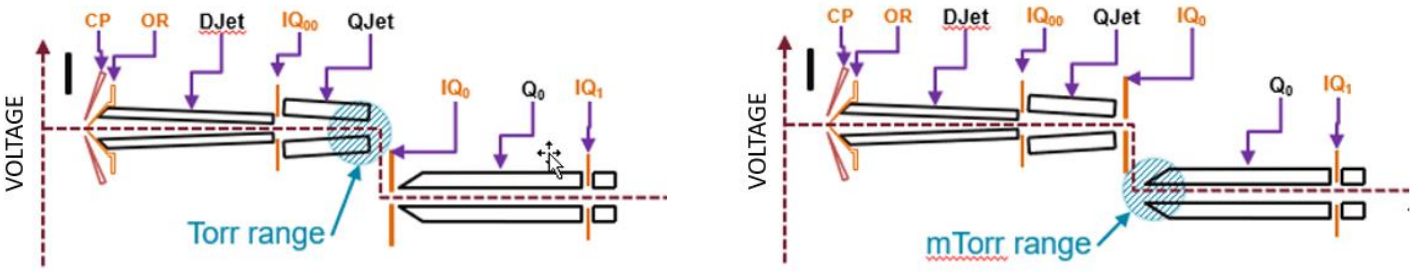


Figure 2. Additional declustering of ions using Q0 dissociation. On the SCIEX 7500 system, additional declustering can be applied during LC-MS analysis using 2 modes: Q0D simple (left) and Q0D enhanced (right). Use of a voltage differential, applied either between the QJet ion guide rods and IQ0 lens or between the IQ0 lens and Q0 on the SCIEX 7500 system, can aid in breaking up clusters, removing interferences and increasing signal to noise.

Methods

Sample preparation: Avermectin (containing 96% avermectin B1a and 4% avermectin B1b), bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerat, λ-cyhalothrin, milbemectin A3 and A4 and permethrin were diluted into mobile phase A for analysis. Calibration curves were constructed in mobile phase A by spiking in compounds at concentrations of 0.02–20 ng/mL.

Organic green tea (1 g) was milled to a fine powder, homogenized and added to 10 mL of water and 10 mL of acetonitrile. For the spices (organic paprika powder), a starting mass of 2 g was used. The sample was shaken for 1 min and added to Macherey-Nagel QuEChERS Mix I (ref 730970). The sample was shaken for another 3 min and centrifuged for 5 min. Next, 8 mL of the organic (upper) phase was added to Macherey-Nagel QuEChERS Mix III (ref 730648). The sample was shaken for 3 min, centrifuged for 5 min and 7 mL was then taken and acidified with 5% formic acid in acetonitrile (10 µL per 1 mL of sample taken). Compounds were spiked into the tea extract at 1 ng/mL and 10 ng/mL, corresponding to 10 ppb and 100 ppb in the original tea and 5 ppb and 50 ppb in the original spice sample. Samples were analyzed on the SCIEX 7500 system without further dilution.

Chromatography: Chromatographic separation was performed using the ExionLC AD system and a Phenomenex Synergi Fusion-RP (4 µm, 50 x 2.1 mm) column at a flow rate of 0.25 mL/min. Mobile phase A was 5 mM ammonium formate in 20% methanol and 80% water (v/v); mobile phase B was 5 mM ammonium formate in 90% methanol and 10% water (v/v). A gradient of 0–100% mobile phase B over 8 min was used. The total analysis time was 17 min, using either a 2 µL or 5 µL injection volume. The column temperature was 40°C.

Mass spectrometry: Multiple reaction monitoring (MRM) analysis was performed using a SCIEX 7500 system. The system was operated in positive electrospray ionization (ESI) mode using the OptiFlow Pro ion source. Data was acquired using SCIEX OS software. Source conditions, which were optimized using the guided optimization tool in SCIEX OS software, were ISV 3500 V, GS1 40, GS2 70, TEM 250ºC and CUR 50.

Data processing: Data was processed using SCIEX OS software.

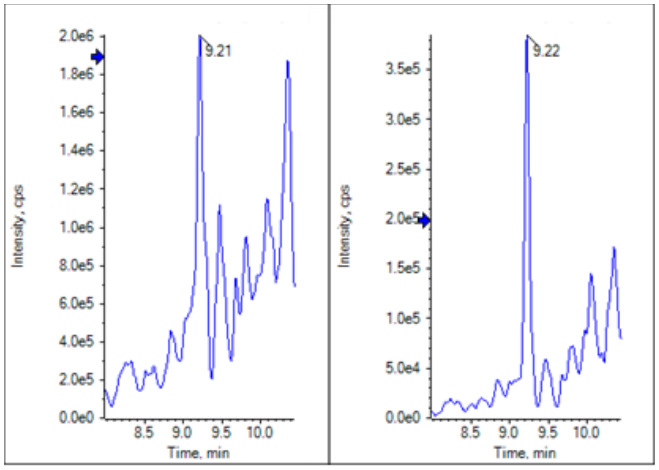


Figure 3. Sensitivity increase resulting from optimization of parameters for Q0D enhanced for milbemectin A3 in spices. XIC for milbemectin A3 using default Q0D value of -10 V (left); XIC for milbemectin A3 using optimized Q0D enhanced mode value of 25 V (right). Milbemectin was analyzed at a concentration of 50 ppb (2 µL injection volume). A signal to noise increase of 3.5-fold was observed after optimization of Q0D.

Method development and optimization

An MRM method was first developed and optimized on the SCIEX Triple Quad 5500 system and targeted more than 500 pesticides and their metabolites. Eleven of the most challenging compounds from the multi-component method were selected for evaluation on the SCIEX 7500 system, and the method was adapted. Using the guided optimization tool in SCIEX OS software, collision energies were re-optimized for the SCIEX 7500 system. Source conditions were also optimized using the guided optimization tool in SCIEX OS software.

Additional declustering of ions can be achieved on the SCIEX 7500 system by using a voltage differential applied either between the QJet ion guide and IQ0 lens (Q0 dissociation, Q0D simple) or between the IQ0 lens and Q0 rods (Q0D enhanced), illustrated in Figure 2. Q0D is useful for breaking up clusters, decreasing interferences and increasing signal to noise in some cases. Q0D was optimized for a subset of the analytes that were the most difficult to detect in green tea and spices. MRM extracted ion chromatograms (XICs) for milbemectin A3 in spices that were acquired using both default settings and optimized Q0D enhanced settings are shown in Figure 3. In this case, a signal to noise increase of 3.5-fold was observed after optimization of Q0D.

The final MRM assay comprised 1 MRM transition per compound. Separation of target compounds is shown in Figure 4.

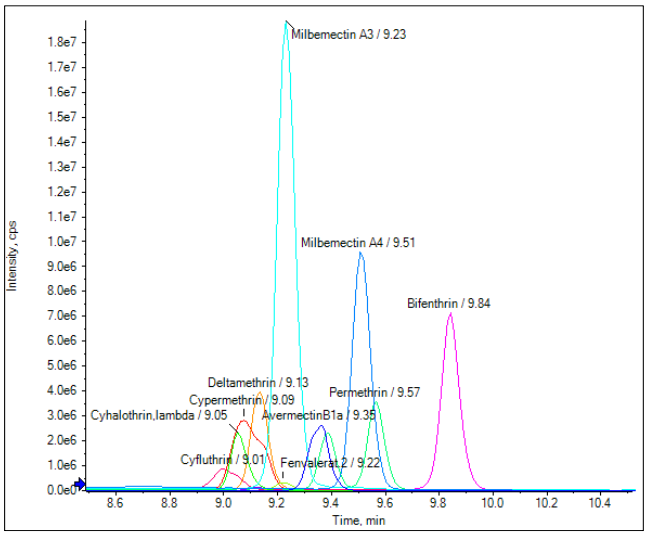


Figure 4. Chromatographic separation of 10 compounds. Avermectin (containing 96% avermectin B1a and 4% avermectin B1b), bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerat, λ-cyhalothrin, milbemectin A3 and A4 and permethrin at a concentration of 10 ng/mL (5 µL injection volume) in mobile phase A.

Dilution series in solvent

The optimized MRM assay was applied to the analysis of a dilution series of target compounds in mobile phase A. Standards were prepared in the range 0.01–20 ng/mL; 5 µL sample was injected per analysis. Each sample was analyzed 3 times. Examples of calibration curves and MRM XICs from the low end of the dilution series for 3 compounds are shown in Figure 5. LLOQs and associated statistics for all compounds are shown in Table 1.

Table 1. LLOQs and associated statistics for a dilution series of 10 compounds in solvent. Avermectin contains 96% avermectin B1a and 4% avermectin B1b. For each compound, linearity was observed from the LLOQ to the 20 ng/mL top standard. A linear regression with 1/x weighting was applied to the data.

Compound	Q1 (*m/z*)	Q3 (*m/z*)	Retention time (min)	LLOQ (ng/mL)	# Values at LLOQ	% Accuracy at LLOQ	% CV at LLOQ	Linearity (r value)
AvermectinB1a	*890.5*	*305.1*	*9.35*	*0.02*	*3 of 3*	*142*	*7.4*	*0.9964*
AvermectinB1b	*876.5*	*291.1*	*9.12*	*0.50*	*3 of 3*	*99.9*	*6.6*	*0.9958*
Bifenthrin	*440.2*	*181.1*	*9.84*	*0.02*	*3 of 3*	*154*	*4.1*	*0.9937*
Cyfluthrin	*450.9*	*434.0*	*8.99*	*0.50*	*3 of 3*	*96.9*	*5.1*	*0.9890*
Cypermethrin	*433.1*	*191.0*	*9.09*	*0.02*	*3 of 3*	*106*	*10.5*	*0.9918*
Deltamethrin	*522.9*	*280.7*	*9.13*	*0.02*	*3 of 3*	*97.4*	*3.4*	*0.9924*
Fenvalerat	*437.0*	*125.0*	*9.22*	*0.20*	*3 of 3*	*93.4*	*5.6*	*0.9915*
λ-cyhalothrin	*467.0*	*225.0*	*9.06*	*0.05*	*3 of 3*	*110*	*12.6*	*0.9897*
Permethrin	*408.2*	*355.1*	*9.57*	*0.05*	*3 of 3*	*107*	*12.6*	*0.9914*
Milbemectin A3	*546.1*	*511.2*	*9.24*	*0.05*	*3 of 3*	*99.3*	*6.6*	*0.9987*
Milbemectin A4	*560.0*	*525.2*	*9.52*	*0.05*	*3 of 3*	*99.5*	*10.4*	*0.9965*

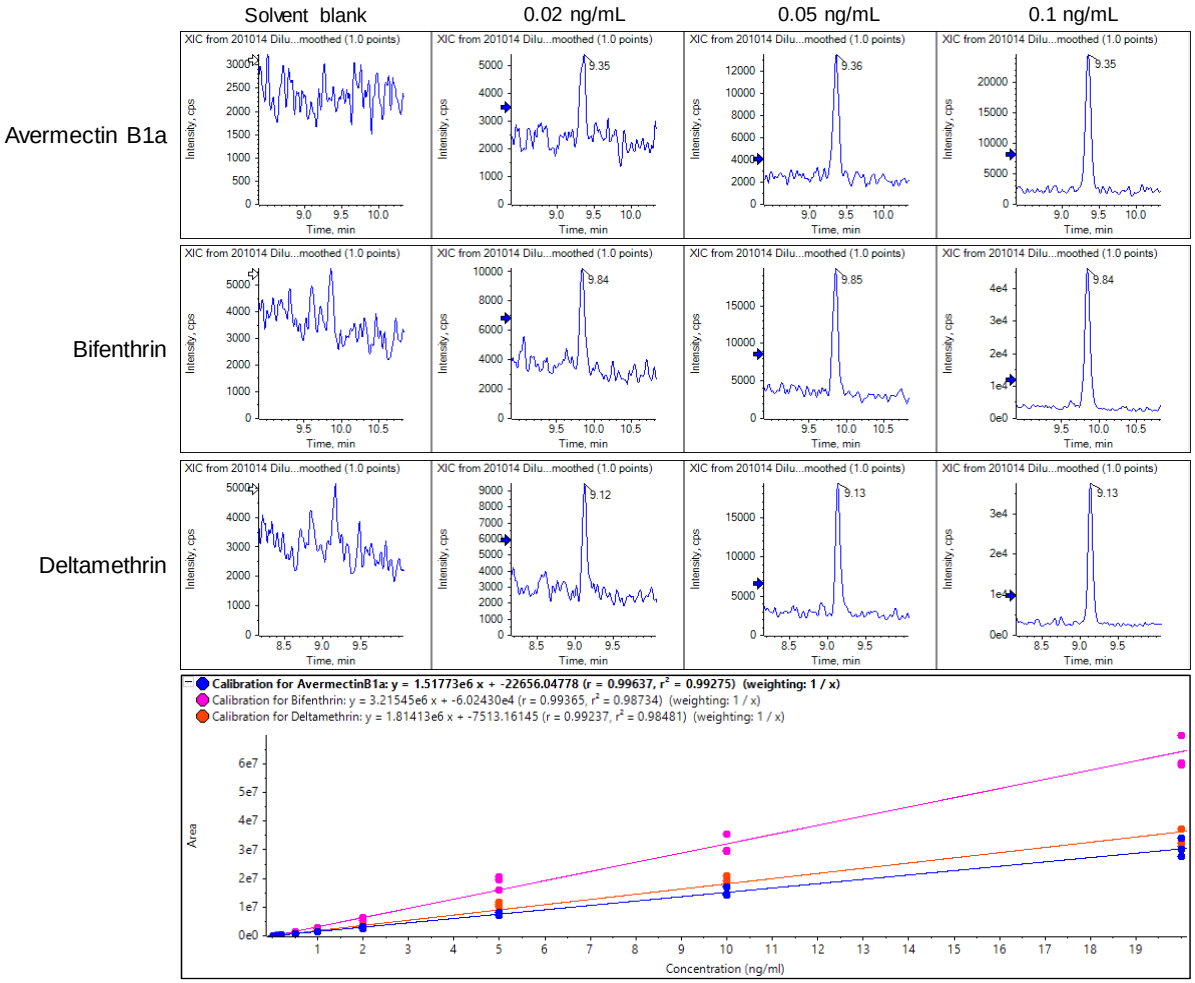


Figure 5. MRM XICs from the low end of the calibration curves for avermectin B1a, bifenthrin and deltamethrin. An LLOQ of 0.02 ng/mL was obtained for each of these 3 compounds in solvent with linearity maintained across the entire dilution series (all r values ≥ 0.9924).

Compounds spiked into spices and green tea

Compounds were next spiked into QuEChERS extracts of spices and green tea at 1 ng/mL and 10 ng/mL, corresponding to 5 ppb and 50 ppb in spices and 10 ppb and 100 ppb in tea. Quantification of target compounds in matrix against the dilution series in solvent was performed using both 2 µL and 5 µL injection volumes to assess the effect of lowering the injection volume on matrix ion suppression. It was found that the reduction in ion suppression observed for a 2 µL injection compared with a 5 µL injection mostly compensated for the reduction in injection volume to the extent that the sensitivity of the assay was not significantly impacted (Table 2 and Figure 6). Figure 7 demonstrates the background signal and ion suppression observed with a 2 µL injection volume; however, the level of ion suppression was much less than the ion suppression observed with higher volume injections (Figure 6).

It is noted that for bifenthrin, an unexpected increase in signal was observed in the QuEChERS spiked samples compared with the dilution series in solvent. The source of this anomalous result is unknown and requires further investigation. See Table 3 and Table 4 for a summary of each compound's ion suppression results and concentrations of detection (mean calculated concentration) in the matrices analyzed.

Compound	Q1	Q3	Spices, 2 µL Injection			Tea, 2 µL Injection		
			Blank	5 ppb	50 ppb	Blank	10 ppb	100 ppb
AvermectinB1a	890.5	305.1	×	✓	✓	×	✓	✓
AvermectinB1b	876.5	291.1	×	×	×	×	×	×
Bifenthrin	440.2	181.1	✓	✓	✓	×	✓	✓
Cyfluthrin	450.9	434.0	×	×	✓	×	×	✓
Cypermethrin	433.1	191.0	×	×	✓	×	✓	✓
Deltamethrin	522.9	280.7	×	✓	✓	×	✓	✓
Fenvalerat	437.0	125.0	×	×	✓	×	✓	✓
Cyhalothrin,lambda	467.0	225.0	×	×	✓	×	✓	✓
Permethrin	408.2	355.1	×	×	✓	×	✓	✓
Milbemectin A3	546.1	511.2	×	×	✓	×	×	✓
Milbemectin A4	560.0	525.2	×	×	✓	×	×	✓

Compound	Q1	Q3	Spices, 5 µL Injection			Tea, 5 µL Injection		
			Blank	5 ppb	50 ppb	Blank	10 ppb	100 ppb
AvermectinB1a	890.5	305.1	×	✓	✓	×	✓	✓
AvermectinB1b	876.5	291.1	×	×	×	×	×	×
Bifenthrin	440.2	181.1	✓	✓	✓	×	✓	✓
Cyfluthrin	450.9	434.0	×	×	✓	×	×	✓
Cypermethrin	433.1	191.0	×	×	✓	×	✓	✓
Deltamethrin	522.9	280.7	×	✓	✓	×	✓	✓
Fenvalerat	437.0	125.0	×	×	✓	×	✓	✓
Cyhalothrin,lambda	467.0	225.0	×	×	✓	×	✓	✓
Permethrin	408.2	355.1	×	×	✓	×	✓	✓
Milbemectin A3	546.1	511.2	×	×	✓	×	×	✓
Milbemectin A4	560.0	525.2	×	×	✓	×	×	✓

Table 2. Detection of spices and green tea. Top: 2 µL sample injected per analysis. Bottom: 5 µL sample injected per analysis. No difference in sensitivity was observed when using a 2 µL injection compared to a 5 µL injection volume. This may be due to the reduction in ion suppression observed for a lower volume injection (see Figure 6).

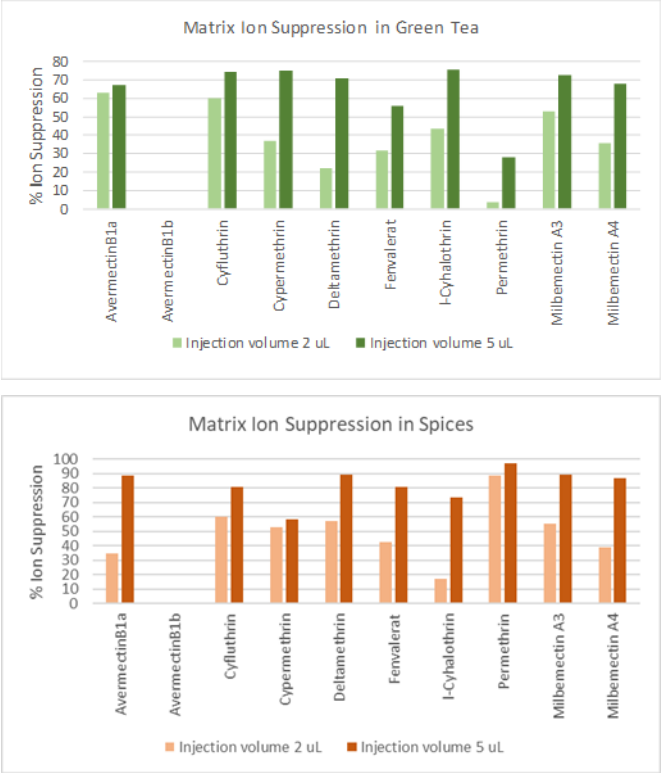


Figure 6. Ion suppression in green tea at 100 ppb (top) and spices at 50 ppb (bottom) versus solvent for injection volumes of 2 µL and 5 µL. Each sample was analyzed 3 times at each injection volume, and concentrations were calculated against a dilution in solvent. The % ion suppression values were calculated based on expected concentrations. See Table 3 and Table 4 for more detail.

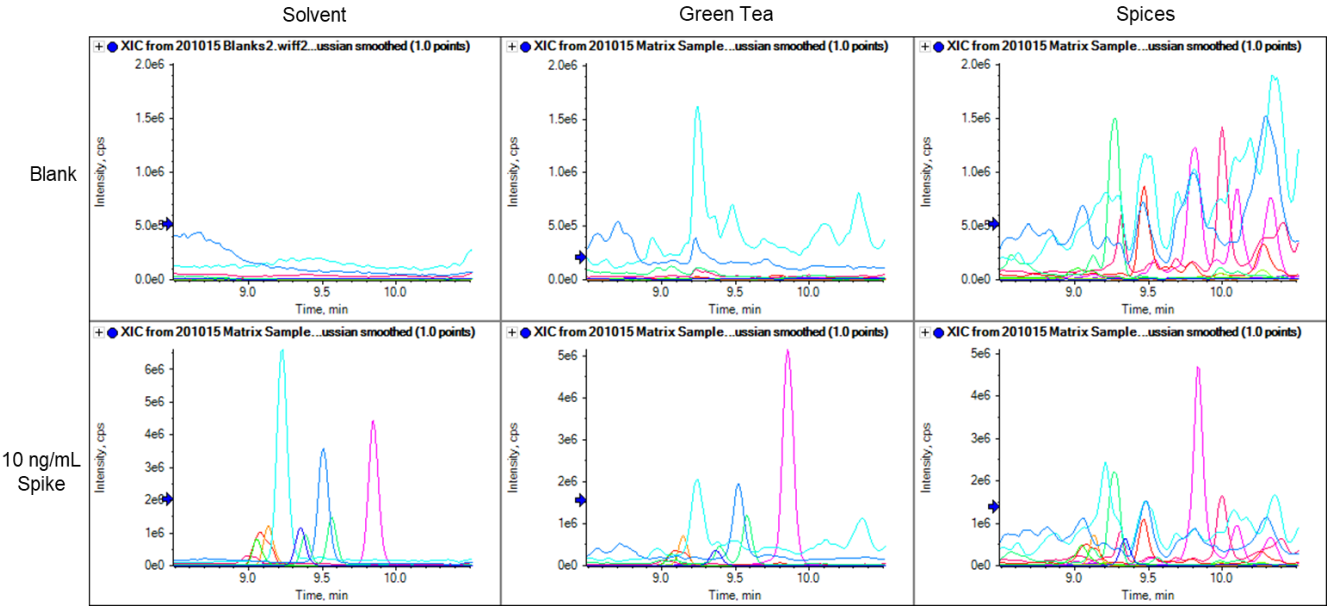


Figure 7. Analysis of 10 compounds in different matrices. MRM XICs for 10 compounds spiked into solvent (left), and into QuEChERS extracts of green tea (middle) and spices (right) using a 2 µL injection volume. Data is from blank samples, shown at the top, and samples with a concentration of 10 ng/mL (100 ppb in green tea and 50 ppb in spices), shown at the bottom. High levels of background signals are present in the green tea and spice QuEChERS extracts compared with analysis of compounds spiked into solvent alone.

Table 3. Quantification of 10 compounds in green tea against a dilution series in solvent. A QuEChERS extract of green tea was spiked at 1 ng/mL and 10 ng/mL (10 ppb and 100 ppb) with each of 10 compounds and analyzed using the SCIEX 7500 system. Two injection volumes (2 µL and 5 µL) were used. Each sample was analyzed 3 times at each injection volume. Calculated concentrations against solvent curves were adjusted for injection volume.

Compound	Solvent RT (min)	Green tea, 10 ppb, 2 µL injection			Green tea, 100 ppb, 2 µL injection			Green tea, 10 ppb, 5 µL injection			Green tea, 100 ppb, 5 µL injection		
		Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression
AvermectinB1a	9.35	9.35	4.1	59.5	9.36	36.4	63.3	9.35	2.7	73.4	9.33	32.7	67.3
AvermectinB1b	9.12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bifenthrin	9.84	9.84	23.9	-138.8	9.85	264.0	-163.9	9.84	14.3	-42.5	9.83	164.0	-63.8
Cyfluthrin	8.99	ND	ND	ND	9.02	39.8	60.3	ND	ND	ND	9.00	25.4	74.6
Cypermethrin	9.09	9.10	3.5	64.8	9.10	63.0	37.1	9.10	1.9	80.7	9.10	24.9	75.1
Deltamethrin	9.13	9.13	4.7	53.3	9.13	77.9	22.1	9.13	3.0	70.2	9.13	29.5	70.5
Fenvalerat	9.22	9.21	6.9	31.5	9.22	68.1	31.9	9.21	3.8	62.2	9.21	44.2	55.8
λ-cyhalothrin	9.06	9.05	3.5	65.5	9.07	56.3	43.7	9.05	2.5	74.9	9.05	24.6	75.4
Permethrin	9.57	9.56	7.4	26.0	9.57	96.5	3.5	9.56	9.8	1.9	9.56	72.1	27.9
Milbemectin A3	9.24	ND	ND	ND	9.23	47.2	52.9	ND	ND	ND	9.21	27.5	72.5
Milbemectin A4	9.52	ND	ND	ND	9.51	64.3	35.8	ND	ND	ND	9.45	32.2	67.9

ND = not detected

Table 4. Quantification of 10 compounds in spices against a dilution series in solvent. A QuEChERS extract of spices was spiked at 1 ng/mL and 10 ng/mL (5 ppb and 50 ppb) with each of 10 compounds and analyzed using the SCIEX 7500 system. Two injection volumes (2 µL and 5 µL) were used. Each sample was analyzed 3 times at each injection volume. Calculated concentrations against solvent curves were adjusted for injection volume.

Compound	Solvent RT (min)	Spices, 5 ppb, 2 µL injection			Spices, 50 ppb, 2 µL injection			Spices, 5 ppb, 5 µL injection			Spices, 50 ppb, 5 µL injection		
		Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression
AvermectinB1a	9.35	9.33	2.6	47.8	9.34	32.6	34.8	9.29	0.7	86.8	9.29	5.8	88.4
AvermectinB1b	9.12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bifenthrin	9.84	9.82	8.8	-75.3	9.84	69.0	-38.0	9.81	1.5	70.6	9.82	15.4	69.2
Cyfluthrin	8.99	ND	ND	ND	9.02	20.0	60.2	ND	ND	ND	9.00	9.6	80.8
Cypermethrin	9.09	ND	ND	ND	9.09	23.6	52.9	ND	ND	ND	9.07	21.0	58.1
Deltamethrin	9.13	9.13	1.7	66.8	9.13	21.6	56.9	9.13	0.6	88.3	9.12	5.6	88.9
Fenvalerat	9.22	ND	ND	ND	9.21	28.9	42.3	ND	ND	ND	9.19	9.7	80.6
λ-cyhalothrin	9.06	ND	ND	ND	9.04	41.6	16.8	ND	ND	ND	9.02	13.3	73.4
Permethrin	9.57	ND	ND	ND	9.56	5.8	88.4	ND	ND	ND	9.54	1.4	97.2
Milbemectin A3	9.24	ND	ND	ND	9.21	22.3	55.5	ND	ND	ND	9.17	5.4	89.2
Milbemectin A4	9.52	ND	ND	ND	9.48	30.6	38.8	ND	ND	ND	9.49	6.7	86.7

ND = not detected

Conclusions

To summarize, the SCIEX 7500 system provides impressive levels of sensitivity, robustness and accuracy for trace quantification of insecticides in food matrices. In this study, excellent sensitivity has been demonstrated with LLOQ values down to 0.02 ng/mL. Quantification of pyrethroids and macrocyclic lactones in both green tea and spices has also been shown. By using a higher sensitivity LC-MS platform, injection volumes could be reduced, lowering ion suppression without impacting the overall assay sensitivity. The reduced amount of matrix injected for large studies can help to improve total system uptime.

References

1. ScienceDirect, Avermectin.
2. Ccanccapa-Cartagena, A.; Masiá, A.; Picó, Y. (2017) Simultaneous determination of pyrethroids and pyrethrins by dispersive liquid-liquid microextraction and liquid chromatography triple quadrupole mass spectrometry in environmental samples. *Anal Bioanal Chem* **409(20),** 4787–4799 (2017).
3. European Commission, Commission Regulation (EU) 2018/1514, October 2018.
4. Enabling new levels of quantification. SCIEX technical note, RUO-MKT-02-11886-A.
5. Sensitivity gains for the evolution of routine bioanalysis. SCIEX technical note, RUO-MKT-02-11885-A.
6. Tomlin, C. The pesticide manual – a world compendium, 13th ed.; British Crop Protection Council (Alton, Hampshire, UK), 2003.
7. European Commission, Food Safety: EU Pesticides Database.
8. European Commission, Food Safety: Maximum Residue Levels.

LC-MS/MS快速檢測植物源性食品中的10種新煙鹼類農藥殘留

Determination of 10 kinds of Neonicotinoid Pesticide Residues in Foods of Plant Origin by High Performance Liquid Chromatography-Mass Spectrometry

李廣甯，孫小傑，劉冰潔，郭立海

Li Guangning, Sun Xiaojie, Liu Bingjie, Guo Lihai

Keywords: SCIEX Triple Quad; Foods; Neonicotinoid

引言

新煙鹼類農藥是一類具有神經活性的殺蟲劑，可與昆蟲中樞神經系統中的煙鹼乙醯膽鹼受體結合，導致其受體阻斷從而起到殺滅蟲害的作用。自上世紀 80 年代問世以來，目前已成為應用最廣泛，市場佔有率最高的一類殺蟲劑。新煙鹼類殺蟲劑噴灑時，可以通過植物根系或莖葉進入其他組織，並以原藥或代謝物的形式存在，若施用不當或過量使用極易造成瓜果蔬菜的殘留超標。中國 GB 2763-2021《食品安全國家標準 食品中農藥最大殘留限量》規定了相關植物源性食品中新煙鹼類農藥的最大殘留限量，但其超標的新聞仍然經常被報導。

根據相關法規，在 SCIEX 液相色譜質譜系統上開發了 10 種新煙鹼類殺蟲劑農藥的檢測方法，該方法具有以下特點：

1. 分析時間短，15分鐘即可完成果蔬基質中10種新煙鹼類殺蟲劑的檢測。
2. 靈敏度高：複雜基質中新煙鹼類農殘的檢出下限低於國家相關標準。
3. 抗基質干擾：SCIEX Turbo V™離子源具有強大的抗基質干擾能力，減少儀器的維護頻次。

儀器設備

SCIEX ExionLC™ 系統 + SCIEX Triple Quad™ 系統

樣品處理：

稱取 10g 試樣（精確至 0.01 g）於 50 mL 塑膠離心管中，加入 10 mL 乙腈振盪 1 min，然後加入 QuEChERS 萃取鹽及 1 顆陶瓷均質子，蓋上離心管蓋，劇烈震盪 1 min 後離心 5 min。吸取 5 mL 上清液至內含 QuEChERS 吸水鹽包于塑膠離心管中，渦旋混勻 1 min，離心 5 min，吸取上清液 0.5 mL 加入 0.5 mL 去離子水，混勻後過微孔濾膜，用於測定。待上機分析。

色譜方法：

色譜柱：HSS T3, 100Å, 1.8 µm, 2.1 mm×100 mm

流動相：A：水（含 5mmol/L 甲酸銨）

B：甲醇

流速：0.3 mL/min；

柱溫：40℃；

進樣量：2 µL

梯度洗脫：

Time [min]	Flow [mL/min]	B[%]
0.00	0.3000	5
0.50	0.3000	5
9.00	0.3000	90
12.50	0.3000	90
12.60	0.3000	5
15.00	0.3000	5

質譜方法：

掃描方式：正模式 MRM

離子源：ESI

離子源參數：

IS 電壓 : 5500 V　　氣簾氣 CUR: 30 psi

霧化氣 GAS1: 65 psi　　輔助加熱器 GAS2: 65 psi

源溫度 TEM: 600℃　　碰撞氣 CAD: Medium

離子對列表見附表

實驗結果

化合物提取離子流色譜圖

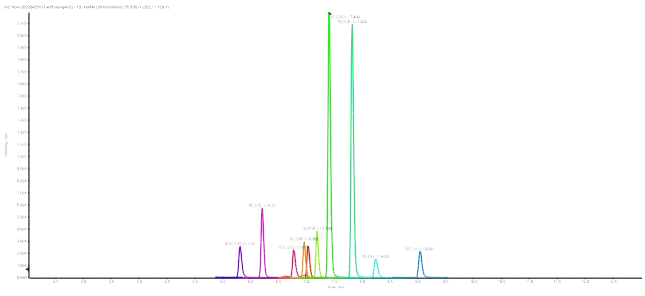


圖**1** 10種新煙鹼類殺蟲劑的提取離子流色譜圖

1. 線性，回歸方程及回歸係數

使用空白基質配置 0.5~250 ng/mL 標準測試液，相關曲線見圖2，回歸係數 r>0.998，方法從低濃度點到高濃度點均具有良好的準確度。

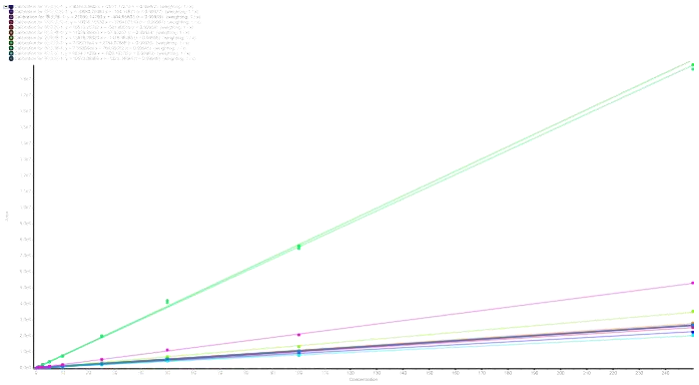


圖**2** 10種新煙鹼類殺蟲劑基質標的校準曲線

2. 靈敏度考察，使用蔬菜和水果基質考察靈敏度，本方法靈敏度可滿足GB 2763-2021《食品安全國家標準食品中農藥最大殘留限量》及相關風險監控的需求。

表**1** 10種新煙鹼類殺蟲劑的檢出限值

化合物	實驗檢出限值（mg/kg)	GB2763限量要求mg/kg		風險監控手冊（mg/kg)
		蔬菜基質	水果基質	
呋蟲胺	0.001	0.1	1	0.002
烯啶蟲胺	0.0005	0.2	0.5	0.002
噻蟲嗪	0.001	0.01	0.01	0.002
噻蟲胺	0.001	0.01	0.01	0.002
吡蟲啉	0.001	0.1	0.2	0.002
氯噻啉	0.001	0.2	0.2	0.002
啶蟲脒	0.0005	0.02	0.2	0.002
噻蟲啉	0.0005	0.02	0.2	0.002
吡蟲啶	0.001	0.1	0.1	0.002

總結

本實驗在 SCIEX Triple Quad™ 系統上，建立了水果及蔬菜中 10 種新煙鹼類殺蟲劑的 LC-MS/MS 方法，方法快速簡便，靈敏度結果顯示其檢出限值低於相關標準規定，可滿足植物源性食品中新煙鹼類殺蟲劑農藥殘留的相關檢測需求。

參考文獻

1. GB 2763-2021《食品安全國家標準 食品中農藥最大殘留限量》

附錄：10種新煙鹼類殺蟲劑的質譜離子對參數

化合物	Q1	Q3	DP	CE	參考保留時間(min)
呋蟲胺	203.1	129.1	53	10	5.28
		157.1		15	5.28
烯啶蟲胺	271.1	126	65	31	5.81
		225.1		15	5.81
噻蟲嗪	292	211	55	15	6.23
		181.1		28	6.23
環氧蟲啶	323.1	126	95	35	6.82
		151.1		28	6.82
噻蟲胺	250	169	53	17	7.04
		132		19	7.04
吡蟲啉	256.1	209.1	65	19	6.97
		175.1		24	6.97
氯噻啉	262	181.1	58	19	7.2
		122.1		37	7.2
啶蟲脒	223.1	126.1	71	25	7.42
		56		17	7.42
噻蟲啉	253	126.1	80	25	7.83
		90.1		50	7.83
呱蟲啶	367.1	321.2	82	17	8.23 / 9.06
		263.1		20	8.23 / 9.06
		306.1		32	8.23 / 9.06
		137.1		32	8.23 / 9.06

LC-MS/MS快速檢測水產類農獸藥殘留

Rapid determination of agricultural and veterinary drug residues in aquatic products by LC-MS / MS

劉青[1]，陳情[2]，黃文傑[2]，李荷香[2]，楊總[1]，劉冰潔[1]，郭立海[1]

Liu Qing[1], Chen Qing[2], Huang Wenjie[2], Li Henxiang[2], Yang Zong[1], Liu Bingjie[1], Guo Lihai[1]

SCIEX Application Support Center, China [1]

Jiangxi Huaxing Testing Co., Ltd [2]

Keywords: Veterinary drug; Aquatic products

引言

隨著經濟快速增長以及生活水準提高，消費者對食品品質安全的關注度日益上升。作為主要食物之一的魚、蝦等水產品的品質安全問題也受到人民大眾的廣泛關注和政府部門的高度重視。水產養殖業的現代化、集約化、規模化發展，使得各類藥物、添加劑、改良劑、微生物製劑等產品在水產養殖業中被廣泛應用,有效降低了水產的發病率、死亡率，降低了養殖成本。但是這些藥物或製劑的使用是否符合規範,是否會導致水產品藥物殘留積累或水體土壤污染等問題值得探究。本文採用高效液相色譜串聯質譜建立了對於中國新頒佈實施的水產類產品的農獸藥檢測標準進行了前處理和方法學驗證工作，該方案的優勢和特點。

1. 方法全面：覆蓋2021版本所有水產類系列標準GB 31656.3-2021、GB 31656.4-2021、GB 31656.5-2021、GB 31656.7-2021、GB 31656.8-2021、GB 31656.9-2021、GB 31656.10-2021、GB 31656.11-2021、GB 31656.12-2021、GB 31656.13-2021中的化合物種類；
2. 方法靈敏度高：水產類基質中所有化合物的靈敏度均滿足以上標準的限量要求；
3. 緊扣標準：所有水產類基質的前處理方法均與標準保持一致，滿足檢驗需求，實用性強；

1 實驗方法

1.1 樣品前處理

本實驗的前處理方法全部按照標準的要求進行；

方法1： GB 31656.3-2021水產品中喹諾酮類殘留量的測定

方法2： GB 31656.4-2021 水產品中氯丙嗪殘留量的測定

方法3： GB 31656.5-2021水產品中安眠酮殘留量的測定

方法4： GB 31656.7-2021水產品中氯硝柳胺殘留量的測定

方法5： GB 31656.8-2021水產品中有機磷類藥物殘留量的測定

方法6： GB 31656.9-2021水產品中二甲戊靈殘留量的測定

方法7： GB 31656.10-2021水產品中四聚乙醛殘留量的測定

方法8： GB 31656.11-2021水產品中土黴素、四環素、金黴素和多西環素殘留量的測定

方法9： GB 31656.12-2021水產品中青黴素類藥物多殘留的測定

方法10：GB 31656.13-2021水產品中硝基呋喃類代謝物多殘留的測定

1.2 液相色譜條件

液相系統：SCIEX ExionLC™ 系統

色譜柱：Phenomenex C18（100×2.1 mm, 1.7 µm）

流動相：A為0.1%的甲酸(5 mmol/L乙酸銨溶液），B為甲醇

流速：0.3 mL/min

柱溫：40℃

洗脫程式：梯度洗脫

1.3 質譜條件

質譜系統：SCIEX 三重四級杆質譜系統

掃描模式：多反應監測MRM；離子源： ESI源；噴霧電壓（IS）：5500/-4500 V；離子源溫度（TEM）：550 ℃；氣簾氣（CUR）：35 psi；碰撞氣（CAD）：Medium；霧化氣（GS1）：55 psi；輔助霧化氣（GS2）：55psi；MRM離子對見（表1）。

表**1.** 離子對資訊

母離子 (m/z)	子離子 (m/z)	化合物名稱	去簇電壓 (V)	碰撞能量 (eV)	標準編號
320.1	276.1	Norfloxacin 1	80	26	31656.3
320.1	233.1	Norfloxacin 2	80	35	31656.3
320.1	302	Norfloxacin 3	41	27	31656.3
360.1	316.1	Enrofloxacin 1	80	28	31656.3
360.1	245.1	Enrofloxacin 2	80	36	31656.3
360.2	342	Enrofloxacin 3	41	27	31656.3
332.1	288.1	Ciprofloxacin 1	80	25	31656.3
332.1	245.1	Ciprofloxacin 2	80	33	31656.3
332.1	314	Ciprofloxacin 3	46	25	31656.3
262	244.1	Oxolinic acid 1	70	26	31656.3
262	216.1	Oxolinic acid 2	70	40	31656.3
262.1	160	Oxolinic acid 3	26	47	31656.3
262.1	244.1	Flumequin 1	77	23	31656.3
262.1	202.1	Flumequin 2	77	42	31656.3
262.1	174	Flumequin 3	26	49	31656.3
362.2	318.1	Ofloxacin 1	80	26	31656.3
362.2	261.1	Ofloxacin 2	80	38	31656.3
319	58	Chlorpromazine 1	66	67	31656.4
319	86.1	Chlorpromazine 2	66	23	31656.4
251.1	91.1	methaqualone 1	131	45	31656.5
251.1	132.1	methaqualone 2	131	35	31656.5
324.7	170.9	Niclosamide 1	-80	-35	31656.7
324.7	288.9	Niclosamide 2	-80	-25	31656.7
299.1	129	Phoxim 1	67	16	31656.8
299.1	77	Phoxim 2	67	46	31656.8
279.1	169	Fenthion 1	78	23	31656.8
279.1	247	Fenthion 2	78	18	31656.8
274	109	Trichlorfon 1	32	25	31656.8
274	88	Trichlorfon 2	31	21	31656.8
221	109	Dichlorvos 1	70	23	31656.8
221	127	Dichlorvos 2	70	25	31656.8
305	169	Diazinon 1	80	27	31656.8
305	153	Diazinon 2	80	28	31656.8
325	183	Azamethiphos 1	26	21	31656.8
325	112	Azamethiphos 2	26	51	31656.8
331	127	Malathion 1	64	17	31656.8
331	99	Malathion 2	64	31	31656.8
240	156.1	Propetamphos-Deisopropyl 1	55	11	31656.8
240	138.1	Propetamphos-Deisopropyl 2	55	21	31656.8
363	227	Coumaphos 1	100	36	31656.8
362.9	307	Coumaphos 2	100	25	31656.8
282.1	212	Pendimethalin 1	45	15	31656.9
282.1	194	Pendimethalin 2	45	25	31656.9
194.2	62.2	Metaldehyde 1	80	20	31656.10
194.2	106.2	Metaldehyde 2	80	23	31656.10
445.1	410.2	Tetracycline 1	80	24	31656.11
445.1	427.1	Tetracycline 2	80	19	31656.11
461.2	426.2	Oxytetracycline 1	80	25	31656.11
461.2	443.2	Oxytetracycline 2	80	17	31656.11
479.1	462	Chlortetracycline 1	80	24	31656.11
479.1	444	Chlortetracycline 2	80	28	31656.11
445	428.1	Doxycycline 1	80	24	31656.11
445	154.1	Doxycycline 2	80	35	31656.11
366.2	349.1	amoxicillin 1	46	13	31656.12
366.2	114.1	amoxicillin 2	46	30	31656.12
366.3	208.3	amoxicillin 3	120	17.5	31656.12
350.3	192.1	ampicillin 1	55	23	31656.12

表**1.** 離子對資訊（續）

母離子 (m/z)	子離子 (m/z)	化合物名稱	去簇電壓 (V)	碰撞能量 (eV)	標準編號
350.3	106.1	ampicillin 2	55	26	31656.12
350.3	160.2	ampicillin 3	120	16	31656.12
351.2	160.1	penicillin V 1	55	16	31656.12
351.2	192	penicillin V 2	55	16	31656.12
335.2	160.1	penicillin G 1	60	18	31656.12
335.2	176.2	penicillin G 2	60	18	31656.12
402.2	160.1	oxacillin 1	60	18	31656.12
402.2	243.1	oxacillin 2	60	18	31656.12
436.2	160.1	cloxacillin 1	60	18	31656.12
436.2	277.1	cloxacillin 2	60	18	31656.12
470.1	160.1	dicloxacillin 1	60	21	31656.12
470.1	311.1	dicloxacillin 2	60	21	31656.12
415.2	199.1	nafcillin 1	64	20	31656.12
415.2	256.1	nafcillin 2	64	51	31656.12
518	160	Piperacillin 1	60	15	31656.12
518	143	Piperacillin 2	60	20	31656.12
462	246	Azlocillin 1	60	17	31656.12
462	218	Azlocillin 2	60	26	31656.12
381	222	Methicillin 1	40	21	31656.12
381	165	Methicillin 2	40	25	31656.12
209.2	166.2	2-NB-SEM 1	80	14	31656.13
209.2	192.1	2-NB-SEM 2	80	16	31656.13
236.1	133.9	2-NB-AOZ 1	80	17	31656.13
236.1	103.9	2-NB-AOZ 2	80	31	31656.13
249.2	134.1	2-NB-AHD 1	80	17	31656.13
249.2	104.1	2-NB-AHD 2	80	27	31656.13
335.2	291.1	2-NB-AMOZ 1	80	17	31656.13
335.2	262.2	2-NB-AMOZ 2	80	23	31656.13

2 實驗結果與討論

2.1 色譜條件優化

針對上述不同的實驗的前處理，實驗詳細優化了色譜條件，比較了不同品牌、不同型號的色譜柱以及流動相，有效的避開基質干擾，定量結果更準確。

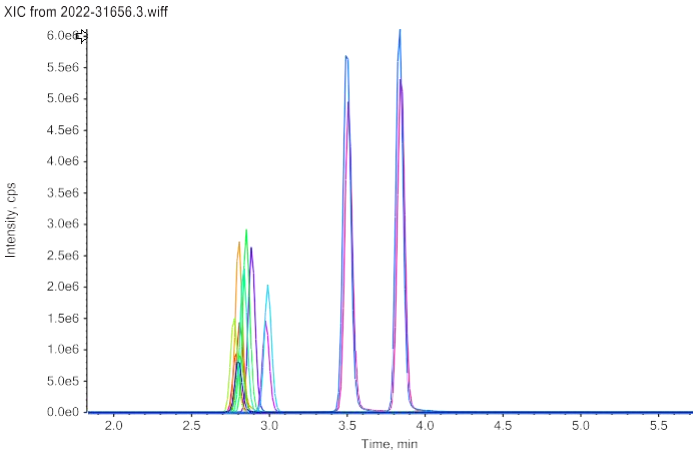


圖**1.** 水產品中喹諾酮類殘留量提取離子流圖(GB 31656.3-2021)

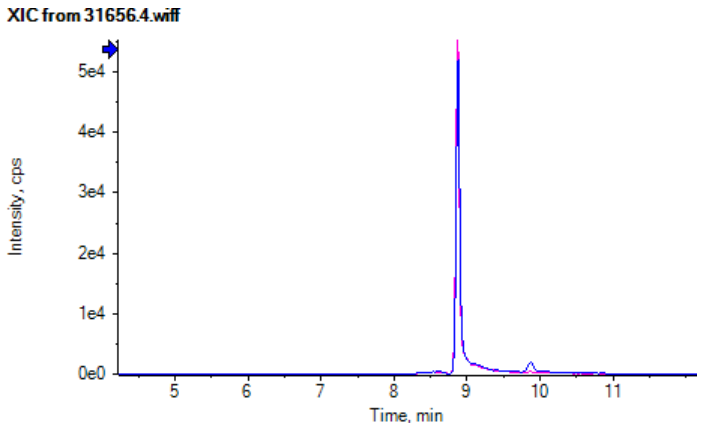


圖**2.** 基質加標氯丙嗪提取離子流圖(GB 31656.4-2021)

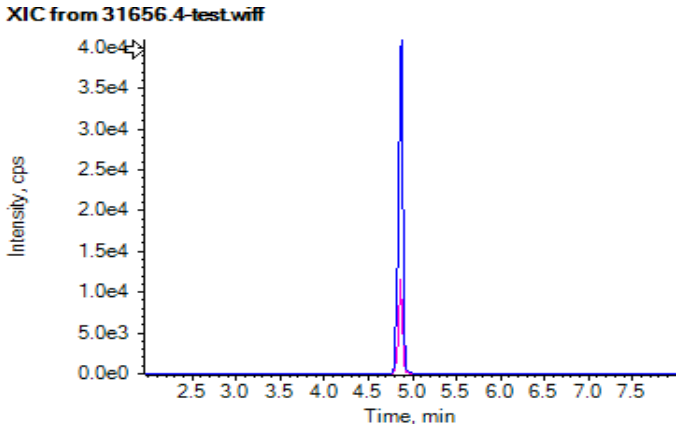


圖3. 基質加標安眠酮提取離子流圖(GB 31656.5-2021)

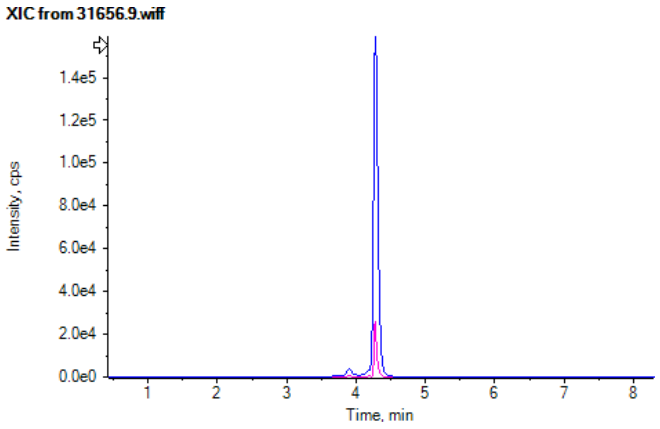


圖6. 基質加標二甲戊靈提取離子流圖(GB 31656.9-2021)

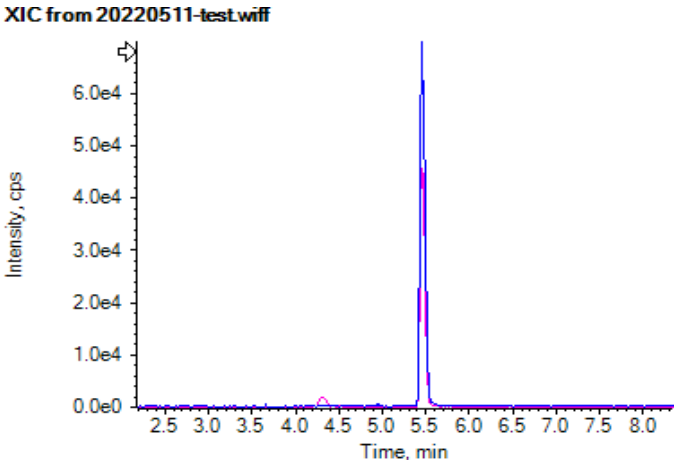


圖4. 基質加標氯硝柳胺提取離子流圖(GB 31656.7-2021)

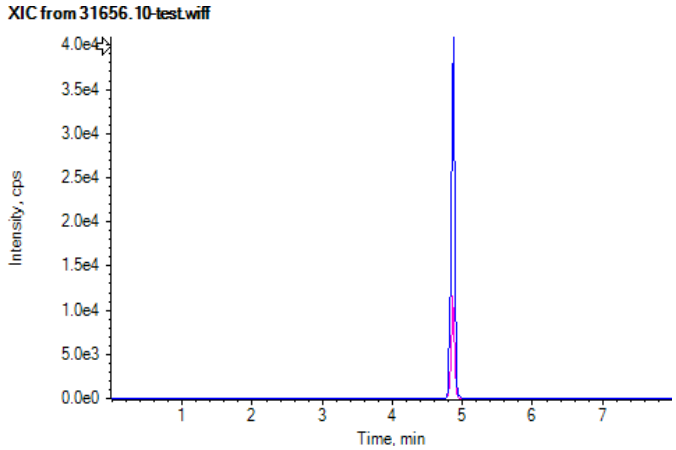


圖7. 基質加標四聚乙醛提取提取離子流圖(GB 31656.10-2021)

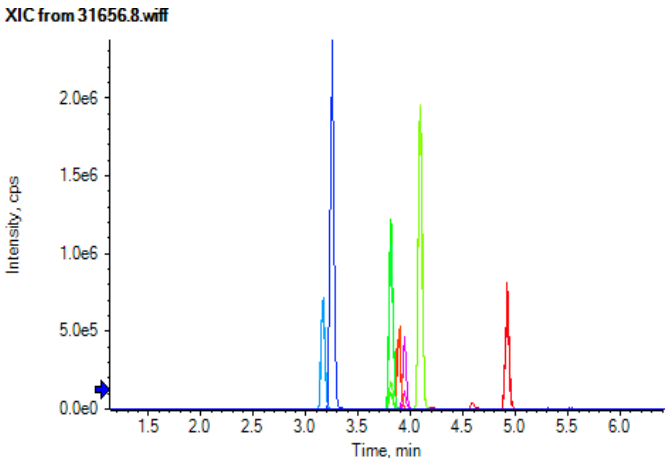


圖5. 基質加標有機磷類藥物提取離子流圖(GB 31656.8-2021)

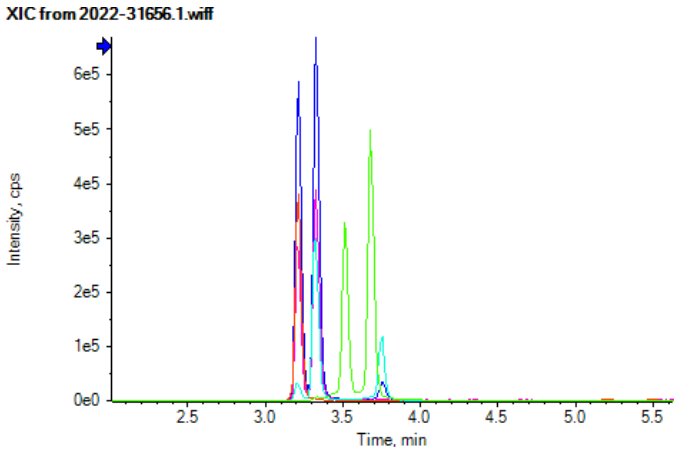


圖8. 基質加標土黴素、四環素、金黴素和多西環素提取離子流圖(GB 31656.11-2021)

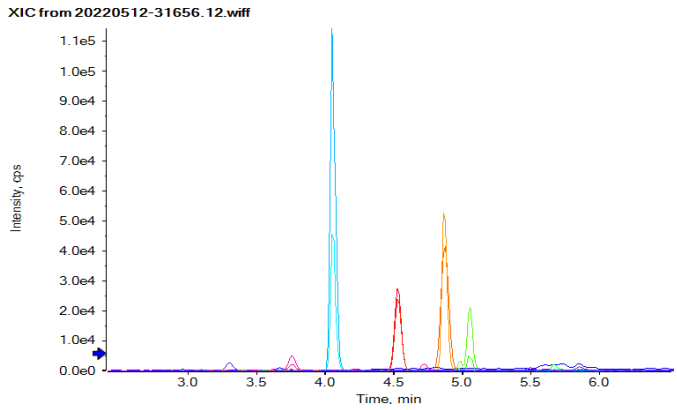


圖**9.** 基質加標青黴素類藥物提取離子流圖(GB 31656.12-2021)

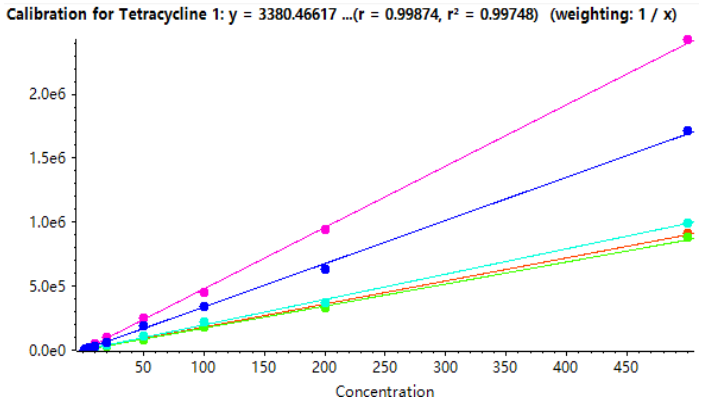


圖**11.** 四環素基質線性回歸曲線GB 31656.11-2021)

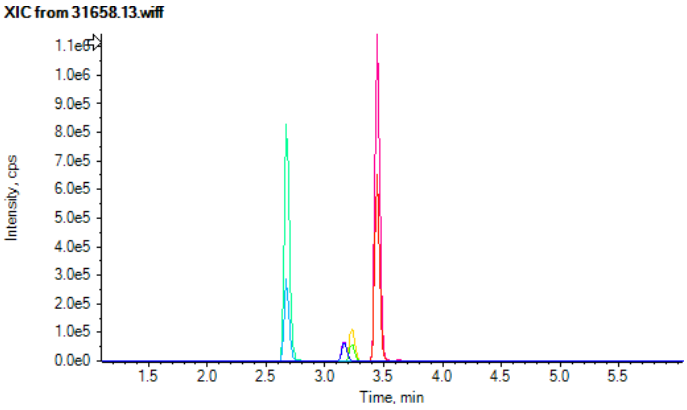


圖**10.** 硝基呋喃類代謝物提取離子流圖（GB 31656.13-2021）

2.2 方法考察了重複性、線性等

實驗分別按照前述前處理方法，選取適用的禽、畜肉空白基質添加1倍和5倍地定量限兩個濃度，每個濃度重複6次，準確度在80.25%-115.23%之間（n=6），相對標準差小於2.76 %（表3），實驗結果表明該方法具有較好的準確度以及良好的穩定性。基質加標曲線相關係數均大於r>0.995（圖2），表明線性良好。該實驗方法完全滿足標準定量檢測的要求。

表**2.** 回收率及重複性實驗（n=6）

化合物名稱	添加濃度 (μg/kg)	平均回收率(%)	相對標準偏差(%)
諾氟沙星	5.0	92.13	1.56
	25.0	89.45	1.93
環丙沙星	5.0	92.45	2,13
	25.0	83.28	2.34
恩諾沙星	5.0	84.65	2.31
	25.0	92.36	1.24
氧氟沙星	5.0	89.23	0.98
	25.0	94.28	0.85
噁喹酸	10.0	105.35	0.64
	50.0	101.26	2.01
氟甲喹	10.0	98.36	2.18
	50.0	94.36	2.07
氯丙嗪	1.0	89.26	1.85
	5.0	93.65	1.94
安眠酮	0.5	88.94	1.83
	2.5	105.68	1.56
氯硝柳胺	0.5	90.35	1.75
	2.5	115.23	2.19
辛硫磷	10.0	90.56	1.84
	50.0	80.64	1.53
倍硫磷	20.0	102.30	2.76
	100.0	92.67	2.24
敵百蟲	10.0	82.56	1.84
	50.0	83.65	1.65
敵敵畏	10.0	105.65	1.87
	50.0	106.85	1.84
二嗪磷	10.0	102.23	1.05
	50.0	99.45	1.27

表**2.** 回收率及重複性實驗（n=6）（續）

化合物名稱	添加濃度 (μg/kg)	平均回收率(%)	相對標準偏差(%)
甲基吡啶磷（甲基吡噁磷）	10.0	108.35	0.99
	50.0	103.75	1.07
馬拉硫磷	10.0	88.93	2.12
	50.0	86.54	2.51
脫異丙基巴胺磷	10.0	89.35	1.96
	50.0	92.65	1.34
蠅毒磷	20.0	106.75	2.23
	100.0	108.35	2.14
辛硫磷	20.0	82.34	2.15
	100.0	86.53	2.51
二甲戊靈	2.0	103.24	1.26
	10.0	101.72	1.54
四聚乙醛	1.0	89.26	2.45
	5.0	84.29	2.56
四環素	10.0	105.37	2.26
	50.0	102.64	1.58
土黴素	10.0	95.35	1.67
	50.0	94.83	1.65
金黴素	10.0	86.94	1.34
	50.0	87.36	1.54
強力黴素(多西環素)	10.0	86.46	1.45
	50.0	88.28	1.86
呫吳四林	25.0	83.18	2.21
	125.0	80.25	2.04
氨卞四林	5.0	83.26	1.97
	25.0	83.05	1.99
青黴素V	5.0	84.56	1.76
	25.0	86.46	1.87
青黴素G	5.0	82.75	2.05
	25.0	85.75	1.87
苯唑西林	5.0	82.98	1.84
	25.0	84.39	1.98
氯唑西林	5.0	88.39	1.87
	25.0	85.63	1.96
雙氯西林	5.0	85.75	1.85
	25.0	92.02	1.75
萘夫西林	5.0	93.06	1.65
	25.0	87.64	1.83
呱拉西林	5.0	93.02	1.63
	25.0	93.04	1.85
阿洛西林	5.0	90.34	1.47
	25.0	91.23	1.64
甲氧西林	5.0	89.04	1.43
	25.0	94.29	1.95
呋喃西林代謝物	1.0	99.08	1.12
	5.0	87.45	1.03
呋喃唑酮代謝物	1.0	88.76	1.25
	5.0	84.67	1.65
呋喃妥因代謝物	1.0	84.28	1.87
	5.0	90.23	1.25
呋喃它酮代謝物	1.0	85.89	1.05
	5.0	84.37	1.02

3 小結

本文建立了高效液相色譜-串聯三重四極杆質譜快速定量分析檢測多類農獸藥的檢測方法。實驗嚴格按照GB31650-2019配套相關標準進行，確保了實驗結果的有效性，定量結果更準確。該方法足以滿足2021系列標準GB 31656.3-2021、GB 31656.4-2021、GB 31656.5-2021、GB 31656.7-2021、GB 31656.8-2021、GB 31656.9-2021、GB 31656.10-2021、GB 31656.11-2021、GB 31656.12-2021、GB 31656.13-2021定量檢測要求，在水產類食品的分析檢測具有重要的參考意義。

SCIEX ZenoTOF™ 7600在柑橘代謝組學研究中的應用

Application of SCIEX ZenoTOF™ 7600 in Citrus Metabolomics

陳西[2]，侯雪[1]，賀光雲[1]，趙祥龍[2]，劉冰潔[2]，郭立海[2]

Chen xi[2], Hou xue[1], He guangyun[1], Zhao xianglong[2], Liu bingjie[2], Guo lihai[2]

[1] 四川省農業科學院農業品質標準與檢測技術研究所；

[2] *SCIEX*中國

[1] *Institute of Quality Standard and Testing Technology for Agro-Products, Sichuan Academy of Agricultural Sciences;*

[2] *SCIEX China*

Keywords: Zeno™ trap; Citrus; Metabolomics; Citric acid

前言

柑橘果實有特殊的色香味，並且富含糖、礦物質、維生素、碳水化合物和抗氧化劑等營養成分，因此深受廣大消費者喜愛[1]。然而，作為商品，消費者在選擇過程中相比營養價值的高低往往更注重其風味。柑橘的甜酸度是消費者選擇的重要依據之一，因此也成為了影響商品柑橘的收穫時間及種植者收入的主要因素[2]。

柑橘的甜度取決於糖含量和總酸度之間的適當比例[3]。除氣候、土質和採收時間的選擇外，合理施用有增甜功效的農藥（肥料）也可以有效改善柑橘的甜酸風味，提高柑橘的品質[4]。由於施加的農藥（肥料）可能會在植物中富集，因此在增甜農藥的選擇中因注意該產品是否會對人類、果樹和土壤環境造成危害。

市場上用於水果增甜的農藥多種多樣，有的增甜劑並無外包裝和商標，為“三無產品”，但經過走訪果農發現其確有較好的增甜效果，因此有必要對其增甜機制和對植物的影響進行研究。

本方案基於SCIEX ZenoTOF™ 7600液質聯用系統，建立了一種非靶向植物成分篩查方法，結合SCIEX天然產物譜庫中的二級譜圖，對柑橘果肉中的成分進行了全面的分析和鑒定。該方法一針進樣可同時完成高品質的一級和二級質譜圖採集，資料全面、覆蓋度高；SCIEX ZenoTOF™ 7600獨特的Zeno MS/MS技術還可以大大提高二級碎片的靈敏度，增加鑒定的準確性。為分析增甜劑的作用機制和對柑橘代謝特徵的影響，鑒定結果被進一步用於對噴施某增甜劑的柑橘及其對照組進行代謝差異分析。此方法的建立不僅有助於本實驗中柑橘成分的研究，也可擴展至其他植物成分分析。

SCIEX ZenoTOF™ 7600

ZenoTOF™ 7600質譜系統的技術特點和優勢：

1、更高的二級掃描（MS/MS）速度和靈敏度

Zeno™ trap通過優化從碰撞池到加速器的離子傳輸，將離子占空比提高到≥90% （常規儀器的占空比為5-25%）[5]，可在高採集速度（最高133Hz）的同時顯著提高MS/MS靈敏度。二級品質的改善可在複雜基質中顯著提升化合物鑒定的可靠性。

2、更高靈敏度的二級定量

ZenoTOF™ 7600質譜系統承襲了SCIEX高分辨質譜系統獨特的MRM^{HR}二級定量方式，並且在開啟Zeno trap後，可獲得約10倍的靈敏度提升。

3、資料全面，可擴展性強

一針進樣同時採集到大量優質的一級和二級數據，不僅方便使用者在後續進行不同角度的資料分析和隨時回溯還可擴展適用於其他工作流程。

4、強大的軟體支撐

OS軟體通過資料的一級質量數、同位素豐度比、保留時間和高分辨二級質譜圖這 “四大關” 進行目標化合物篩查，專業的天然產物二級譜庫（包括有機酸、黃酮、多酚等在不同能量下的二級譜圖），快速高效的提供更準確的定性篩查結果；MarkerView™軟體可根據需要進行多元統計分析。

實驗方法

1. 液相條件：

流動相： A相： 0.05%甲酸水溶液
B相：甲醇-乙腈（1:1，含0.05%甲酸）

流速：0.3 mL/min；

進樣量：1 µl

洗脫程式：

Time(min)	A (%)	B (%)
0.0	95	5
1.0	95	5
7.0	50	50
13.0	30	70
17.0	2	98
22.0	2	98
22.1	95	5
25.0	95	5

2. 質譜方法：

離子源：ESI源，正/負離子模式

掃描模式：TOF MS-IDA-TOF MS/MS; Zeno on

掃描範圍：TOF MS: 50-1200 Da；TOF MS/MS: 30-1200 Da

氣簾氣 CUR: 35 psi　　碰撞氣 CAD: 8

霧化氣 GS1: 50 psi　　輔助氣 GS2: 55 psi

IS電壓: 5500 V / -4500 V　　源溫度 TEM: 500℃

DP電壓: 80 V / -60 V　　碰撞能量: 40±20 / -40±20

3. 樣品的收集和製備

四川某地春見（耙耙柑）果樹，分為施加增甜劑和未施加增甜劑（對照組）兩組，待果實成熟後每組隨機採集約三公斤為一個樣本，每組採集20個樣本。從每個樣本中隨機選出3個果實，剝皮後取果肉勻漿。

取勻漿後的果肉約5 g至50 mL離心管中，加入15 mL甲醇（含0.1%甲酸），渦旋混勻，再超聲提取30 min，8000 r/min離心5 min，取出上清液轉移至另一50 mL離心管中，殘餘物按上述操作重複提取2次，合併上清液並用提取溶劑定容至50 mL，搖勻，經0.22 µm微孔過濾膜過濾後，4℃保存待分析。

質控樣品（QC）取所有待分析樣品各100 µl至5 mL離心管中，渦旋混勻。

結果與討論

1. 資料品質

好的資料重複性是組學研究的前提，本實驗通過在樣品間穿插QC樣品，對資料的重複性進行了監測，從圖1可以看出，不同極性和質荷比的化合物在保留時間和峰面積兩個維度均有良好的重複性。

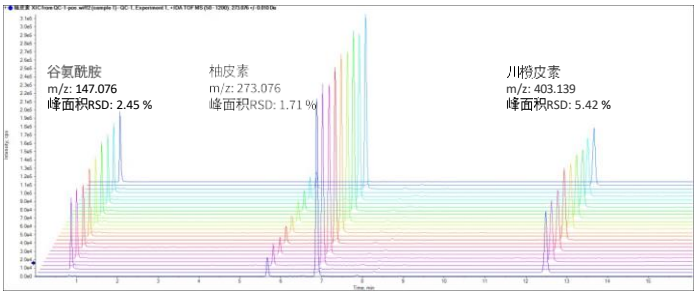


圖1. 正離子模式8個QC資料中三個典型化合物的提取離子流色譜圖（XIC）

資料的全面性是有效成分篩查和鑒定的保障，ZenoTOF™ 7600的核心創新是在Q2碰撞池元件的末端增加了一個Zeno™ trap(Zeno阱)，通過提高離子占空比到90%以上使二級質譜圖品質得到改善。如圖2所示，在掃描速度均為10Hz的條件下，一針進樣，即能採集到超過10000張二級譜圖，當Zeno阱開啟時，二級質譜圖回應提高了10倍以上。

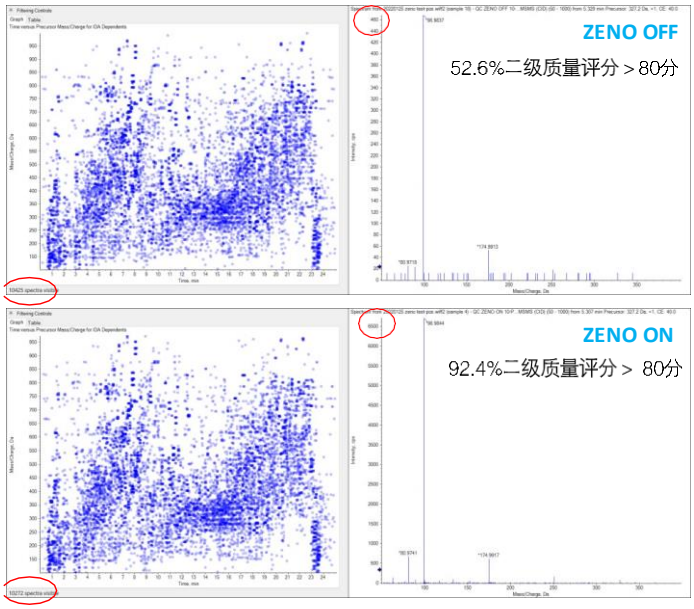


圖**2.** 正離子模式QC樣品的IDA示意圖：在掃描速度相同（均為10Hz）的情況下，均能得到1w張以上的二級質譜圖，且在開啟Zeno trap時，二級質譜圖品質明顯更優。

2. 化合物鑒定

植物中成分複雜多樣，且有較多同分異構體，因此，僅有準確的一級無法對化合物進行確證。針對大量樣本， SCIEX OS軟體可自動進行峰提取和搜庫，通過一級質量數、同位素豐度和二級碎片的匹配對化合物進行鑒別（圖4），使篩查流程快速準確。

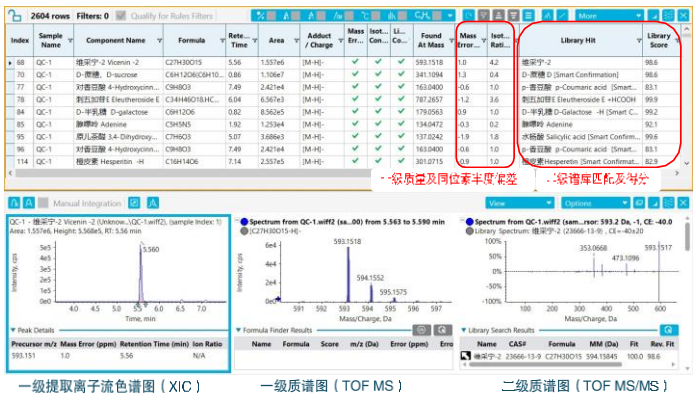


圖**4.** SCIEX OS軟體典型篩查流程展示

ZenoTOF™ 7600新增的Zeno trap功能，在開啟時可以有效提高化合物的二級品質，使低濃度化合物也能夠準確匹配譜庫，從而提高化合物鑒定的個數和準確度（圖5）。鑒定出的化合物還可在同一台儀器上進行二級定量（MRM^{HR}），在開啟Zeno trap時，同樣可以提高二級定量的靈敏度（圖6）。本次實驗從柑橘果肉中共鑒定出了包括有機酸、黃酮類、脂類和糖在內的142個天然成分。

圖**5.** 某樣本中咖啡醯蔗糖在開啟和關閉Zeno trap時的二級譜庫匹配結果對比：在母離子回應相同的情況下，Zeno trap將二級回應提高了12倍以上，譜庫匹配得分也由79分上升到96分，增加了對鑒定結果的信心。

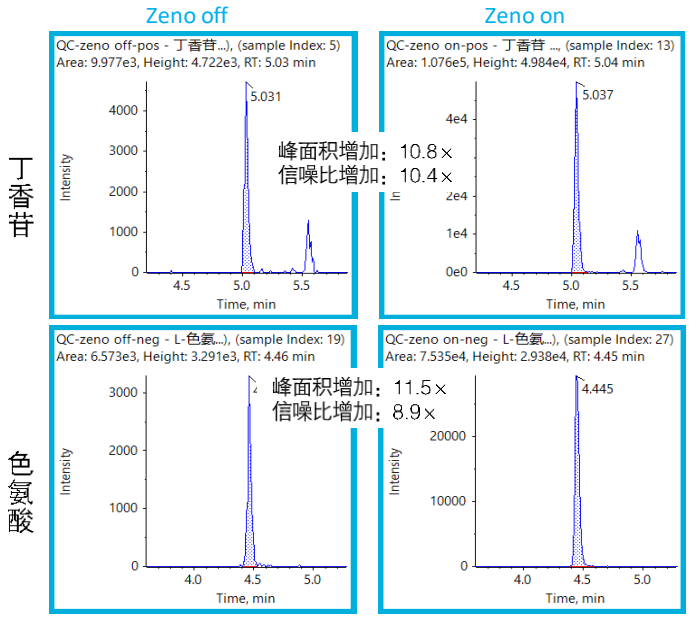


圖**6.** 丁香苷（正）和色氨酸（負）在開啟和關閉Zeno trap時，MRM色譜圖的靈敏度均提高10倍左右。

3. 統計分析

實驗利用Markerview™ 1.3.1軟體對處理組（Sample）、對照組（Control）和質控組（QC）樣品資料進行PCA-DA分析，從圖7

可以看出，處理組和對照組的資料聚類良好並且明顯分離。說明經過增甜劑處理後的果肉成分與對照組比確實發生了明顯變化。

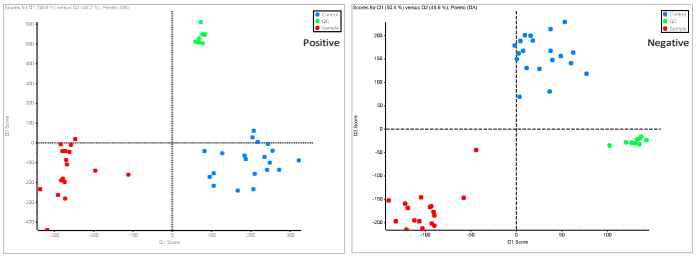


圖7：正（左）、負（右）離子模式下，處理組（紅）、對照組（藍）和QC（綠）樣品的PCA-DA分析圖。

將鑒定出的化合物導入軟體，進行差異分析，以 t檢驗p < 0.01 且fold change＞0.75為標準，篩選出了37個差異物，處理組中除咖啡酸等少數幾個化合物相較於對照組上調以外，包括檸檬酸和脂質在內的大量化合物均下調（圖8）。

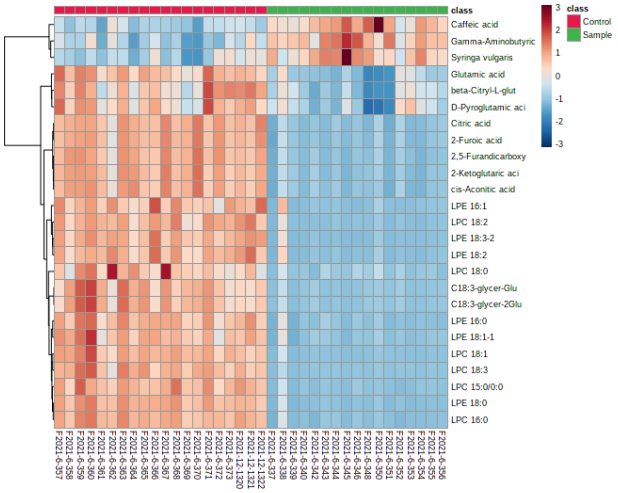


圖8：差異最大的前25個化合物的熱圖。

柑橘的甜酸風味主要受糖、酸含量及糖酸比的影響。柑橘中最主要的有機酸為檸檬酸[6]，最主要的糖為果糖、葡萄糖和蔗糖[7]。圖9顯示了兩種單糖的總量、蔗糖和檸檬酸含量的對比結果，經過增甜劑處理的果肉樣品中檸檬酸含量明顯低於對照組，單糖（葡萄糖和果糖）含量略高於對照組，而蔗糖含量沒有明顯區

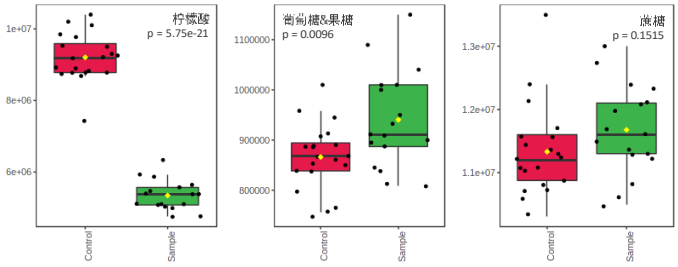


圖9：正（左）、負（右）離子模式下，處理組（紅）、對照組（藍）和QC（綠）樣品的PCA-DA分析圖。

別。檸檬酸在果實生長發育過程中，是由可溶性糖經三羧酸迴圈合成的[8]說明該增甜劑的主要作用機制為通過調控三羧酸迴圈，降低檸檬酸含量來提高糖酸比。

總結

本方案基於SCIEX ZenoTOF™ 7600 液質聯用系統建立了一種高效的柑橘中營養物質的鑒定及代謝組學研究方法，並將該方法應用於研究某水果增甜劑的增甜機制和對柑橘代謝的影響。

ZenoTOF™ 7600在高掃描速度的基礎上通過Zeno™ trap技術使二級靈敏度增加約10倍，使化合物鑒定更加可靠，且碎片離子的回應提高還可以用於更高靈敏度的準確定量。OS軟體結合Markerview™軟體，可輕鬆實現從鑒定、定量到統計分析的完整工作流程。

參考文獻

[1] 丁曉波, 張華, 劉世堯, 等. 柑橘果品營養學研究現狀[J]. 園藝學報

[2] Syed Bilal Hussain, Cai-Yun Shi, et al., Recent Advances in the Regulation of Citric Acid Metabolism in Citrus Fruit. Critical Reviews in Plant Sciences

[3] Baldwin, E. A. Citrus fruit. In: Biochemistry of Fruit Ripening (pp. 107–137).

[4] 羅曾玲，徐素珍. 柑橘的甜酸風味及生產管理措施分析; 羅曾玲 徐素珍. 鄉村科技

[5] Qualitative flexibility combined with quantitative power -Using the SCIEX ZenoTOF 7600 LC-MS/MS system, powered by SCIEX OS software. SCIEX technical note, RUO-MKT-02-13053-A.

[6] Etienne, A., G_enard, M., et al., What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. J. Exp. Bot. 64: 1451–1469.

[7] 曾祥國. 不同種類和產區柑橘糖酸含量及組成研究〔D〕.華中農業大學，2005.

[8] LIN Qiong, et al. Low temperature induced changes in citrate metabolism in Ponkan (Citrus reticulata Blanco cv · Ponkan) fruit during maturation · Plos One ，2016 ·

SCIEX

Quantification of Whey Protein Content in Infant Formula by CE-SDS

Gao Tie,[1] Chen Hongxu,[1] Wu Jianhua,[1] Feng Ping[2]
1 CE & Biopharma, SCIEX, China
2 Wyeth Nutrition, China

Introduction

Milk is almost always the first source of protein for human growth and development. Mammalian milk includes two major groups of proteins: whey proteins, a family of globular proteins, and casein proteins, a family of phosphoproteins. They are found in a roughly 60:40 ratio in human milk and in other ratios in other mammalian milks. Whey proteins are also a standard component of most infant formulas. According to the People's Republic of China National Food Standard GB 10765, milk-based infant formula refers to liquid or powdered products made mainly from milk and milk protein products; with addition of adequate amount of vitamins, minerals, and/or other ingredients; and produced and processed by physical methods only, that is suitable for normal infants.

Figure 2. PA 800 Plus Pharmaceutical Analysis System

To verify the claims of infant formula vendors and ensure the quality, safety, and efficacy of infant formula, it is essential to have an accurate method for measuring whey content. Previously, however, there was no reliable method to separate the two major protein types and quantify the whey protein

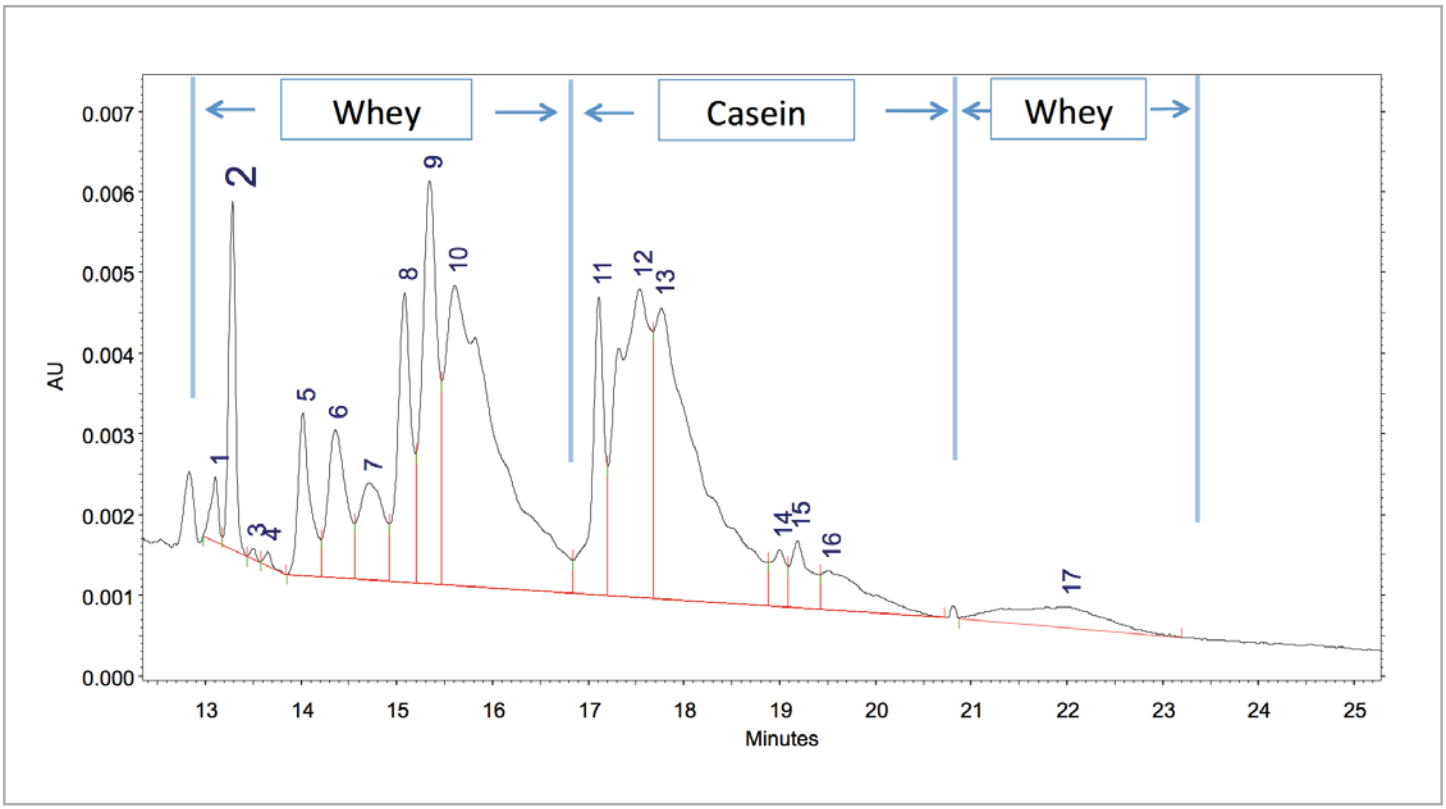


Figure 1. Electropherogram of infant formula.

content. The challenge was compounded by pasteurization, during which the Maillard reaction can cause proteins to form complexes with sugars. These complexes make effective separation of the various proteins even more difficult.

This note explores an effective, practical method based on capillary electrophoresis—sodium dodecyl sulfate (CE-SDS)—for separating and quantifying the whey protein content in infant formula. Analysis using the CE-based SCIEX PA 800 Series Pharmaceutical Analysis System clearly separated whey, casein proteins, and immunoglobulins (Figure 1), and provided accurate, reliable quantitation of the whey proteins. The method was validated across multiple laboratories and demonstrated to be reliable and reproducible.[1] This method was approved by the AOAC Expert Review Panel for Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN).[1]

Key Features

- CE-SDS on the PA 800 Plus system provides excellent separation resolution, clearly separating the whey proteins, casein proteins, and immunoglobulins in both protein standard and infant formula samples
- Separations are relatively fast; under 30 minutes in the case of this analysis
- Validation across multiple laboratories found the method to be reliable and reproducible

Experimental

Reagents and Supplies

The SDS-MW Analysis Kit (SCIEX, P/N 390953) supplied the capillary, SDS gel buffer, 10 kDa marker, MW marker, and sample buffer. Contents are listed in Table 1. Other reagents and supplies included: β- mercaptoethanol (SIGMA P/N M7154 or M6250); milk protein standards (SIGMA: α-lactalbumin, L6010; β-lactoglobulin, L3908; IgG, I5506; BSA, A1933; β-casein, C6905; αS-casein, C6780; κ-casein, C0406. ALAR Ingredients: CGMP). Infant formula was provided by a local dairy manufacturer. Deionized water (Millipore).

Sample Preparation

Milk protein standard mixture

Milk proteins were weighed and dissolved in water according to the infant formula ratio.

Infant formula

Infant formula (100 mg ± 4 mg) was added to 1.5 mL centrifuge tubes. Water (1 mL) was added to each tube. Tubes were vortexed and oscillated until the infant formula was fully dissolved.

Sample running pre-solution

Sample pre-running solution was prepared by mixing sample buffer and the 10 kDa Marker (internal standard), both from the SDS-MW Analysis Kit, in an 84:1 ratio.

Denaturation

10 µL of each sample solution was pipetted into separate 1.5 mL microcentrifuge tubes. 85 µL of sample running pre-solution and 5 µL of β-mercaptoethanol were added to each microcentrifuge tube. Each tube was mixed well and then heated in a water bath at 100° ±5° C for 10 minutes. The tubes were allowed to cool to room temperature and then vortexed. Finally, each sample was transferred to a separate injection vial.

Items	Part Number
50 µm ID capillary column	338451
SDS Gel Buffer	A30341
10 kDa marker	A26487
MW marker	A22196
SDS sample buffer	
0.1N NaOH	
0.1N HCl	

Table 1. Items in SDS-MW Analysis Kit

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	10.00 min	BI:D1	BO:D1	forward	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	20.0 psi	5.00 min	BI:E1	BO:E1	forward	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse - Pressure	20.0 psi	2.00 min	BI:F1	BO:F1	forward	ddH2O rinse to remove the acid residue
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward	SDS Gel rise to fill the capillary
5	0.00	Separate - Voltage	15.0 KV	10.00 min	BI:C1	BO:C1	5.00 Min ramp, reverse polarity, both	SDS Gel for voltage equilibration
6								

Figure 3. Column conditioning method.

Separation and Detection

All separations were carried out on a capillary electrophoresis-based SCIEX PA 800 Plus Pharmaceutical Analysis System equipped with a photodiode array (PDA) detector operated at 220 nm, 2 Hz. Aperture was 2 (100 x 200 µm). The system was equipped with a fused-silica capillary column (50 µm ID, 30 cm total / 20 cm effective length). Sample storage and capillary temperatures were both 25° C. Conditioning and separation methods are show in Figures 3 and 4, respectively.

Data Analysis

Data analysis was performed using SCIEX 32 Karat Software.

Integration

Integration was carried out with Caesar integration off.

The electropherogram of the protein standard mixture was integrated. Migration of the whey and casein proteins are defined by the comparison to the E-grams of every single protein standard shown in Figure 5.

For the infant formula electropherogram, the autointegration start point was after the solvent peak and approximately 0.4 minutes before the 10 kDa marker peak. The auto integration end point was the end of the casein protein peaks group, the position of which was defined according to the standard mixture. Manual integration was performed for high MW whey proteins that eluted after the casein proteins.

Calculation

Whey protein content in the infant formula was calculated using the following equations:

$$Aw.c. = 1.4 \times Aw \quad (1)$$

where: Aw.c. = corrected integrated area of the whey components

Aw = total integrated area of the whey components

1.4 = correction factor

$$\text{Whey protein \%} = Aw.c./(Aw.c.+Acn)x100 \quad (2)$$

where: Acn = integrated area of the casein components

The correction factor is used to adjust the response factor difference of whey proteins and casein proteins when detected at UV 220nm, since caseins contain more aromatic amino acids (t) which contribute higher response for the amount to area than whey proteins do.

Results and Discussion

Milk Protein Standard Mixture

The milk protein standard composed of whey proteins (α-lactalbumin, β-lactoglobulin, CGMP, IgG, and BSA) and casein proteins (β-casein, αS1-casein, αS2-casein, κ-casein) were analyzed by CE-SDS. All the casein proteins migrated between the IgG light chain and IgG heavy chain. The whey proteins and casein proteins were completely separated from each other (Figure 5).

Infant Formula

The electropherogram of the infant formula sample was previously shown in Figure 1. Whey protein content was calculated to be 61.97%, in line with the whey protein content of natural human milk.

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	3.00 min	BI:D1	BO:D1	forward, In / Out vial inc 8	0.1 N NaOH rinse to clean capillary surface - Automatic increment every 8 runs
2		Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	forward, In / Out vial inc 8	0.1 N HCl rinse to neutralize capillary surface silanol group - Automatic increment every 8 runs
3		Rinse - Pressure	70.0 psi	1.00 min	BI:F1	BO:F1	forward, In / Out vial inc 8	Water rinse to remove the acid residue - Automatic increment every 8 runs
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward, In / Out vial inc 8	SDS Gel rinse to fill the capillary with SDS gel - Automatic increment every 8 runs
5		Wait		0.00 min	BI:A1	BO:A1	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip - Automatic increment every 8 runs
6		Wait		0.00 min	BI:A4	BO:A4	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip - Automatic increment every 8 runs
7		Inject - Voltage	5.0 KV	20.0 sec	SI:A1	BO:C1	Override, reverse polarity	Sample injection
8		Wait		0.00 min	BI:B4	BO:B4	In / Out vial inc 8	ddH2O, use for dipping to avoid sample carry over - Automatic increment every 8 runs
9	0.00	Separate - Voltage	15.0 KV	30.00 min	BI:C1	BO:C1	1.00 Min ramp, reverse polarity, both, In / Out vial inc 8	SDS Gel for separation - Automatic increment every 8 runs
10	5.00	Autozero						
11								

Figure 4. Separation method.

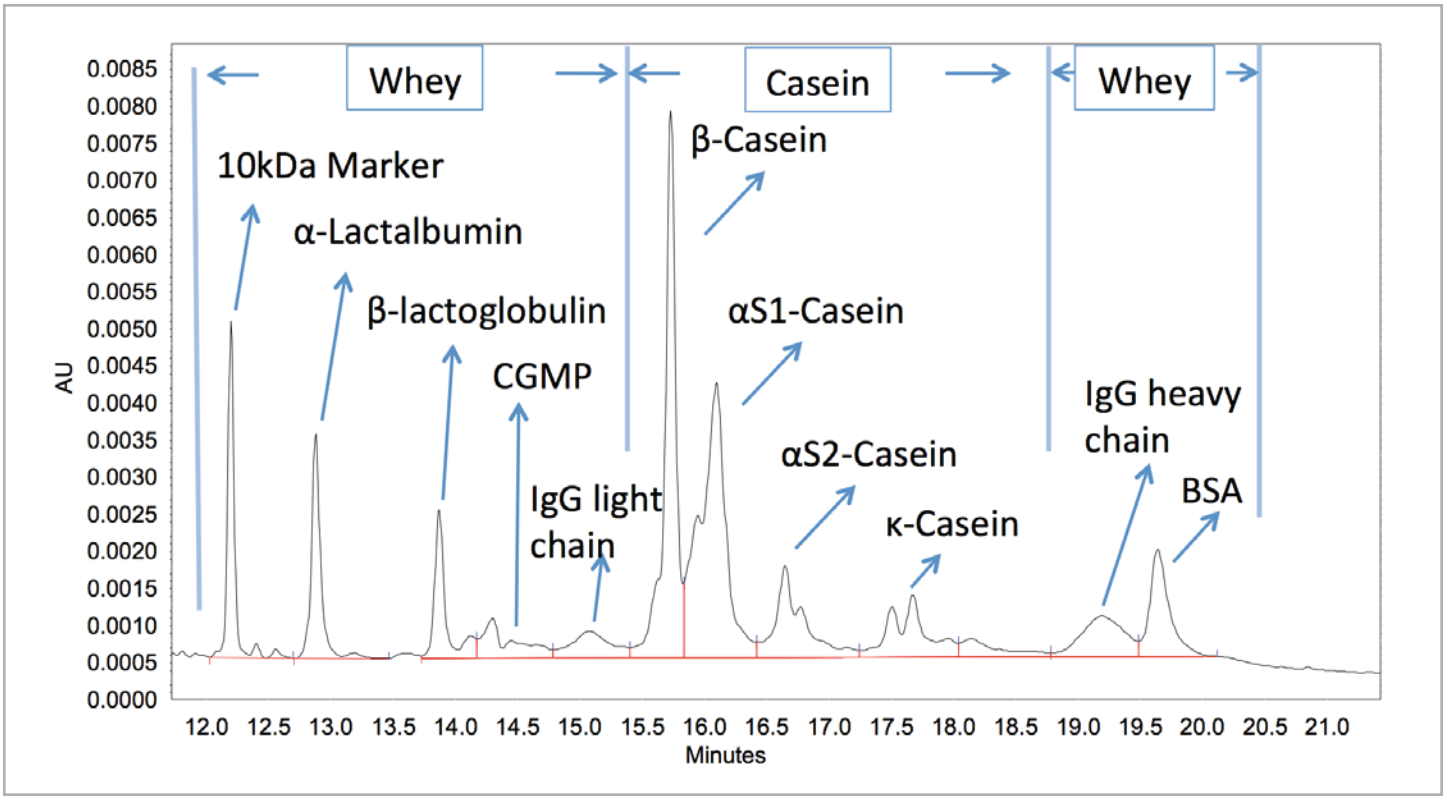


Figure 5. Electropherogram of protein standard mix. The first integrated peak is the 10 kDa marker.

Conclusion

Capillary electrophoresis with SDS was successfully applied to the quantification of whey protein content in infant formula. The CE-SDS method fully separated the whey proteins, casein proteins, and immunoglobulins, facilitating quantification of the whey proteins. Validation in multiple laboratories has shown the method to be an accurate, reliable solution to the challenging problem of verifying whey content.

References

1. Ping Feng, Christophe Fuerer, Adrienne McMahon. Quantification of Whey Protein Content in infant Formulas by Sodium Dodecyl Sulfate-Capillary Gel Electrophoresis (SDS-CGE): Single-Laboratory Validation, First Action 2016.15; *Journal of AOAC International*, Vol. 100, No. 2, 2017

Who is SCIEX? SCIEX company's global leadership and world- class service and support in the capillary electrophoresis and liquid chromatography-mass spectrometry industry have made it a trusted partner to thousands of the scientists and lab analysts worldwide who are focused on basic research, drug discovery and development, food and environmental testing, forensics and clinical research.

Contact Us: sciex.com/contact-us

Publication number: RUO-MKT-02-8133-B 07/2018

SCIEX Triple Quad™ 3500系統對大米中固醇類物質的定性與定量分析

Identification and Quantitation of Sterols in rice by SCIEX Triple Quad™ 3500 system

李志剛，孫小傑，劉冰潔，郭立海

Li ZhiGang, Sun Xiaojie, Liu Bingjie, Guo Lihai

*SCIEX*應用技術中心，北京

Keywords: Sterols, Rice, Triple Quad™ 3500

前言

固醇是天然固族化合物中的一類物質，按照來源可分為動物固醇、植物固醇和菌固醇。 植物固醇是一種天然活性物質，通常為無色、無味、不溶于水、易溶於有機物，結構上與膽固醇相似。植物固醇主要來源於植物類食物，且大部分存在於油料種子中。食用植物油中含有多種固醇，固醇含量相對較多的為稻米油、玉米油、葵花籽油、菜籽油，主要固醇種類為菜籽固醇、菜油固醇、豆固醇、β-穀固醇。植物固醇應用範圍很廣，添加到醫藥品中具有降低血清總膽固醇、低密度脂蛋白膽固醇以及血清甘油三酯濃度的功效，添加到化妝品中可提高乳化穩定性，添加到飼料中可以提高禽畜的增重率。

液相色譜串聯質譜法測定食品中固醇具有前處理方法簡單、檢出限低的特點。可以高效、快速的完成檢測工作。基於此，我們開發了三重四極杆對九種固醇的進行定性與定量分析方法。

試驗方法

1. 樣品前處理

取1 g樣品於250 mL圓底燒瓶中，加入70 mL內標溶液，稱重，充分混勻後放入回流冷凝器中，加熱並保持提取液微沸，30 min後停止加熱並冷卻至室溫，用無水乙醇補足失重，混勻，吸取5 mL於8000 r/min下離心5 min，取2 mL上清液過0.22 μm尼龍濾膜，待測。

2. 液相條件

液相：SCIEX Exion LC系統

分析柱及流動相條件：Phenomenex Kinetex F5（3.0×50 mm, 2.6 μm），流速0.5 mL/min，流動相A：水（0.1%甲酸）；B：甲醇，梯度見表1。

柱溫：40 ℃

梯度洗脫條件：

表**1.** 流動相洗脫程式

時間	A %	B %
0	40	60
1	40	60
6	5	95
8	5	95
8.1	40	60
10	40	60

3. 質譜條件

SCIEX Triple Quad™ 3500系統

離子源：APCI源

離子源參數：

NC電壓：3 V

氣簾氣：20 psi

霧化氣GS1：40 psi

源溫度TEM：500 ℃

碰撞氣CAD：8

表**2.** 10種固醇的質譜參數

化合物名稱	英文名稱	保留時間 RT,min	母離子	子離子	去簇電壓 DP, V	碰撞能量 CE, v
麥角固醇	Ergosterol	4.10	379.3	69	90	45
			379.3	125	90	20
菜籽固醇	Brassicasterol	4.16	381.3	69	66	40
			381.3	147	66	30
鹽藻固醇	Fucosterol	4.24	395.4	81	52	50
			395.4	147	52	30
羊毛固醇	Lanosterol	4.21	409.4	109	109	36
			409.4	149	109	38
膽固烷醇	β -Cholestarol	4.21	371.4	81.1	100	50
			371.4	109	100	37
豆固醇	Stigmasterol	4.24	395.4	81	52	50
			395.4	147	52	30
菜油固醇	Campesterol	4.24	383.4	161.1	100	36
			383.4	95.1	100	40
β -穀固醇	β -Sitosterol	4.31	397.4	161	60	41
			397.4	135.1	60	37
豆固烷醇	Stigmastanol	4.34	399.4	81.1	90	68
			399.4	149	90	32
6-膽固烷醇	6-Ketocholestanol	4.17	385.3	367.3	100	40
			385.3	159.2	100	41

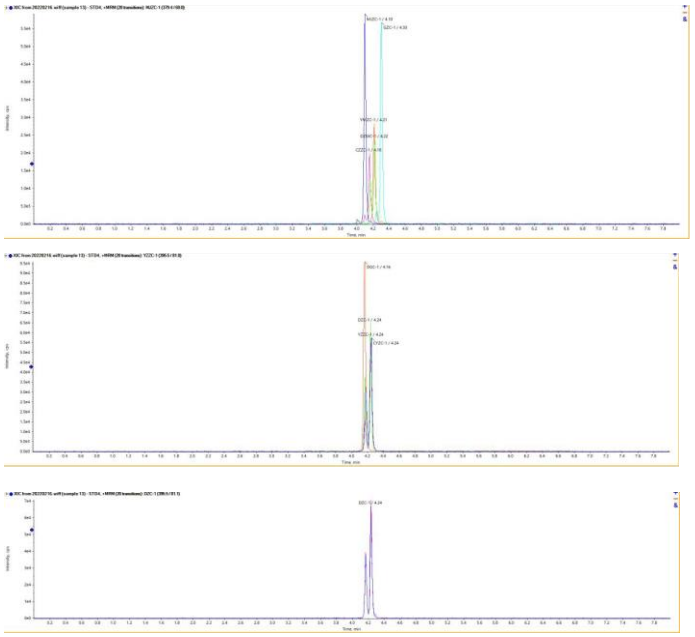


圖**1.** 10種固醇的典型色譜圖

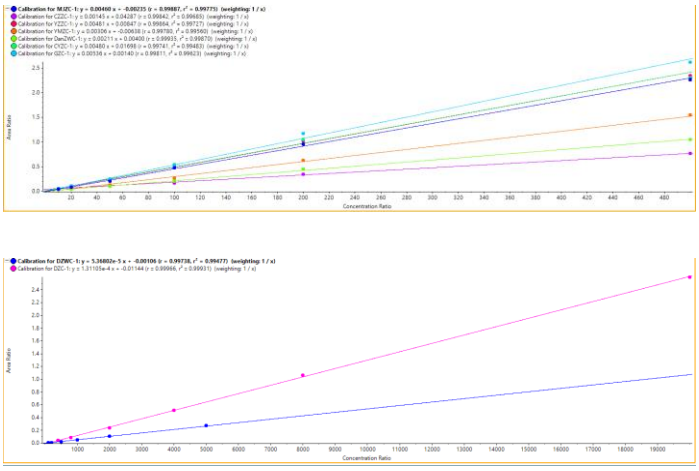


圖**2.** 9種固醇的線性關係曲線

4. 實驗結果

4.1 固醇的典型色譜圖（見圖***1***）

4.2 標準曲線及靈敏度結果（見圖***2***，表***3***）

採用空白溶劑加標，配置濃度在10-500 μg/L範圍內的系列標準曲線，全部9種化合物線性關係良好，見圖2。

將50 μg/L的標準品溶液逐級用無水乙醇稀釋，以信噪比S/N≥10為定量限標準，確定9種化合物的儀器靈敏度，見表3。

表**3.** 9種固醇的線性方程及定量限結果

化合物名稱	線性方程	相關係數	線性範圍（**μg/L**）
麥角固醇	y = 0.0046 x + 0.00235	0.9989	10-500
菜籽固醇	y = 0.00145x + 0.04287	0.9984	10-500
鹽藻固醇	y = 0.00481x + 0.00847	0.9986	10-500
羊毛固醇	y = 0.00306x + 0.00638	0.9978	10-500
膽固烷醇	y = 0.00211x + 0.00400	0.9994	10-500
菜油固醇	y = 0.00480x + 0.01698	0.9974	10-500
穀固醇	y = 0.00536x + 0.00140	0.9981	10-500
豆固烷醇	y = 5.36082e-5x -0.00106	0.9974	100-5000
豆固醇	y =1.31105e-4x -0.01144	0.9997	400-20000

4.3 重複性及回收率結果

針對本方法中涉及的9種化合物，在1、10、100 ng/L三個濃度下進行加標回收試驗，大米的實際加標回收率在90.5-104.9%之間，滿足方法學要求。同時進行三個濃度點的重複性試驗，所有化合物的相對標準差RSD在0.50-1.61%之間，方法及儀器的穩定性良好。

總結

建立了一種SCIEX Triple Quad™ 3500分析大米中9種固醇的分析方法。該方法前處理操作簡單，可有效地節約時間和人力成本，提高工作效率；方法的靈敏度高、重複性好、準確度高，經過多批次的實際樣品測定，結果穩定可靠。

基於ZenoTOF™ 7600系統的不同品種藜麥食品組學研究

Foodomic Study of Different Varieties of Quinoa Based on ZenoTOF™ 7600 System

陳慧敏[1]；王淑芳[2]；楊總[1]；劉冰潔[1]；郭立海[1]

Chen Huimin[1], Wang Shufang[2], Yang Zong[1], Liu Bingjie[1], Guo Lihai[1]

SCIEX, China[1]

Jiangsu Academy of Agricultural Science, China[2]

Key words: Metabolomics; Lipidomic; Chenopodium quinoa; Zeno™ trap; EAD; ZenoTOF™ 7600

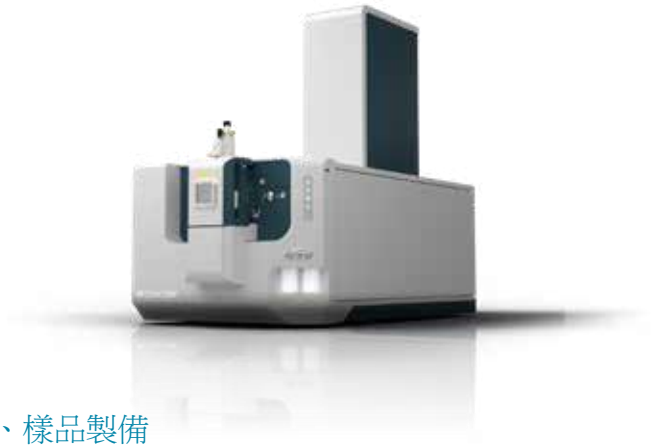
引言

藜麥（拉丁名： Chenopodium quinoa Willd.）是藜科藜屬植物。富含蛋白質、不飽和脂肪酸、維生素和礦物質等營養成分，可以滿足人體所需的基本營養，被譽為“太空食品”和 “素食之王”[1]。此外，藜麥具有耐寒、耐旱、耐瘠薄、耐鹽鹼等生理特點，對未來農業系統的發展具有十分重要的意義。我國於上世紀80 年代引入藜麥，近些年開始呈現規模化種植。基於代謝組學和脂質組學進行藜麥的營養成分鑒定，品種產地鑒別很有意義。

本實驗採用ZenoTOF™ 7600系統的非靶向脂質組學和代謝組學方法進行5個不同品種藜麥成分鑒定和品種鑒別。使用SCIEX OS軟體對藜麥完成資料獲取和資料分析工作，鑒定出藜麥中小分子代謝物及脂類成分，並進行五個品種的差異分析。

實驗方案特點：

1. 實驗對藜麥脂質組學和代謝組學進行了系統研究，綜合分析藜麥品種的差異
2. SCIEX ZenoTOF™ 7600系統展示出良好穩定性，輕鬆應對大批量組學樣本實驗
3. Zeno™ trap (Zeno阱）幫助提升二級譜圖品質，保證組學鑒定結果的準確性
4. 電子啟動解離（EAD）碎裂技術方式，一針進樣得到脂質全面的資訊，脂類的頭基、脂質骨架、特徵診斷離子、脂肪酸鏈和雙鍵位置資訊，對脂質精細結構鑒定提供了有力的手段



1、樣品製備

5個品種的藜麥各10份，共50份。加入液氮磨粉，分別提取脂質和小分子代謝物用於脂質組學和代謝組學研究。製備好的樣品每個取10ul混合為質控樣（QC）。

2、實驗條件

質譜系統： SCIEX ZenoTOF™ 7600系統；

掃描方式：ESI+/-TOF MS-IDA-30TOF MS/MS; 動態背景扣除開啟；Zeno™ Trap 開啟；掃描範圍：代謝組學：一級m/z 60-1200，二級m/z 30-1200；脂質組學：一級m/z 200-1250，二級m/z 100-1250；

噴霧電壓IS: 5500 V	源溫度 TEM: 500 ℃
氣簾氣 CUR: 30 psi	碰撞氣 CAD: 7
霧化氣 GS1: 50 psi	輔助氣 GS2: 50 psi

3、結果與討論

3.1 資料重現性和可靠性

SCIEX ZenoTOF™ 7600系統穩定性和抗污染能力保證資料重現性及真實性。每10針穿插一針QC，脂質組學樣品及代謝組學樣品在正離子模式和負離子模式下各自的6針QC總離子流圖（TIC）重現性良好，見圖1和圖2。表明信號強度和保留時間穩定，採集到的大批量組學樣本結果可靠，可以用來進行差異分析。

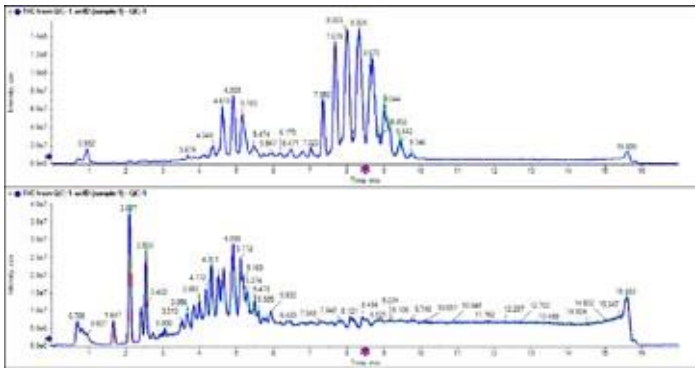


圖**1.** 脂質組學樣品6針QC疊加的正離子模式（上）和負離子模式（下）總離子流圖

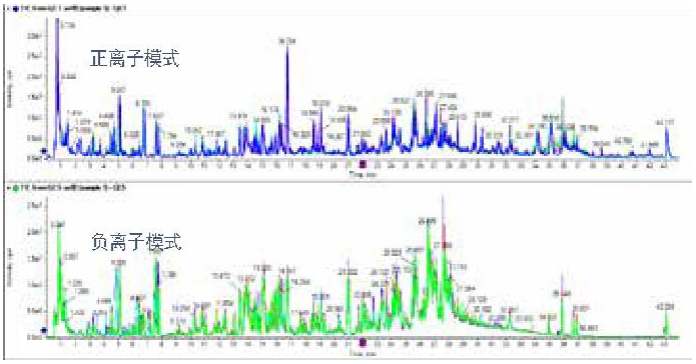


圖**2.** 代謝組學樣品6針QC疊加的正離子模式（上）和負離子模式（下）總離子流圖

*3.2 EAD*技術在食品組學的應用

脂質鑒定過程中,以卵磷脂（PC）為例，傳統CID碎裂模式需要正離子模式採集資料確定脂質的類別，負離子模式採集一針資料得到脂肪酸鏈資訊。共同確證才能確定兩條脂肪酸鏈的具體資訊，若存在不飽和雙鍵無法確定不飽和雙鍵位置（圖3）。採用EAD碎裂模式可以一針進樣得到脂質豐富的碎片資訊，通過m/z 184.0801、m/z224.1065、m/z 226.0858判斷為PC類脂質，診斷離子m/z 489.3200判斷第二條脂肪酸鏈為FFA18:2，根據EAD模式碎裂單鍵而不碎裂雙鍵的特點，脂肪酸鏈的單鍵斷裂會有規律的差-CH2質量數差14，可在二級譜圖上看到連續差15個14da的碎片，這就是脂肪酸鏈16：0產生的碎片。而雙鍵出現的位置即為質荷比差值為26（-C2H2）的位置，因此可以確認脂肪酸鏈18：2上C8和C10之間以及C11和C13之間為雙鍵位置，準確的鑒定出脂質化合物精細結構為PC sn1 16:0/sn2 18:2(n-12, n-9)（圖4）。

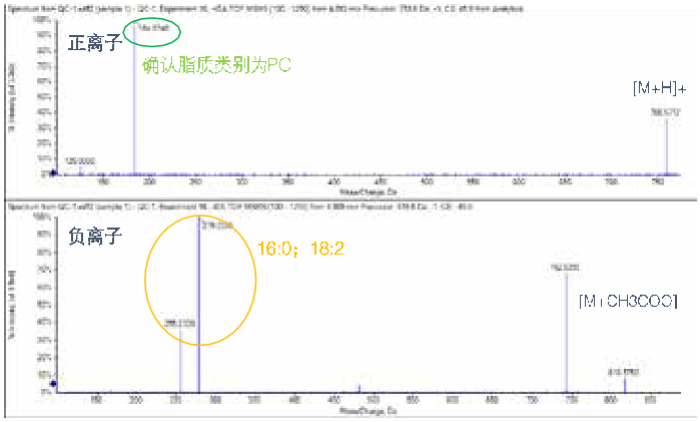


圖**3.** 脂質PC16:0-18:2(n-12,n-9) 的CID模式下的二級質譜圖

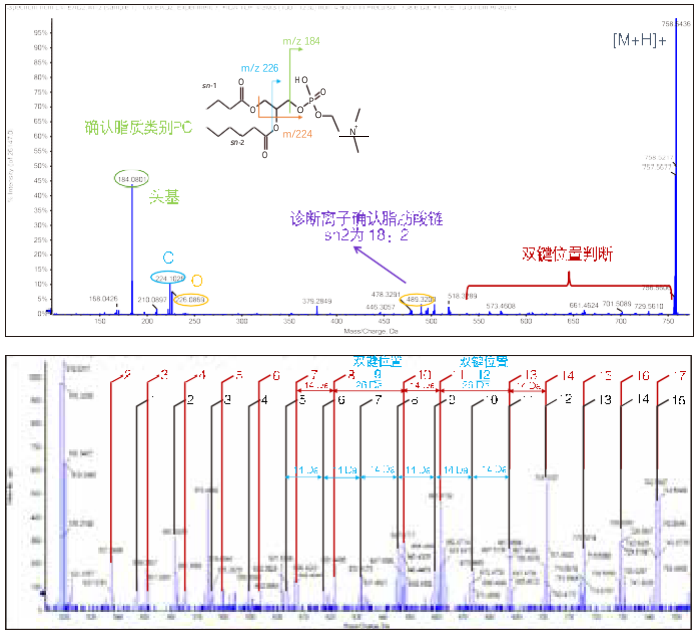


圖**4.** 脂質PC16:0-18:2(n-12,n-9) 的EAD模式下的二級質譜圖及結構確認（上）；二級譜圖部分放大及雙鍵位置確認（下）

*3.3 Zeno™ trap*在食品組學的應用

Zeno™ trap的阱集能力可以使得即使很低的碎片依舊可以得到，提高所有檢測到的化合物二級品質，從而幫助更準確的判斷化合物結構提高鑒定到的化合物數目。我們採集到的代謝組學資料表明，開啟Zeno™ trap可以使二級譜圖品質評估>80分的比例從56% 提升至96% 。橘皮素的鑒定情況也可以展示出Zeno™ trap對於二級譜圖的回應增強使得鑒定結果的可靠性顯著提高（圖5）。

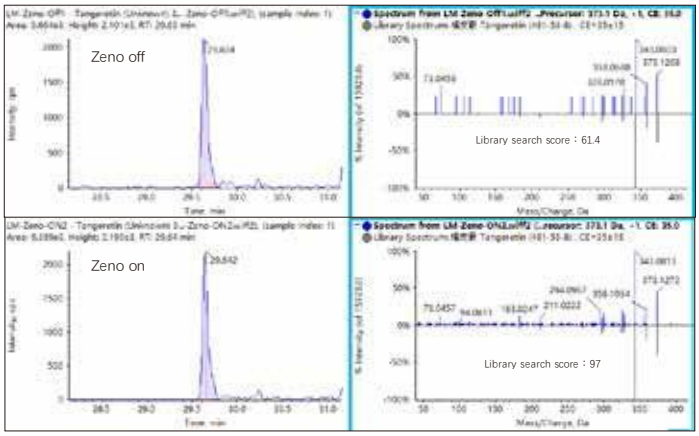


圖5. 橘皮素鑒定時二級譜圖庫匹配結果在Zeno off（上）與Zeno on（下）對比

3.4 脂質組學鑒定結果

基於SCIEX ZenoTOF™ 7600系統通過非靶向脂質組學技術共鑒定到16大類，384種脂質（圖6）。藜麥中脂質含量最多的是甘油酯，卵磷脂和游離脂肪酸。對五個品種藜麥中鑒定到的脂質的峰面積進行統計學分析篩選出154個差異脂質分子可以用於不同品種藜麥的區分。偏最小二乘法判別分析（PLS-DA）結果見圖7，可以看出品種4在脂類化合物與其他品種有較大差異。

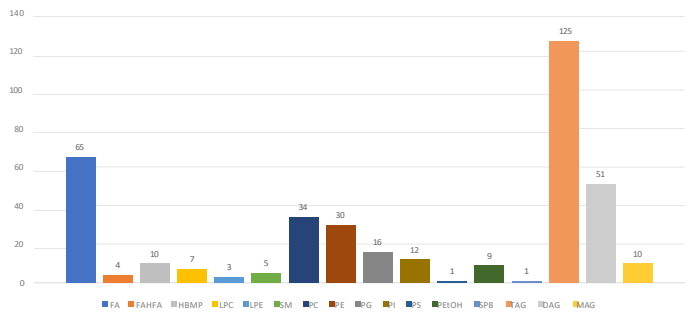


圖6. 藜麥的脂類化合物鑒定結果

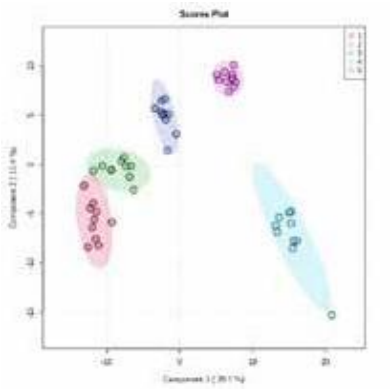


圖7. 五個品種藜麥中代謝物的PLS-DA圖

3.5 代謝組學鑒定結果

結合SCIEX代謝物資料庫和天然產物資料庫共鑒定到藜麥中246個化合物，個數占比最高的為皂苷類，黃酮類和氨基酸相關化合物（圖8）。利用五個品種藜麥中鑒定到的代謝物峰面積進行PLS-DA分析，圖9可以看出幾個品種藜麥代謝組成具有顯著差異，但品種4和5差異較小；並且篩選出55個差異組分可以用於不同品種藜麥的區分。

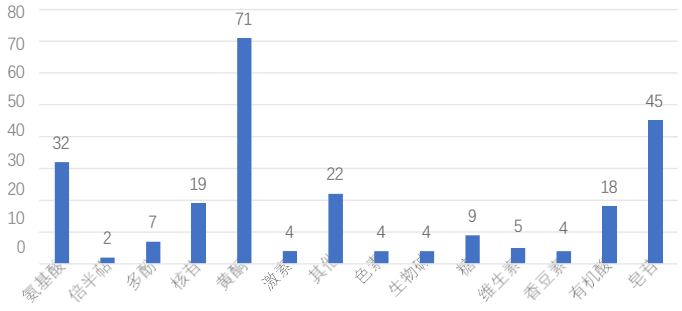


圖8. 藜麥的代謝物鑒定結果分佈圖

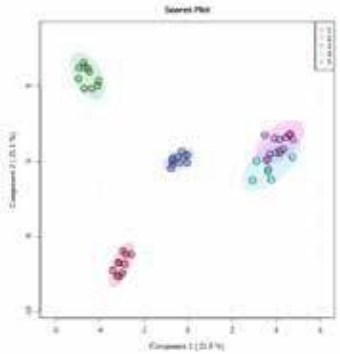


圖9. 五個品種藜麥中代謝物的PLS-DA圖

4、總結

本文使用SCIEX ZenoTOF™ 7600系統建立了藜麥非靶向脂質組學和代謝組學方法。Zeno™ trap技術顯著提升了組學樣本二級品質，提高定性結果的準確性。EAD碎裂技術產生區別於CID碎裂方式的二級碎片資訊,說明鑒定脂質精細結構。ZenoTOF™ 7600系統採集的資料保證得到全面豐富的鑒定結果，幫助進行藜麥成分鑒定和品種鑒別。

參考文獻

1. 肖正春，張廣倫. 藜麥及其資源開發利用[J]. 中國野生植物資源,2014, 33(2): 62-66.

應用X500R QTOF系統基於代謝組學進行黃酒的酒齡鑒別

Application of SCIEX X500R QTOF in Chinese Rice Wine Metabolomics

陳慧敏[1]， 胡建剛[2]， 郇宇[1]， 楊總[1]， 劉冰潔[1]， 郭立海[1]

Chen Huimin[1], Hu Jiangang[2], Huan Yu[1], Yang Zong[1], Liu Bingjie[1], Guo Lihai[1]

[1] *SCIEX, China*

[2] *Shaoxing Food And Drug Administration, China*

Keywords：Metabolomics; Chinese rice wine; X500R

引言

黃酒在陳釀老化過程中，酒體中各種成分相互作用發生大量的化學變化和物理變化，改善了酒體的口感和穩定性，因而黃酒的陳釀時間（即酒齡）是其品質的主要標誌。目前，酒齡鑒別是黃酒行業存在的諸多問題之一，各個生產廠家庫存的陳年黃酒有多有少，勾兌技術不統一，少數企業有隨意標注酒齡的現象,不利於黃酒的推廣和銷售。因此需要較明確的品質指標作為酒齡判斷依據。

本文通過X500R QTOF系統採用非靶向代謝組學方法進行不同陳釀時間的黃酒成分差異研究。使用SCIEX OS軟體對黃酒完成資料獲取和資料分析工作，鑒定出黃酒中小分子代謝物，並進行五個酒齡的黃酒的差異分析，幫助探究酒齡鑒別的指標。

實驗方案特點：

1. 本實驗利用代謝組學思路對紹興黃酒進行成分鑒定，並進行了陳釀年份對黃酒品質風味影響的研究；
2. SCIEX X500R QTOF系統展示出良好穩定性，輕鬆應對大批量組學樣本實驗；
3. SCIEX OS軟體紅綠燈式篩查設置結合高品質二級譜庫幫助簡單快速進行化合物鑒定；
4. 研究結果篩選出黃酒中80種差異化合物可以用來進行陳釀年份的判別。

1、樣品準備

5個酒齡的紹興黃酒（2009年份、2011年、2014年份、2019年份和2021年份相當於13年份、11年份、8年份、4年份和1年份酒齡）各10份，共50個樣品。50%甲醇水(v/v)稀釋五倍後過0.22 μm濾膜進樣。製備好的樣品每個取10 μL混合為質控樣（QC）。

2、實驗條件

質譜系統： SCIEX X500R QTOF系統（圖1）；

圖1. SCIEX X500R QTOF 系統

掃描方式：ESI+/-TOF MS-IDA-10 TOF MS/MS； 動態背景扣除開啟；掃描範圍：一級m/z 70-1250 Da，二級m/z 40-1250 Da；

噴霧電壓IS: 5500 V/-4500 V　　源溫度 TEM: 500 ℃

氣簾氣 CUR: 30 psi　　碰撞氣 CAD: 7 psi

霧化氣 GS1: 50 psi　　輔助氣 GS2: 50 psi

3、結果與討論

3.1 資料重現性和可靠性

SCIEX X500R QTOF系統穩定性和抗污染能力保證資料重現性及可靠性。每5針穿插一針QC, 代謝組學樣品在正離子模式和負離子模式下各自的11針QC總離子流圖（TIC）重現性良好, 見圖2。表明信號強度和保留時間穩定，採集到的大批量組學樣本結果可靠，可以用來進行差異分析。

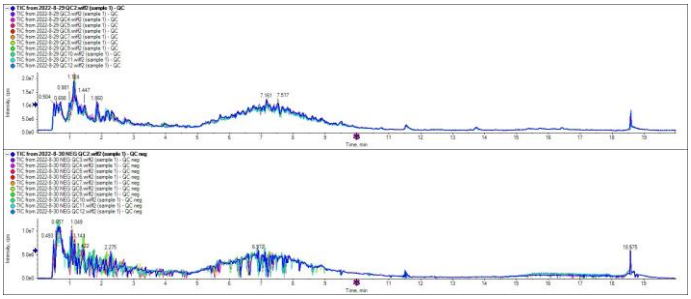


圖**2.** 樣品11針QC疊加的正離子模式（上）和負離子模式（下）總離子流圖

*3.2 SCIEX OS*軟體說明快速鑒定黃酒成分

利用SCIEX X500R QTOF系統對QC進行正離子模式和負離子模式分別一針進樣便可以獲得全面一級二級資訊，用於準確定性。結合高品質二級譜庫，利用SCIEX OS軟體通過一級品質精度、同位素分佈和二級庫匹配快速進行化合物篩查鑒定，紅綠燈式篩查結果顯示如圖3。並且可以呈現詳細色譜圖積分情況、一級質譜圖的同位素豐度比對情況以及二級質譜圖與二級譜庫中標準譜圖匹配情況，以丁二酸為例見圖4。

Index	Component Name	Area	Reten... Time	Adduct / Charge	Formula	Precur... Mass	Mass Error (...	Mass Error Conf...	Isotope Confidence	Library Confide..
111	Leu-Leu	3.397e6	7.09	[M+H]+	C12H24N2O3	245.1860	-0.8	✓	✓	✓
113	脯氨酸 Proline	3.220e6	0.65	[M+H]+	C5H9NO2	116.0706	-0.2	✓	✓	✓
115	Norharmane	2.705e6	7.09	[M+H]+	C11H8N2	169.0760	-0.7	✓	✓	✓
116	Glycerophosphocholi...	2.531e6	0.61	[M+H]+	C8H20NO6P	258.1101	0.8	✓	✓	✓
118	Ile-Leu	2.348e6	6.30	[M+H]+	C12H24N2O3	245.1860	-0.3	✓	✓	✓
117	Acetophenone	2.339e6	1.24	[M+H]+	C8H8O	121.0648	-0.7	✓	✓	✓
119	Ile-Phe	2.165e6	7.48	[M+H]+	C15H22N2O3	279.1703	-0.6	✓	✓	✓
120	Ile-Val	2.099e6	3.55	[M+H]+	C11H22N2O3	231.1703	-0.6	✓	✓	✓
168	PyroGlu-Pro	2.026e6	2.73	[M+H]+	C10H14N2O4	227.1026	0.0	✓	✓	✓
122	Val-Pro	1.973e6	2.00	[M+H]+	C10H18N2O3	215.1390	0.1	✓	✓	✓
121	Val-Val	1.877e6	1.86	[M+H]+	C10H20N2O3	217.1547	0.1	✓	✓	✓
123	Val-Ile	1.747e6	4.21	[M+H]+	C11H22N2O3	231.1703	-0.8	✓	✓	✓
124	L-焦谷氨酸 L-Pyroglut...	1.734e6	1.12	[M+H]+	C5H7NO3	130.0499	-1.0	✓	✓	✓

圖**3.** 紅綠燈式篩查結果顯示

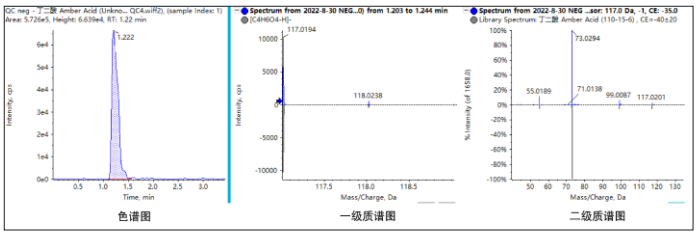


圖**4.** 丁二酸色譜圖、一級質譜圖和二級質譜圖

3.3 代謝組學鑒定結果及差異分析

結合SCIEX代謝物資料庫和天然產物資料庫共鑒定到黃酒中208個化合物，個數占比最高的為氨基酸相關化合物。另外還鑒定到多酚、有機酸、核苷酸、醛類、酯類以及糖等化合物（圖5）。利用五個年份黃酒中鑒定到的代謝物峰面積進行PLS-DA分析，圖6可以看出五個年份黃酒代謝物組成具有顯著差異。利用ANOVA P<0.05 & VIP>1篩選差異化合物，篩選出黃酒的不同陳釀年份產生的80個差異化合物。

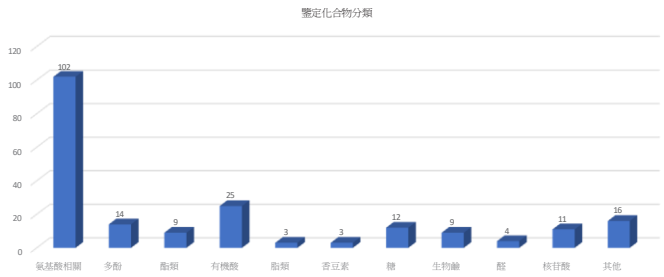


圖**5.** 黃酒的代謝物鑒定結果分佈圖

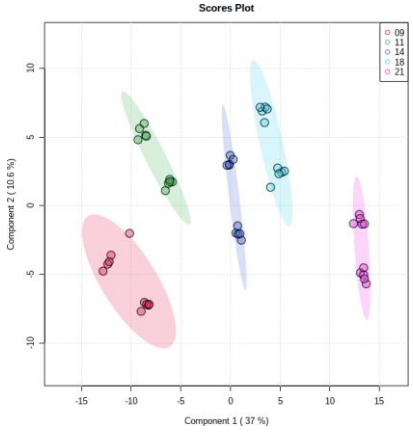


圖**6.** 五個酒齡黃酒中代謝物的PLS-DA圖

結果表明，不同的氨基酸及小肽隨著陳釀年份增加有上調和下調變化, 其中4種環二肽隨著年份增加均呈現顯著增長，2011年釀造的11年的黃酒含量最高而酒齡達到13年時含量稍有下降。另外幾種有機酸、醛類和酯類顯著上調，有研究表面酯類的升高這一趨勢普遍存在於紹興黃酒的陳釀過程中[1]。最後我們還發現7種化合物只在酒齡為一年的黃酒中檢出（見表1），釀造時間更長的黃酒中無檢出。均可考慮作為黃酒酒齡鑒別依據。圖6為環(纈氨酸-脯氨酸) 和水楊酸在不同酒齡黃酒中含量變化的箱狀圖。

圖**7.** 五個酒齡黃酒中環(纈氨酸-脯氨酸) 和水楊酸含量變化箱狀圖

表**1.** 僅2021年釀造的黃酒檢測到的化合物

序號	中文名
1	小麥黃素
2	甲硫氨酸
3	山奈酚-3-葡萄糖苷-7-木糖苷
4	5′-去氧-5′-(甲硫基)腺苷
5	3-吲哚丙烯酸
6	4-甲基傘形酮
7	苯乙酸

4、總結

本文使用SCIEX X500R QTOF系統建立了黃酒非靶向代謝組學方法。由於X500R超快掃描速度兼顧靈敏度和解析度，一針進樣即可得到複雜基質樣品中待測物的全面的高品質一級與二級質譜資料，且一級品質精度均小於1 ppm。並且利用SCIEX OS軟體配合一級質譜、同位素分佈和二級質譜圖可快速準確地提供定性結果。X500R QTOF系統採集的資料保證得到全面豐富的鑒定結果，幫助進行黃酒成分鑒定和陳釀年份鑒別。

參考文獻

1. 江超，林峰，鄒慧君等．陳年紹興黃酒成分分析和品質鑒定．食品與發酵工業，2009，35(10): 18-22.

Determination of Irganox compounds extracted from food packaging using 4 food simulants

Quantification of Irganox chemicals using the QTRAP 6500+ system

Lakshmanan Deenadayalan[1], Sashank Pillai[1], Jessica Smith[2], Jack Steed[2] and Jianru Stahl-Zheng[3]
[1]SCIEX, India; [2]SCIEX, UK; [3] SCIEX, Germany

Introduction

This technical note demonstrates the accurate and precise quantification of 16 Irganox compounds spiked into food simulants and in a plastic food packaging extract. Most Irganox compounds met the acceptable accuracy (70-130%) and precision criteria (%CV <15%) when quantifying from a single solvent external calibration curve, eliminating the need for matrix-matched standards. The lowest calibration standard measured ranged from 0.025 to 0.50 ng/mL, demonstrating the sensitivity of the QTRAP 6500+ system.

Human health can be impacted by chemicals that migrate into foods and beverages from food contact materials (FCMs), such as wrappers and containers. Understanding the risk of FCM chemicals requires sensitive and accurate analytical methods. Extractables are chemicals that are released when the FCMs are stressed, such as using solvents, elevated temperatures, solvent exposure time and agitation. Conversely, leachables are chemicals that migrate under ambient conditions.

The method presented in this technical note focuses on extractables found in food packaging. The method was developed for the quantification of a diverse suite of 16 Irganox compounds found in food packaging using 4 food simulants, as outlined by the EU regulations.[1]

Key features of the QTRAP 6500+ system for the analysis of Irganox compounds

- Two external calibration curve preparations that were dependent on compound-specific solubility were used to analyze food packaging samples in 4 different food simulants, based on EU regulations
- The sensitivity of the QTRAP 6500+ system enabled accurate quantification below the 10 ng/mL EU regulatory level
- Linearity was observed between 0.025 and 50 ng/mL with a r^2 value >0.99
- Post-spike sample accuracy was observed between 70% and 130% with %RSD <15% when quantified using an external calibration curve (n=6)

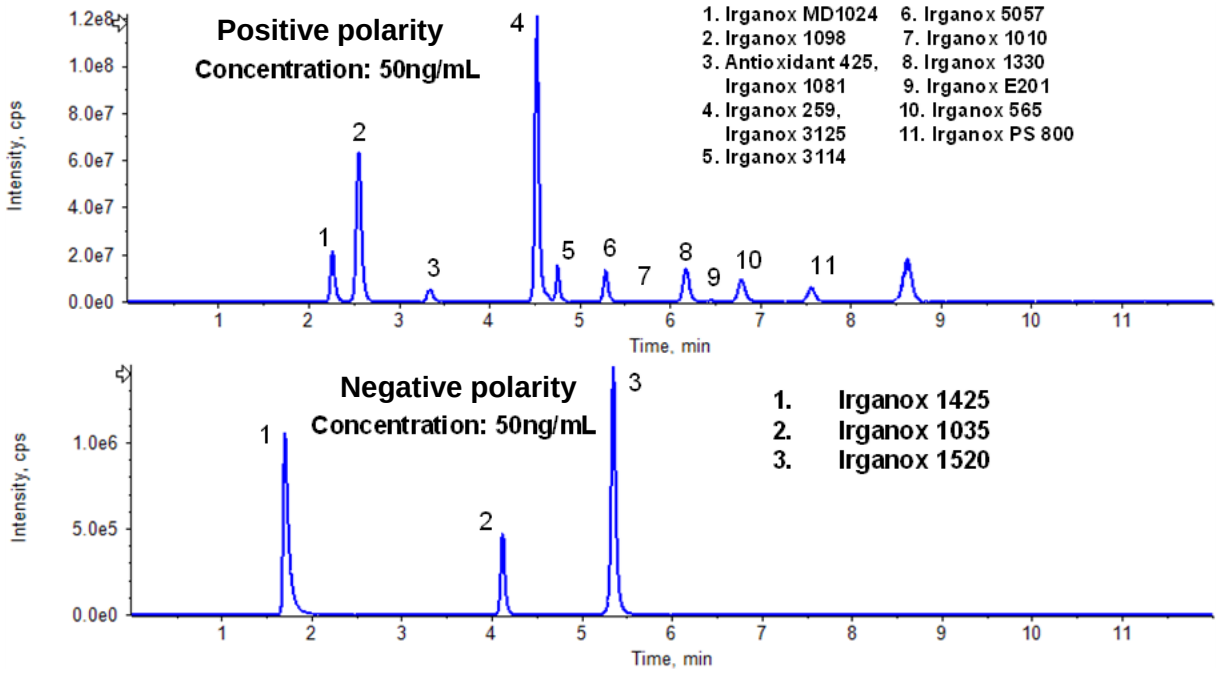


Figure 1. Overlaid extracted ion chromatograms (XICs) of 16 Irganox compounds. Positive ion (top panel) and negative ion (bottom panel) analytes were acquired in electron spray ionization (ESI) mode and are shown for a 50 ng/mL standard.

Methods

Standard preparation: All standards were weighed to approximately 1 mg, dissolved in 1 mL of methanol and sonicated for 5 min. Irganox 1529 (n-hexane) and Irganox E201 (ethanol) were available as neat standard solutions. A 50 µg/mL mixed standard was prepared from the individual stock solutions with a final solvent composition of 10:90 (v/v), water/methanol.

Calibration curve preparation: Stock solutions were prepared in methanol and spiked into calibration solution 1 or 2, depending on compound-specific solubility. Calibration solutions were composed of 50:50 (v/v), water/ethanol with 3% acetic acid by volume (calibration curve 1) and 50:50 (v/v), water/ethanol (calibration curve 2). Thirteen of the 16 Irganox compounds were prepared as described for calibration curve 1. The final calibration curves ranged from 0.025 to 50 ng/mL.

Food simulant spikes: Compounds were spiked into each of the 4 food simulants to yield final in-sample concentrations of 0.10, 1.00 and 2.5 ng/mL but were then diluted using the appropriate solvent in a 1:2 ratio to ensure complete solubility (Table 1). Therefore, the final in-vial concentrations were 0.033, 0.33 and 0.83 ng/mL. The food simulant spike samples were quantified using both calibration curves 1 and 2.

Food packaging material extract preparation: A 1 g sample of a plastic food packaging material (yogurt container) was weighed into each 10 mL glass centrifuge tube and 10 mL of each simulant was added (Table 1). The tubes were vortexed for 5 minutes and incubated at 40°C for 24 hours. Samples were filtered through a 0.22 µm syringe filter and diluted using the appropriate dilution solvent in a 1:2 ratio (Table 1).

Post-extraction spike of food packaging material: Extracts were prepared as described above. The final extract was spiked with the mixed Irganox standard to yield an in-sample concentration of 10 ng/mL. The resulting solution was then diluted with the appropriate solvent in a 1:2 ratio (Table 1).

Table 1. Food simulants and dilution solvents used to evaluate plastic materials or articles intended for food contact based on the EU commission regulation (10/2011).

Food simulant	Dilution solvent
3% Acetic acid (w/v)	*100% Ethanol*
10% Ethanol (w/v)	*50 % Ethanol with 3% acetic acid*
20% Ethanol (w/v)	*50 % Ethanol with 3% acetic acid*
50% Ethanol (w/v)	*Samples were injected without dilution*

Chromatography: The ExionLC AD system was used with a Phenomenex Luna Omega Polar C18 analytical column (C18 100Å, 3 µm, 100 x 4.6 mm). In addition, an Agela Ghost Hunter column (50 x 4.6 mm) was used as a delay column to separate the analytical peak from the LC pump contamination. The LC gradient conditions used are shown in Table 2. Mobile phase A was water with 10mM ammonium formate and 0.1% formic acid by volume. Mobile phase B was methanol. The flow rate was 0.8 mL/min and the column oven temperature was 40°C.

Table 2. LC gradient used for the separation of 16 Irganox compounds.

Time (min)	%A	%B
0.01	*10*	*90*
1.0	*10*	*90*
2.0	*2*	*98*
10.5	*2*	*98*
10.6	*10*	*90*
12.0	*10*	*90*

Mass spectrometry: Samples were analyzed using the SCIEX QTRAP 6500+ system with electrospray ionization (ESI) and polarity switching. Data were acquired using the Scheduled MRM algorithm. The target scan time was 0.80 sec for positive polarity and 0.30 sec for negative polarity. The optimized source and gas parameters and compound-specific MRM parameters used are shown in Tables 3 and 4, respectively.

Table 3. Optimized source and gas parameters.

Parameter	Value
Curtain Gas	*40 psi*
CAD Gas	*Medium*
Ion Spray Voltage	*4500/-4500 V*
Temperature	*500°C*
GS1	*90 psi*
GS2	*35 psi*

Data processing: All data were processed using SCIEX OS software, version 2.1.6. For the consistency with the EU regulations, concentrations are reported on an in-sample basis which is representative the method sensitivity, whereas the in-vial concentrations represent the instrument sensitivity.

Table 4. Optimized compound-specific parameters used for analysis.

Compound	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (V)	CXP (V)
Irganox PS 800	*515.4*	*329.2*	*150*	*20*	*13*
Irganox 1010	*1194.8*	*785.4*	*130*	*73*	*13*
Irganox 1330	*792.6*	*219.1*	*125*	*37*	*12*
Irganox MD1024	*553.4*	*237.1*	*120*	*38*	*10*
Irganox 565	*589.3*	*250.0*	*160*	*62*	*15*
Irganox 5057	*394.3*	*134.1*	*195*	*50*	*10*
Irganox 3125	*1059.6*	*762.4*	*100*	*44*	*14*
Irganox 1098	*637.5*	*581.4*	*130*	*32*	*11*
Irganox 259	*656.6*	*415.3*	*80*	*34*	*10*
Irganox 1081	*359.0*	*139.1*	*40*	*28*	*8*
Irganox E201	*431.1*	*165.1*	*120*	*37*	*10*
Antioxidant 425	*386.3*	*135.1*	*20*	*26*	*16*
Irganox 3114	*801.5*	*784.5*	*88*	*20*	*12*
Irganox 1425	*327.1*	*299.1*	*-115*	*-33*	*-11*
Irganox 1035	*641.3*	*423.2*	*-130*	*-42*	*-13*
Irganox 1520	*423.2*	*145*	*-60*	*-21*	*-11*

Chromatographic separation

Good separation was achieved for the 16 Irganox compounds using an optimized chromatography method (Figure 1). A fast polarity switching method was used to acquire data for both positive and negative ion compounds in a single injection. A delay column was introduced after the mixing chamber to reduce the background interference from the mobile phases, thereby improving the method detection limits.

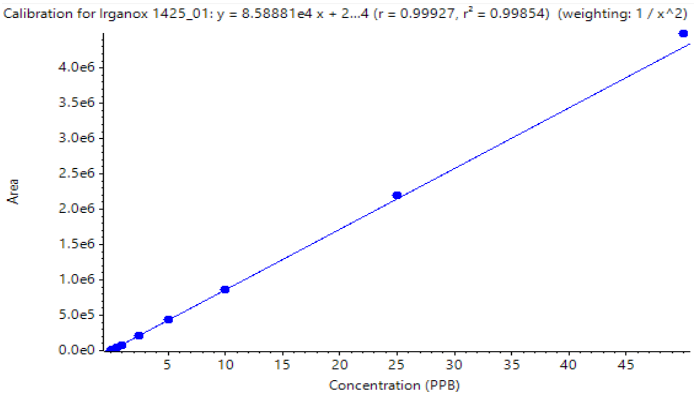


Figure 2. Illustrative calibration curve for Irganox 1425. The calibration curve was prepared using 50:50 (v/v), water/ethanol with 3% acetic acid (calibration curve 1) as diluent over the range of 0.025-50 ng/mL. An r^2 value >0.99 was achieved.

Calibration curve sensitivity and linear dynamic range

Calibration curves were generated for all compounds analyzed across a concentration range of 0.025 to 50 ng/mL. Standards were prepared in the calibration 1 diluent (50:50 (v/v), water/ethanol with 3% acetic acid by volume), unless specified. Table 5 shows the accurate quantification achieved across this range with an r^2 value >0.99. The lower limit of quantification (LLOQ) is represented by the lowest standard analyzed in the calibration range and this value varied from 0.025 to 0.5 ng/mL across Irganox compounds. Figure 2 shows an example calibration curve using the quantifier transition for Irganox 1425, which demonstrates the linear dynamic range of 0.025 to 50 ng/mL and r^2>0.99.

Table 5. Linearity range and correlation coefficient (r^2) achieved for the 16 Irganox compounds.

Compound	Linearity range (ng/mL)	Correlation coefficient (r^2)
Irganox PS 800	*0.250–50*	*0.991*
*Irganox 1010**	*0.500–50*	*0.993*
Irganox 1330	*0.050–10*	*0.990*
Irganox MD1024	*0.100–50*	*0.995*
*Irganox 565**	*0.025–50*	*0.992*
Irganox 5057	*0.050–25*	*0.993*
Irganox 3125	*0.100–25*	*0.990*
Irganox 1098	*0.100–50*	*0.999*
Irganox 259	*0.025–50*	*0.996*
Irganox 1081	*0.500–50*	*0.998*
Irganox E201	*0.250–50*	*0.996*
Antioxidant 425	*0.050–50*	*0.997*
*Irganox 3114**	*0.500–50*	*0.992*
Irganox 1425	*0.025–50*	*0.999*
Irganox 1035	*0.025–50*	*0.991*
Irganox 1520	*0.050–25*	*0.991*

Note: *All Irganox compounds were quantified using calibration curve 1 (50:50 (v/v), water/ethanol with 3% (v/v) acetic acid) except for those denoted with an asterisk, which were quantified using calibration curve 2 (50:50 (v/v), water/ethanol).*

Accurate and precise quantification of Irganox compounds in food simulant spikes using a single solvent calibration curve

Excellent accuracy (70-130%) and precision (%CV <15%) were achieved for 13 of the 16 Irganox compounds in all 4 food simulant spikes when quantified with the calibration curve 1 solutions (Table 6). The remaining 3 compounds (Irganox 1010, 565 and 3114) showed good accuracy and precision with either calibration curve 1 or calibration curve 2 solutions, depending on the food simulant matrix. Excellent precision based on 6 replicates was achieved at the LLOQ of all compounds spiked in each food simulant. These results demonstrate that good data quality can be achieved for most Irganox compounds using a single solvent curve across the diversity of food simulant matrices specified in the EU regulations. Removing the need to run individual matrix-matched curves can save significant sample preparation and analysis time.

Food packaging material spikes

Overall, the 10 ng/mL post-extraction spikes of the representative plastic food material (for example, yogurt container) showed good accuracy among the 4 food simulants, indicating minimal quantitative bias due to the matrix (Figure 3). The individual data points in Figure 3 represent the average of 6 replicates per Irganox compound. The 10% and 20% ethanol food simulants showed 70-130% accuracy for all compounds except for Irganox 1010 in the 10% ethanol simulant. The 50% ethanol and 3% acetic acid extracts showed 3 and 4 Irganox compounds outside the 70-130% acceptable range, respectively. No Irganox compounds were detected in the yogurt container, prior to spiking.

It is recommended to analyze the Irganox compounds with accuracies that failed to meet the 70-130% accuracy criteria using matrix-matched calibration or with mass-labeled internal standards. Overall, these findings demonstrate that the majority of the Irganox compounds in the 4 food simulants can be accurately quantified without the need for internal standards.

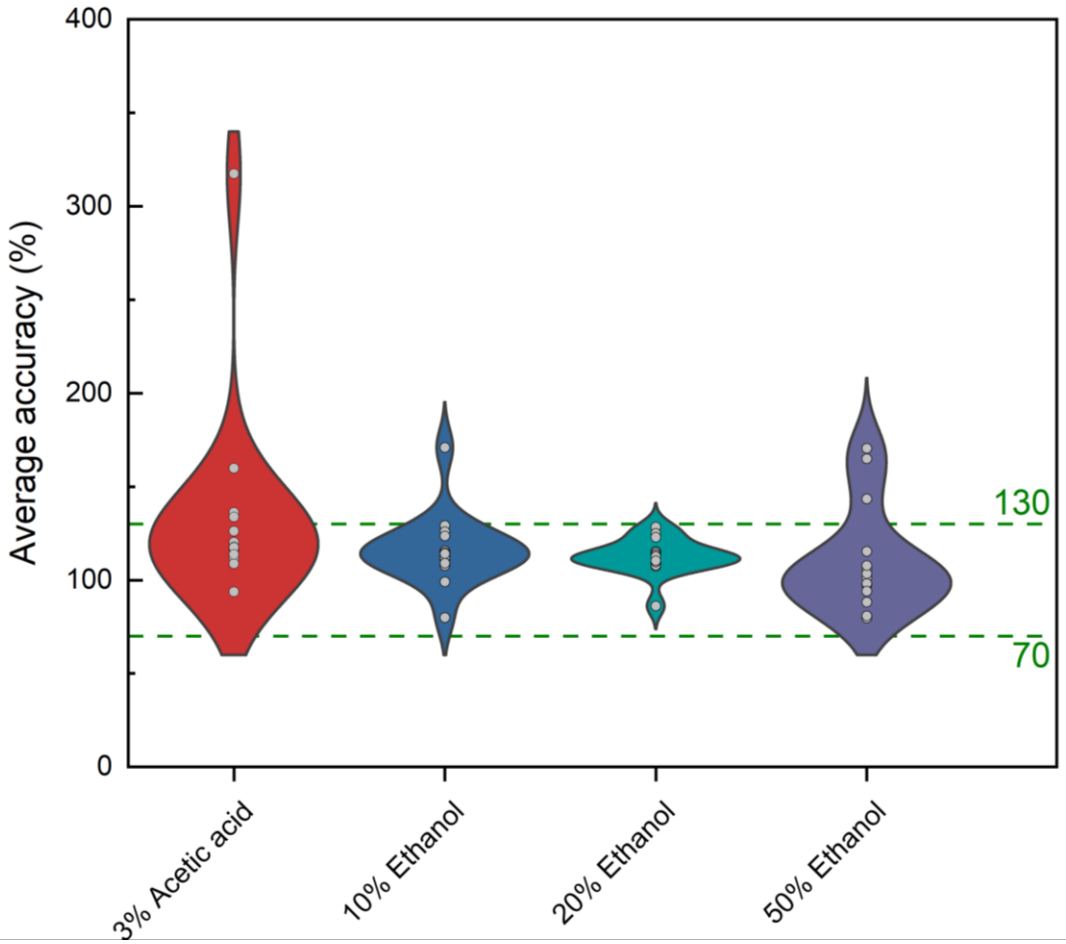


Figure 3. Average accuracy (n=6) of post-extraction spikes from the plastic yogurt container. Final extracts were spiked to yield 10 ng/mL in-sample and then diluted 2:1 with the compatible diluent solvent. Dots represent averages for individual Irganox compounds and the curve width represents approximate frequency of data points.

Table 6. Average accuracy and %CV (n=6) for all 16 Irganox compounds at the lowest measured concentration. Concentrations are reported on an in-sample basis.

Simulant	3% Acetic acid		10 % Ethanol		20 % Ethanol		50% Ethanol	
Analyte	In-sample concentration (ng/mL)	Avg. accuracy (%CV)	In-sample concentration (ng/mL)	Avg. accuracy (%CV)	In-sample concentration (ng/mL)	Avg. accuracy (%CV)	In-sample concentration (ng/mL)	Avg. accuracy (%CV)
Irganox PS 800	1.00	119 (11)	1.00	120 (13)	1.00	124 (13)	1.00	94 (5.6)
Irganox 1330	1.00	114 (3.0)	1.00	121 (2.8)	1.00	120 (3.1)	0.025	112 (3.3)
Irganox MD1024	0.10	113 (1.9)	0.10	108 (6.8)	0.10	109 (2.7)	0.025	100 (5.9)
Irganox 5057	0.10	115 (3.7)	0.10	117 (8.4)	0.10	122 (4.0)	0.025	122 (9.1)
Irganox 1098	0.10	118 (12)	0.10	115 (7.9)	0.10	107 (1.5)	0.025	100 (1.8)
Irganox 259	0.10	114 (2.4)	0.10	111 (2.2)	0.10	110 (2.4)	0.025	108 (0.8)
Irganox 1081	1.00	105 (13)	1.00	101 (11)	1.00	112 (11)	1.00	105 (5.0)
Irganox E201	1.00	103 (4.8)	2.50	113 (3.1)	2.50	113 (9.7)	1.00	125 (3.4)
Antioxidant 425	0.10	95 (13)	0.10	88 (6.4)	0.10	86 (12)	0.025	70 (3.3)
Irganox 1425	1.00	108 (4.1)	1.00	112 (2.9)	1.00	108 (3.4)	0.10	79 (11)
Irganox 1035	0.10	111 (9.6)	0.10	109 (11)	1.00*	95 (5.3)	0.025	106 (13)
Irganox 3125	1.00	106 (6.2)	1.00	104 (5.6)	1.00	103 (5.6)	0.10	91 (6.9)
Irganox 1520	1.00	117 (3.0)	1.00	103 (2.5)	1.00	105 (2.3)	0.025	117 (6.0)
Irganox 1010	2.50*	106* (8.4)	2.50	106 (12)	2.50	96 (2.4)	1.00*	118* (7.3)
Irganox 565	1.00	96 (7.9)	1.00*	95* (2.6)	1.00*	85* (7.4)	0.025*	90* (5.9)
Irganox 3114	1.00*	114* (5.4)	1.00*	121* (2.6)	1.00*	117* (3.3)	1.00*	89* (2.9)

Note: *All Irganox compounds were quantified using calibration curve 1 (50:50 (v/v), water/ethanol with 3% (v/v) acetic acid) except for those denoted with an asterisk, which were quantified using calibration curve 2 (50:50 (v/v), water/ethanol).*

Conclusions

- A fast polarity switching method was developed to analyze 16 Irganox compounds in a single method
- The SCIEX QTRAP 6500+ system provided excellent sensitivity, reproducibility and linearity when quantifying Irganox compounds in different food simulants and post-extraction spikes in a plastic food container
- Two solvent-based external calibration curves were used to evaluate Irganox compounds in different simulants. Matrix-matched calibration curves were not required.
- R^2 values >0.99 were achieved for all compounds with %CV <15% across 6 replicates at the lowest measured concentration in all simulants, meeting the EU regulation requirements

References

1. Commission regulation (EU) No 10/2011 of 14th January 2011 on plastic materials and articles intended to come into contact with food (EUR-Lex - 32011R0010 - EN - EUR-Lex (europa.eu).

QTRAP® 技術在包裝材料可提取物篩查中的應用

Application of the QTRAP® system in the screening of extractable substance of packaging materials

陳西，趙祥龍，李立軍，郭立海
Chen xi, Zhao xianglong, Li lijun, Guo lihai

SCIEX China

Key words: SCIEX OS; Packaging materials; Screening

引言

包裝材料是商品的重要組成部分，可以保護商品不受外來生物、物理和化學因素的影響，尤其在食品和藥品行業應用十分廣泛。根據材質的不同，可以將包裝材料分為塑膠、橡膠、金屬和玻璃等類別。包裝材料根據其用途，應兼具安全性、適應性、穩定性、功能性和便利性等特點。為了達到上述目的，在包裝材料的生產過程中，往往需要使用不同種類的添加劑，如增塑劑、抗氧化劑、穩定劑、硫化促進劑等[1,2]。此外，在包材的生產和儲藏過程中，由於溫度或者光照的影響，這些添加劑有可能發生降解，產生新的物質[3]。作為與食品或藥品直接接觸的包裝材料，這些化合物有可能遷移到藥物或食物中，威脅人類健康。如常用的酚類抗氧劑BHT，對有肺、肝等組織有直接損害作用，並有致癌風險[4]；高分子材料合成中常用的雙酚A為雌激素類似物，對肝臟和生殖系統都有損害[5]。因此需要對包裝材料中的各類化合物進行檢測，控制用量和種類。如化學藥品注射劑與塑膠包裝材料相容性研究技術指導原則（徵求意見稿）中規定，注射劑包裝中添加抗氧劑不能超過三種，總量不得超過0.3%。

市面上包裝材料的材質、廠家各異，其中的添加劑也種類繁多，因此在檢測中無法固定對某一化合物進行限量檢測，而需要先確定化合物種類，也就是定性。一般的定性思路為2個MRM離子對，並與標準品對比離子比率和保留時間。但由於添加劑種類繁多，獲得全面的標準品比較困難，導致化合物篩查範圍較窄。本文結合實際介紹了一種利用QTRAP®技術進行包材篩查的通用工作流程（圖2），無需標準品，通過與二級譜庫匹配，直接得到化合物的定性資訊。

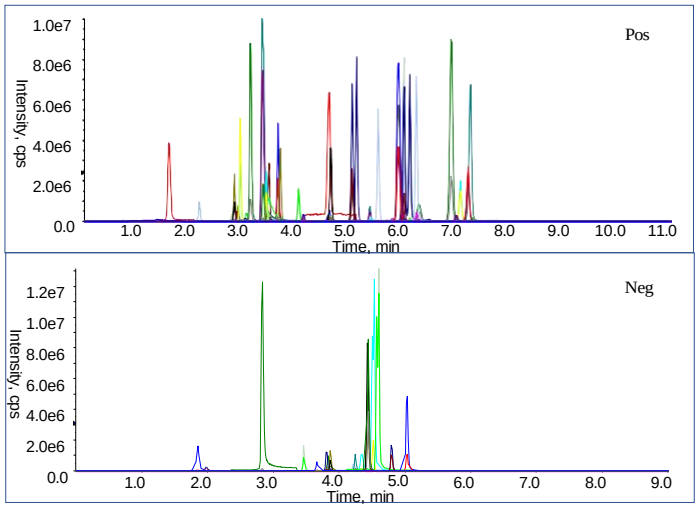


圖1. 包材混標在正離子和負離子模式下典型色譜圖。

實驗流程

本實驗在QTRAP®質聯用系統上進行，利用QTRAP®特有的MRM-IDA-EPI功能，只需在方法中輸入待測化合物離子對，軟體在採集過程中根據預設的二級質譜採集條件，對滿足條件的化合物進行二級掃描，同時得到化合物的MRM色譜圖和二級質譜圖（原理示意圖見圖2）。採集到的資料導入SCIEX OS軟體，即可自動與包材專屬二級譜庫進行匹配，得出化合物名稱和匹配度得分，操作流程見圖3。考慮到不同電離模式的化合物對流動相的要求不同，為兼顧靈敏度，因此將化合物分成正、負兩組，分別用兩種不同流動相進行洗脫。正負兩組化合物的色譜洗脫時間分別為11 min和9 min，即總共只需20 min就可以完成樣品採集。資料獲取典型圖譜請見圖1.

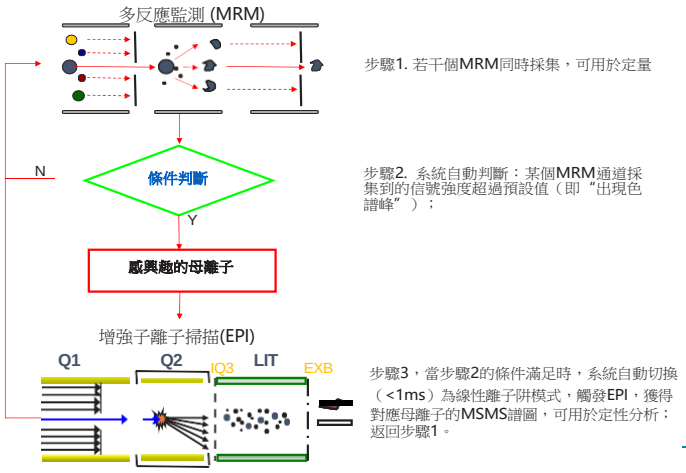
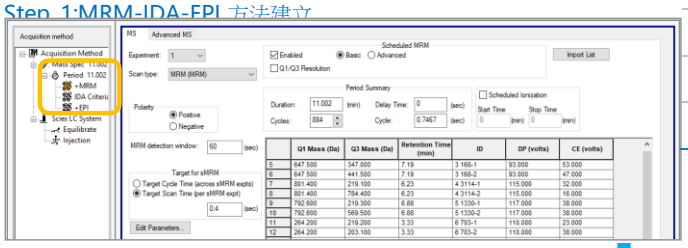
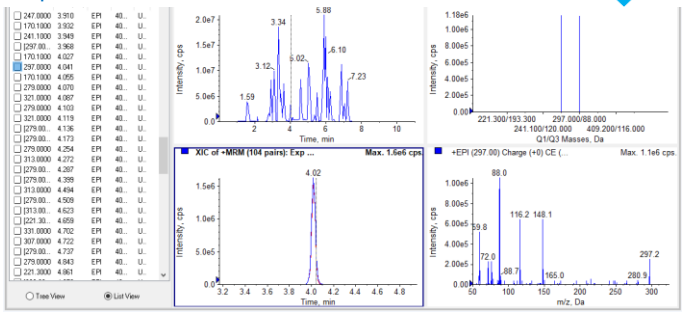


圖2 MRM-IDA-EPI原理示意圖。

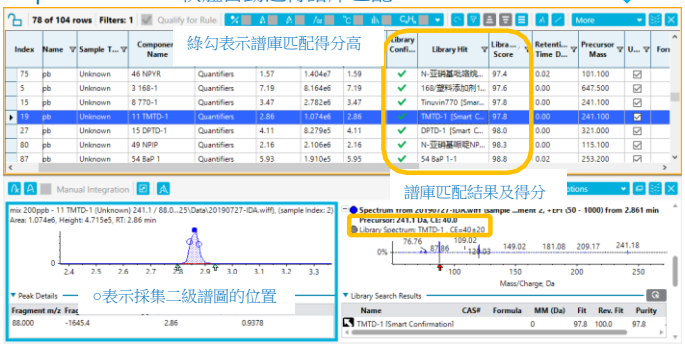
Step 1:MRM-IDA-EPI 方法建立



Step 2:同時採集色譜和二級質譜圖



Step 3:SCIEX OS 軟體自動進行譜庫匹配



MRM 色譜圖　　二級質譜圖及譜庫匹配結果

圖3. QTRAP®系統化合物篩查流程。

1. 液相條件：

色譜柱：Phenomenex Kinetex F5, 2.6 µm, 2.1 mm×100 mm;

流動相（正模式）：A相： 0.1%甲酸水　B相：甲醇

流動相（負模式）：A相： 0.01%氨水　B相：甲醇

流速：0.5 mL/min；

色譜柱溫度：40 ℃；

進樣量：2 µL；

洗脫程式（正模式）：

Time(min)	A (%)	B (%)
0.0	80	20
1.5	35	65
5.0	5	95
8.5	5	95
8.6	80	20
11	80	20

洗脫程式（負模式）：

Time(min)	A (%)	B (%)
0.0	75	25
3.5	5	95
6.5	5	95
6.6	75	25
9	75	25

2. 質譜方法：

掃描模式：MRM-IDA-EPI

氣簾氣 CUR: 30 psi　　碰撞氣 CAD: High

霧化氣 GS1: 50 psi　　輔助氣 GS2: 55 psi

IS電壓: 5500 V　　源溫度 TEM: 500 ℃

EPI碰撞電壓：40±20

EPI掃描範圍：50-1000 Da（正模式），50-800 Da（負模式）

3. 樣品測定

利用上述方法及包材譜庫，對某氯化丁基橡膠塞的甲苯浸提物進行篩查，結果檢出包括抗氧化劑和鄰苯二甲酸酯類增塑劑在內的

20種化合物（表1）。樣品正負離子模式色譜圖見圖4.

表**1.** 氯化丁基橡膠塞可提取物篩查結果。

化合物名稱	（min）	峰面積	得分
抗氧化劑1076	7.27	6.47E+07	100
鄰苯二甲酸二丁酯	4.59	1.46E+06	100
N-亞硝基二甲胺	1.34	1.09E+06	100
抗氧劑1010	6.97	1.77E+05	99.5
3114	6.23	1.55E+06	98.3
芥酸醯胺	5.95	3.20E+07	97.7
油酸醯胺	5.35	1.21E+07	97.1
二苯胺	3.63	8.74E+04	79.4
鄰苯二甲酸酯二甲酯	2.89	7.91E+05	95.4
Ethanox703-1	3.33	4.13E+05	94.8
Irganox 1098-1	5.67	7.37E+06	93.1
2246	4.52	1.30E+06	100
BHT-CHO	3.82	1.95E+06	100
BHT-1	4.35	2.79E+06	98.2
對特辛基苯酚	4.25	5.50E+07	97.3
BHT-OH	3.87	5.05E+06	95.5
1310	1.97	1.37E+06	94.9
棕櫚酸	2.83	2.13E+07	91.2
對叔丁基苯酚	3.67	5.59E+05	90.9
BHT-COOH	1.81	3.44E+05	86.8

總結

本文利用QTRAP®液質聯用系統建立了一個簡單、快速的包裝材料可提取物的篩查方法並用於實際樣品的測定。該方法充分考慮了包材可提取物的種類多樣性，分為正、負離子兩個部分，兼顧了化合物靈敏度和方法的簡便性，並且擁有良好的可擴展性，可以隨時新增待測物而幾乎無需對方法做改動。實驗證明，QTRAP®液質

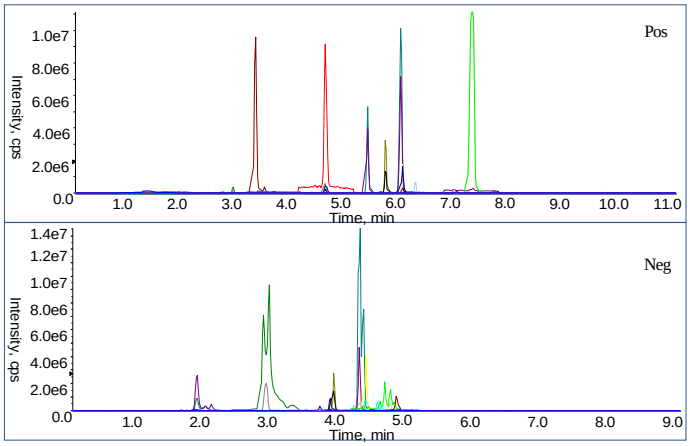


圖**4.** 氯化丁基橡膠塞甲苯提取物篩查色譜圖。

聯用系統與SCIEX OS軟體結合，擁有強大定性採集功能和資料處理功能，非常適合於各行業包裝材料的可提取物測定。

參考文獻

[1] 馬玉楠，馬磊，蔣煜．藥品與包裝材料相容性研究相關指導原則解讀－試驗結果的評估 [J]．中國新藥雜誌，2014，23（8）：940-943．

[2] Corredor C, Tomasella FP, Young J. Drug interactions with potential rubber closure extractables. Identifi cation of thiol-disulfi de exchange reaction products of captopril and thiurams [J]. J Chromatogr A, 2009, 1216(1): 43-48

[3] 蔣煜，馬玉楠，霍秀敏，馬磊. 化學藥品注射劑與塑膠包裝材料相容性研究技術指導原則解讀-提取遷移試驗方法設計 [J].中國新藥雜誌，2014

[4] 李濤，趙聲蘭，餘旭亞，等. 抗氧化劑的研究與應用現狀 [J]. 食品研究與開發，2003,24（2）：23-25.

[5] 紀紅蕊，陳家駒，張茜，等。雙酚A的毒性作用機制 [J]. 瀋陽工業大學學報，2015，37（6）：710-715.

X500R QTOF在包裝材料提取物篩查中的應用

Application in Screening of Packaging Material Extracts Based on X500R QTOF High Resolution Mass Spectrometry

趙祥龍，李立軍，郭立海
Zhao Xianglong, Li Lijun, Guo Lihai
SCIEX China

Key words: X500R QTOF, packaging material, extracts, screening

引言

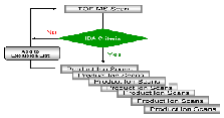
食品/藥品的品質和安全性很大程度上需要依賴包裝材料進行保存和保護，與此同時包裝材料也被稱作特殊“添加劑”，這是因為包裝材料與食品/藥品在長期直接接觸的過程中，包裝材料中遷移出的化合物會污染其中內容物，從而影響其安全性和品質，這些有害物質可能通過飲食或給藥進入人體，為消費者的健康和生命安全帶來潛在的威脅[1]。近年來，已有相關文獻報導在尿液和唾液等體液中檢測到常見包裝材料添加劑，如抗氧化劑、光穩定劑、增塑劑、光敏劑、雙酚類、阻燃劑等[2]。

目前，已報導的有關包裝材料中提取物的檢測方法囊括了氣相色譜法（GC）、高效液相色譜法（HPLC）、氣相色譜-質譜法（GC-MS）及液相色譜-串聯質譜法（LC-MS/MS）等多種手段[3-4]；但是現有方法只針對特定的某類或某幾類添加劑，而在實際的工作中，食品和藥品包裝材料中的常見添加劑/可溶出物種類繁多、數量可達數百種，除外之外還可能引入其他非有意添加物等未知成分。因此，建立一種快速高效針對食藥包裝材料的溶出物/提取物篩查手段，對保障食品和藥品安全具有有十分重要的現實意義。SCIEX基於新型高分辨質譜X500R QTOF系統建立了食藥包裝材料中常見添加物/溶出物的篩查方案，依靠專屬的E&L高分辨譜庫和SCIEX OS軟體簡便、高效篩查流程，能夠實現對食品和藥品包裝材料中溶出物進行快速篩查和準確鑒定。

SCIEX包裝材料溶出物/提取物篩查方案技術優勢：

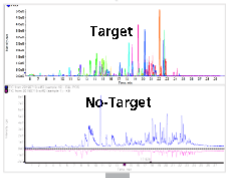
- 基於X500R系統優異的抗污染能力，常規包材提取物可直接上樣分析，能夠有效避免複雜前處理過程導致低濃度溶出物漏篩。
- X500R高分辨質譜以業界最快掃描速度，結合專利動態背景扣除技術，實現一針進樣，同時採集到包材提取物鑒定所需的高分辨TOF MS和TOF MS/MS資料，尤其對於低豐度易降提取物鑒定，無需二次進樣分析。
- SCIEX OS軟體結合專屬的高分辨E&L譜庫，能夠自動進行目標化合物的快速鑒定及非目標化合物的定性篩查和結構解析，簡便的工作流程讓包材提取物篩查更高效。
- 基於X500R QTOF建立的200多種常見添加物的篩查方案，能夠針對食品和藥品包裝材料中的溶出物進行精准鑒定，實際樣品檢測表明該方法簡單、快速、準確，為食品和藥品包材的篩查和研究提供有效參考。

基本工作流程：



1. 資料獲取：採用TOF MS-IDA-TOF MS/MS採集模式，一針進樣採集鑒定所需 MS及MS/MS資料

2. 資料分析：基於SCIEX OS智慧模組化軟體，自動進行資料分析和定性篩查



3. 目標性和非目標性篩查：通過目標性資料處理獲得所關注化合物資訊，非目標性資料處理獲得樣品中完整的物質資訊

4. 篩查結果鑒定：通過一級品質偏差、保留時間、同位素分佈、二級譜圖匹配等四大維度對未知物進行定性確證，對於新型提取物結合二級資訊進行成分鑒定和結構解析

實驗方法

1. 液相方法：

流動相A：正模式：0.1 % FA 水　負模式A：0.5 mM NH_4AC

流動相B：甲醇

色譜柱：Phenomenex Kinetex F5 (100×3.0 mm, 2.6 µm)

流速：0.4 mL/min；柱溫：40 ℃；

進樣量：5 µL；

洗脫程式：梯度洗脫

2. 質譜方法：

掃描方式：IDA採集方式

採集模式：ESI+源/ESI-源

CDS自動校正

IS電壓: 5500 V / -4500 V

源溫度 TEM: 550 ℃

氣簾氣 CUR: 35 psi　　碰撞氣 CAD: 7

霧化氣 GS1: 55 psi　　輔助氣 GS2: 60 psi

去簇電壓DP：±80 V　　碰撞能量CE：35±15 V

TOF MS Range：70-1200 Da

MS/MS MS Range：50-1200 Da

3. 樣品前處理

依照食品和藥品包材相關類比條件溶劑和溫度對塑膠、塑膠塞、紙質包裝材料進行溶出提取，提取液直接過膜上機檢測。

結果與討論

目標化合物定性篩查

該方法能夠對包裝材料中200多種添加劑（部分化合物資訊見表1）、可提取物/溶出物進行快速目標性篩查，包括增塑劑、抗氧化劑、硫化劑、螢光劑、光敏劑、紫外穩定劑、全氟類、雙酚類、芳香胺、亞硝胺、分散染料等十幾大類，部分抗氧化劑保留較強，需要對色譜條件進行優化調整，200多種化合物的提取離子流圖如圖1；SCIEX OS軟體自動根據目標化合物的高分辨一級品質誤差、保留時間、同位素分佈和二級碎片譜圖進行篩查確證，保證篩查鑒定結果的準確性（如圖2）。

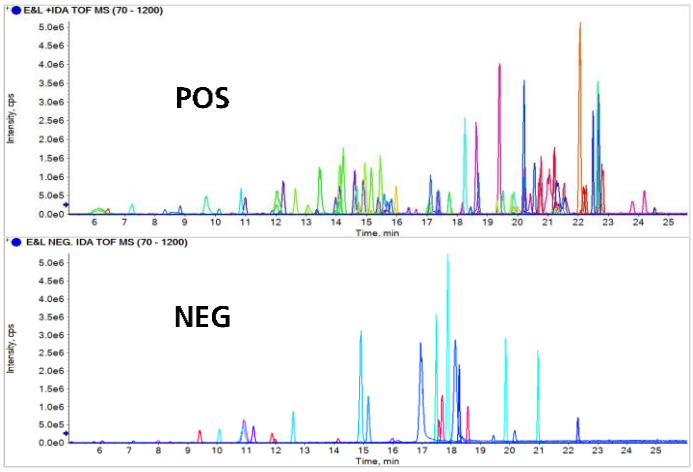


圖1. 包裝材料中200多種可提取物/溶出物提取離子流圖（XIC圖）。

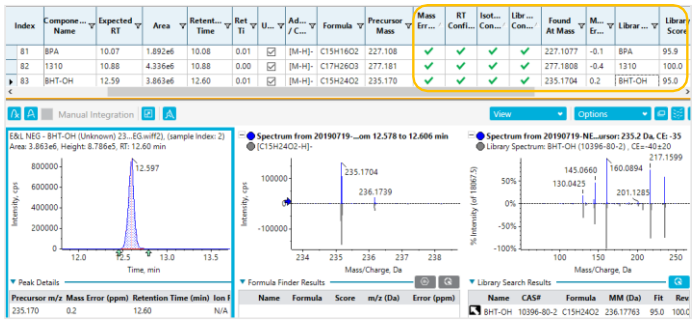


圖2. SCIEX OS軟體目標性篩查圖示。

從圖2中篩查資料示例可以表明，大部分化合物在該方法中能夠保證優異的品質精度和穩定性，品質偏差均在1 ppm以內，同位素分佈、二級譜庫匹配度高，篩查結果準確可靠。

非目標化合物篩查

針對非目標性未知物篩查，通過將樣品和空白資料導入SCIEX OS軟體自動完成化合物峰提取和二級譜圖匹配，Formula Finder功能根據未知物的TOF MS資料和同位素分佈自動計算可能的分子組成，確證後的分子式可自動連結ChemSpider檢索可能的結構，同時關聯MS/MS進行結構解析；

表1. 包裝材料中提取物/溶出物部分化合物資訊。

化合物名稱	分子式	化合物名稱	分子式
2-Cl-BTH	C_7H_4NSCl	Disperse Yellow G	$C_{15}H_{15}N_3O_2$
2-Me-BTH	C_8H_7NS	DMEP	$C_{14}H_{18}O_6$
5-Methyl-benzotriazole	$C_9H_9N_3$	DMP	$C_{10}H_{10}O_4$
BBP	$C_{19}H_{20}O_4$	DPP	$C_{18}H_{26}O_4$
Benzyl butyl phthalate	$C_{19}H_{20}O_4$	Ethanox702	$C_{29}H_{44}O_2$
BHT-CHO	$C_{15}H_{22}O_2$	Irganox 1310	$C_{17}H_{26}O_3$
BHT-OH	$C_{15}H_{24}O_2$	Irganox 2246	$C_{23}H_{32}O_2$
BPA	$C_{15}H_{16}O_2$	Irganox 245	$C_{34}H_{50}O_8$
BTH	C_7H_5NS	Irganox 246	$C_{18}H_{30}O$
Cyanox425	$C_{25}H_{36}O_2$	Irganox 259	$C_{40}H_{62}O_6$
DBP	$C_{16}H_{22}O_4$	Isobutylcyclohexyl phthalate	$C_{18}H_{24}O_4$
DHXP	$C_{20}H_{30}O_4$	NITROSODIETHYLAMINE	$C_4H_{10}N_2O$
Diallyl phthalate	$C_{14}H_{14}O_4$	PFBA	$C_4HO_2F_7$
DIBP	$C_{16}H_{22}O_4$	PFDA	$C_{10}HO_2F_{19}$
Dibutyl adipate	$C_{14}H_{26}O_4$	PFDoA	$C_{12}HF_{23}O_2$
Dibutyl phthalte	$C_{16}H_{22}O_4$	PFHpA	$C_7HF_{13}O_2$
Diethyl phthaltate	$C_{12}H_{14}O_4$	PFHxA	$C_6HO_2F_{11}$
Diisobutyl adipate	$C_{14}H_{26}O_4$	PFNA	$C_9HO_2F_{17}$
Diisobutyl phthalate	$C_{16}H_{22}O_4$	PFOA	$C_8HF_{15}O_2$
Dimethyl phthalate	$C_{10}H_{10}O_4$	PFPeA	$C_5HF_9O_2$
Disperse Blue 106	$C_{14}H_{17}N_5O_3S$	PFUdA	$C_{11}HF_{21}O_2$
Disperse Blue 124	$C_{16}H_{19}N_5O_4S$	NMOR	$C_4H_8N_2O_2$
Disperse Blue 3	$C_{17}H_{16}N_2O_3$	NMEA	$C_3H_8N_20$
Disperse Blue 35	$C_{20}H_{14}N_2O_5$	NPYR	$C_4H_8N_2O$
Disperse Orange 3	$C_{12}H_{10}N_4O_2$	NDEA	$C_4H_{10}N_2O$
Disperse Orange 37	$C_{17}H_{15}Cl_2N_5O_2$	NPIP	$C_5H_{10}N_{210}$
Disperse red 1	$C_{16}H_{18}N_4O_3$	NDPA	$C_{16}H_{14}N_2O$

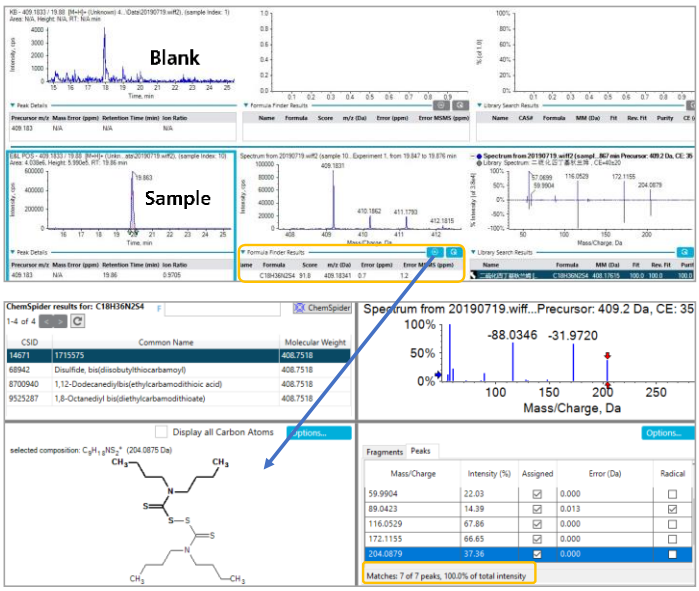


圖3. SCIEX OS軟體非目標性篩查過程。

從圖3示例中顯示Formula Finder根據未知物M/Z409.1833的高分辨一級和同位素資訊推測出其分子式：$C_{18}H_{36}N_2S_4$，接著連結到ChemSpider進行結構檢索，結合該化合物二級資訊進行結構匹配和解析，解析結果顯示其中排首位的化合物的7個主要碎片都能準確匹配且偏差較小。

包裝材料提取物實際樣本檢測

在模擬條件下的對塑膠和紙盒包裝材料進行溶出提取，提取溶劑為95%乙醇和50%乙醇，提取溶液經過膜直接上樣分析，基於優化後高分辨篩查方法，可從塑膠、紙質包材中快速篩查鑒定出13種、19種提取物（見表2），主要為常見的塑化劑、抗氧化劑等常見添加劑，實際樣品正負模式鑒定化合物提取離子流圖見圖4。

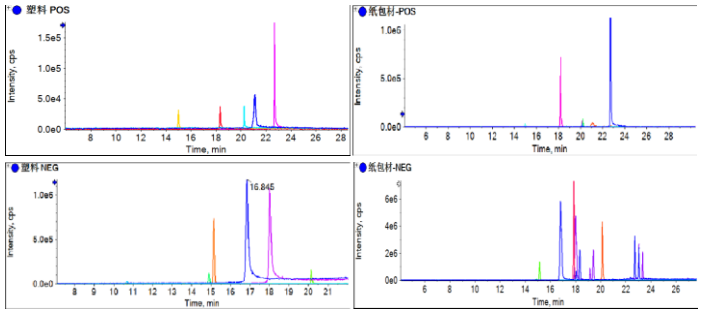


圖4. 塑膠和紙盒包材提取物篩查化合物提取離子流圖。

表2. 塑膠和紙盒包材提取物篩查化合物資訊。

化合物名稱 (紙包)	分子式	化合物名稱 (塑膠)	分子式
鄰苯二甲酸二異壬酯	$C_{26}H_{42}O_4$	芥酸醯胺	$C_{22}H_{43}NO$
抗氧化劑168	$C_{42}H_{63}O_3P$	抗氧劑168	$C_{42}H_{63}O_3P$
油酸醯胺	$C_{18}H_{35}NO$	抗氧劑264	$C_{15}H_{24}O$
鄰苯二甲酸二正辛酯	$C_{24}H_{38}O_4$	油酸醯胺	$C_{18}H_{35}NO$
軟脂酸	$C_{16}H_{32}O_2$	鄰苯二甲酸二壬酯	$C_{26}H_{42}O_4$
硬脂酸	$C_{18}H_{36}O_2$	鄰苯二甲酸二辛酯	$C_{24}H_{38}O_4$
2,4-二叔丁基苯酚	$C_{14}H_{22}O$	抗氧劑 330	$C_{54}H_{78}O_3$
對特辛基苯酚	$C_{14}H_{22}O$	軟脂酸	$C_{16}H_{32}O_2$
芥酸醯胺	$C_{22}H_{43}NO$	硬脂酸	$C_{18}H_{36}O_2$
抗氧化劑1310	$C_{17}H_{26}O_3$	鄰苯二甲酸二(2-乙基己)酯	$C_{24}H_{38}O_4$
3,5-二叔丁基-4-羥基苯甲醛	$C_{15}H_{22}O_2$	2,2'-亞甲基雙-(4-甲基-6-叔丁基苯酚)	$C_{23}H_{32}O_2$
磷酸三乙酯	$C_6H_{15}O_4P$	抗氧化劑2246	$C_{23}H_{32}O_2$
		2,4-二叔丁基苯酚	$C_{14}H_{22}O$
		對特辛基苯酚	$C_{14}H_{22}O$
		3,5-二叔丁基-4-羥基苯甲醛	$C_{15}H_{22}O_2$
		BHT-OH	$C_{15}H_{24}O_2$
		抗氧劑DSTP	$C_{42}H_{82}O_4S$
		三苯基氧磷	$C_{18}H_{15}OP$

總結：

本實驗基於SCIEX新一代高分辨質譜X500R QTOF系統針對食品和藥品包裝材遷移物/提取物開發的高分辨篩查方法，能夠覆蓋常見200多種包材添加劑和提取物，X500R卓越的抗污染能力和系統穩定性，可有效減少前處理步驟同時保證資料的可靠性；SCIEX高分辨質譜超快的掃描速度（100 HZ），可實現一針進樣同時採集到高分辨一級和高分辨二級完整資料，避免了部分提取物易降解難以檢測問題，OS軟體的簡單易用性，讓高分辨資料處理更加簡單、快捷，目標和非目標性的資料處理流程可實現對未知化合物的快速鑒定和解析，為包裝材料提取物篩查和研究提供高效的工作流程和完整方案。

參考文獻

[1] 張雲. 基於色譜質譜技術的食藥塑膠包裝材料中添加劑的分析及其遷移行為的研究[D]. 北京化工大學, 2017.

[2] 邢雪彬, 張雲, 孫姝琪等. 基於聚合物添加劑譜庫的緩衝鹽中塑膠藥品包裝材料難揮發可溶出物的快速分析方法研究[J]. 分析測試學報, 2017, 36(1): 25-30.

[3] N. T., Crosby. Food packaging materials[M]. Applied, 1981.

[4] Pattarin,Leelaphiwat, Janice. Effects of packaging materials on the aroma stability of seasoning powder as determined by descriptive sensory analysis and gas chromatography-mass spectrometry[J]. Journal of the science of food and agriculture, 2017, 97(6): 1854-1860.

RUO-MKT-02-10106-ZH-A

食品接觸材料中的12種酚類化合物的LC-MS/MS快速定量方法

A Rapid Quantitative Method of LC-MS /MS for 12 kinds of phenolic compounds in Food packaging material

李星霖[1]，金莉莉[2]，楊總[1]，劉冰潔[1]，郭立海[1]

Li Xinglin[1], Jin Lili[2], Yang Zong[1], Liu Bingjie[1], Guo Lihai[1]

SCIEX China1

國家食品接觸材料檢測重點實驗室（常州）[2]

National Reference Laboratory for Food Contact Material (Changzhou)[2]

Keywords: SCIEX Triple Quad; Food packaging material; phenolic compound

引言

雙酚A、壬基酚和辛基酚是典型的酚類環境雌激素。雙酚A是生產聚碳酸酯塑膠和環氧樹脂的重要原料，涉及的產品包括食品包裝材料、粘合劑等，也可用於生產增塑劑、阻燃劑、抗氧化劑等精細化工產品。2008年，加拿大衛生部宣佈雙酚A為危害物質，禁止進口和銷售含有雙酚A的聚碳酸酯嬰兒奶瓶。壬基酚和辛基酚被廣泛用作紡織整理劑、塑膠增塑劑等，涉及的產品包括食品包裝材料、粘合劑等。壬基酚和辛基酚作為內分泌干擾物質，通過食物鏈進入人體，會在生物體內積累，對人體癌細胞生長及生殖能力均會造成嚴重影響，被歐盟列為優先危害物質。因此酚類化合物的測定對於保證人體健康有重要意義，也是目前食品領域的研究熱點問題。本方案基於中國食品安全國家標準GB 31604.10-2016《食品接觸材料及製品2,2-二(4-羥基苯基)丙烷(雙酚A)遷移量的測定》和GB 31604.50- 2020《食品接觸材料及製品壬基酚遷移量的測定》等標準，採用 SCIEX ExionLC™ 2.0+和Triple Quad™ System建立了食品包裝材料中 12種酚類化合物的快速定量方法，該方法具有以下幾個特點：

1. 本方法覆蓋度廣，總共包含12種酚類化合物，遠超于相關包材標準要求的種類。
2. 本方法靈敏度高，檢測靈敏度可以達到pg級別以下，足以滿足國標的要求。
3. 本方法效率高、重複性好、回收率高，正負切換同時檢測12種酚類化合物只需要7分鐘，以空白基質為溶劑，分別添加1 ng/mL、5 ng/mL和10 ng/mL三個濃度樣品，每個濃度重複6份，加標回收率均在82.1%~109.1%之間，相對標準差在5%（n=6）以內。

1. 實驗部分

1.1 樣品前處理：

本方法遷移實驗採用水基食品模擬物進行遷移，遷移試驗的條件選擇和操作步驟按照GB 31604.1和GB 5009.156的規定，選中空心製品（礦泉水瓶）採用全浸沒法，加入80%容量的水，在40℃下遷移10天，移取遷移試驗後得到的水基食品模擬物，通過0.2 μm濾膜過濾後，以供液相色譜-串聯質譜儀測定。

1.2 色譜方法：

色譜柱：Phenomenex Kinetex F5, 2.6 μm, 100 mm×3.0 mm

流動相：A：水（5 mM乙酸銨）；B：甲醇

柱溫：40 ℃

洗脫程式：梯度洗脫（表1）

表**1.** 液相洗脫梯度

Time (min)	Flow(mL/min)	B (%)
0	0.3	50
1	0.3	70
2	0.3	90
5	0.3	90
5.1	0.3	50
7	0.3	50

1.3 質譜方法：

掃描方式：電噴霧電離（electrospray ionization, ESI），正/負離子模式

離子源參數：

氣簾氣（CUR）: 30 psi； 碰撞氣（CAD）: 9 ；

噴霧電壓（IS）: 5500V/-4500 V； 離子源溫度（TEM）: 550 ℃；

霧化氣（GAS 1）: 50 psi； 輔助加熱氣（GAS 2）: 55 psi；

MRM離子對見附表

2. 實驗結果：

2.1. 色譜條件優化

實驗詳細優化了色譜條件，比較了不同品牌、不同型號的色譜柱以及流動相，最終選擇的色譜柱是Phenomenex Kinetex F5，2.6 µm，100 mm×3.0 mm，流動相為A為5 mmol/L乙酸銨溶液，B為甲醇，兼顧了各化合物的峰型和靈敏度（如圖1所示），並且有效的避開基質干擾，使定量結果更準確。

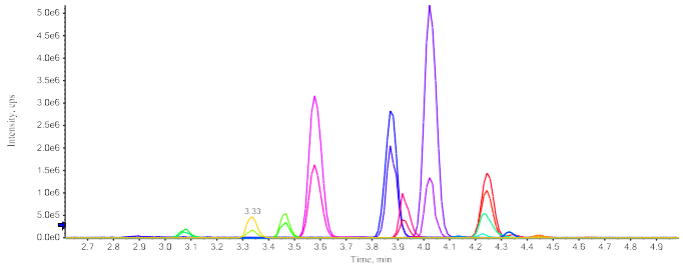


圖**1.** 12種酚類化合物的提取離子流色譜圖

2.2. 線性、回收率和重複性考察

以空白水基食品模擬物為溶劑，分別添加1 ng/mL、5 ng/mL和10 ng/mL三個濃度樣品，每個濃度重複6份，加標回收率均在82.1%~109.1%之間，相對標準差（RSD%）在5%（n=6）以內，實驗結果表明該方法具有較好的回收率以及良好的穩定性（如表2所示）。12種酚類化合物的基質加標曲線回歸係數均達到0.995以上（如圖2所示），表明線性良好。該實驗方法足以滿足標準GB 31604.50-2020和GB 31604.10-2016的定量檢測要求。

表**2.** 12種酚類化合物在不同濃度下的加標回收率結果

序號	化合物名稱	1 ng/mL		5 ng/mL		10 ng/mL	
		回收率%	RSD %	回收率%	RSD %	回收率%	RSD %
1	雙酚F-二縮水甘油醚	88.7	4.67	109.1	2.97	103.5	2.80
2	雙酚A-二縮水甘油醚	96.0	2.33	103.2	3.02	91.9	2.58
3	雙酚A-(2,3-二羥基丙基)縮水甘油醚	87.3	3.73	90.9	2.66	105.4	2.35
4	雙酚A-雙(3-氯-2-羥丙基)甘油醚	101.0	4.60	95.7	3.25	98.7	1.11
5	3-ring noge	87.9	3.19	83.6	3.94	107.9	1.47
6	4-ring noge	-	-	108.9	4.41	93.5	2.69
7	雙酚A	86.5	3.45	97.8	2.32	102.2	1.36
8	雙酚B	108.8	2.91	87.4	2.85	85.9	2.60
9	雙酚F	105.9	4.30	83.6	3.45	91.3	2.87
10	壬基酚	91.2	4.77	82.1	4.59	97.6	2.76
11	辛基酚	94.5	3.46	87.2	3.26	102.6	1.54
12	對特辛基酚	-	-	89.1	4.39	103.8	2.19

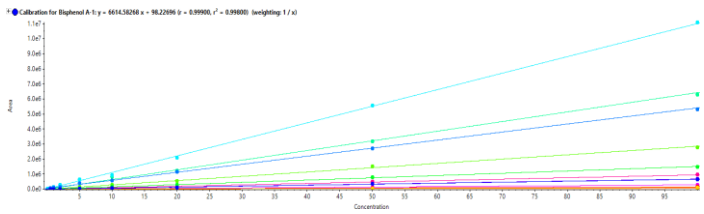


圖**2.** 12種酚類化合物的標準曲線

3. 小結

本文採用SCIEX ExionLC™ 2.0+和SCIEX Triple Quad™ System建立了食品包裝材料中12種酚類化合物的快速定量方法，該方法詳細優化了色譜質譜條件，具有覆蓋範圍廣，靈敏度高，分析速度快，重現性好等特點。該方法足以滿足標準GB 31604.50-2020和GB 31604.10-2016的定量檢測要求，對於食品包材中酚類化合物的分析檢測具有重要的參考意義。

附表. 12種酚類化合物質譜離子對列表

序號	化合物名稱	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (eV)
1	雙酚F-二縮水甘油醚	330.1	163.2	31	19
		330.1	133.2	31	10
2	雙酚A-二縮水甘油醚	358.1	191.2	36	18
		358.1	135.1	36	10
3	雙酚A-(2,3-二羥基丙基)縮水甘油醚	376.1	209.1	37	19
		376.1	135.1	37	10
4	雙酚A-雙(3-氯-2-羥丙基)甘油醚	430.1	227	32	18
		430.1	135.2	32	10
5	3-ring noge	492.2	163	90	27
		492.2	145	90	31
6	4-ring noge	654	163	100	34
		654	207	100	33
7	雙酚A	227.1	212.1	-90	-24
		227.1	133	-90	-31
8	雙酚B	241.1	212.1	-100	-22
		241.1	110.9	-100	-37
9	雙酚F	199	93	-100	-28
		199	105	-100	-28
10	壬基酚	219.2	133.1	-100	-40
		219.2	147.1	-100	-34
11	辛基酚	205.1	106.1	-70	-24
		205.1	133.1	-70	-29
12	對特辛基酚	205.1	133.1	-70	-29
		205.1	106.1	-70	-24

參考文獻：

1. 食品安全國家標準 食品接觸材料及製品遷移試驗通則，GB 31604.1-2015
2. 食品安全國家標準 食品接觸材料及製品遷移試驗預處理方法通則，GB 5009.156-2016
3. 食品接觸材料及製品2,2-二(4-羥基苯基)丙烷(雙酚A)遷移量的測定，GB 31604.10-2016
4. 食品接觸材料及製品壬基酚遷移量的測定，GB 31604.50-2020

食品接觸材料中的29種芳香胺的LC-MS/MS快速定量方法

A Rapid Quantitative Method of LC-MS /MS for 29 kinds of Aromatic Amines in Food packaging material

李星霖[1]，金莉莉[2]，楊總[1]，劉冰潔[1]，郭立海[1]

Li Xinglin[1], Jin Lili[2], Yang Zong[1], Liu Bingjie[1], Guo Lihai[1]

SCIEX China[1]

國家食品接觸材料檢測重點實驗室（常州）[2]

National Reference Laboratory for Food Contact Material (Changzhou)[2]

Keywords: SCIEX Triple Quad; Food packaging material; Aromatic Amines

引言

芳香胺是一類化合物，其中最簡單的形式是苯胺。大部分芳香胺都不會威脅人類的健康，但某些物質卻是已知的人類致癌物。比如苯胺對人體具有毒性作用，僅少量就能引起中毒。它主要通過皮膚、呼吸道和消化道進入人體內，不僅能使氧合血紅蛋白變為高鐵血紅蛋白從而降低血液的載氧能力，使組織細胞缺氧而窒息，造成中樞神經系統、心血管系統和其它臟器損害，而且苯胺類化合物還具有致癌作用。因此芳香胺類化合物的測定對於保證人體健康有重要意義，也是目前食品領域的研究熱點問題。本方案基於中國食品安全國家標準GB31604.52-2021《食品接觸材料及製品芳香族伯胺遷移量的測定》標準，採用SCIEX ExionLC™ 2.0+和Triple Quad™ system建立了食品包裝材料中29種芳香胺類化合物的快速定量方法，該方法具有以下幾個特點：

1. 本方法覆蓋面廣，總共包含29種芳香胺化合物，足以滿足相關標準的要求。
2. 本方法靈敏度高，靈敏度達到pg級別以下，足以滿足相關標準的要求。
3. 本方法效率高、重複性好、回收率高。檢測29種芳香胺類化合物只需要10分鐘，以空白基質為溶劑，分別添加0.5 ng/mL、1 ng/mL和10 ng/mL三個濃度樣品，每個濃度重複6份，加標回收率均在81.2%~107.1%之間，相對標準差在5%（n=6）以內。

1. 實驗部分

1.1. 樣品前處理：

本方法遷移實驗採用4%乙酸食品模擬物進行遷移，遷移試驗的條件選擇和操作步驟按照GB 31604.1和GB 5009.156的規定，選中空心製品（礦泉水瓶）採用全浸沒法，加入80%容量的4%乙酸，在40℃下遷移10天，移取遷移試驗後得到的食品模擬物，用針式尼龍篩檢程式過濾後，以供液相色譜串聯質譜儀測定。

1.2. 色譜方法：

色譜柱：Phenomenex Kinetex F5, 2.6 µm，150 mm × 3.0 mm

流動相：A：水（0.05%甲酸）；B：乙腈

柱溫：40 ℃

洗脫程式：梯度洗脫（表1）

表**1.** 液相梯度洗脫

Time (min)	Flow(mL/min)	B (%)
0	0.4	10
2	0.4	70
5	0.4	95
7	0.4	95
7.1	0.4	10
10	0.4	10

1.3. 質譜方法：

掃描方式：電噴霧電離（electrospray ionization, ESI），正離子模式

離子源參數：

氣簾氣（CUR）：30 psi； 碰撞氣（CAD）：9 ；

噴霧電壓（IS）：4000 V； 離子源溫度（TEM）：600 ℃；

霧化氣（GAS 1）：65 psi； 輔助加熱氣（GAS 2）：60 psi；

MRM離子對見附表

2. 實驗結果：

2.1. 色譜條件優化

實驗詳細優化了色譜條件，比較了不同品牌、不同型號的色譜柱以及流動相，最終選擇的色譜柱是Phenomenex Kinetex F5，2.6 µm，100 mm × 3.0 mm，流動相為A為0.05%甲酸水溶液，B為乙腈，兼顧了各化合物的峰型和靈敏度（如圖1所示），並且有效的避開基質干擾，使定量結果更準確。

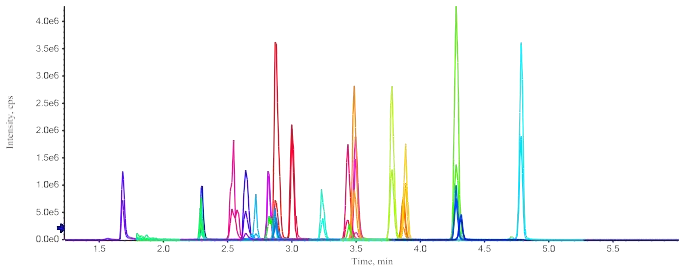


圖**1.** 29種芳香伯胺的提取離子流色譜圖

2.2. 線性、回收率和重複性考察

以空白4%乙酸食品模擬物為溶劑，分別添加0.5 ng/mL、1 ng/mL和10 ng/mL三個濃度樣品，每個濃度重複6份，加標回收率均在81.2%~107.1%之間，相對標準差（RSD%）在5%（n=6）以內，實驗結果表明該方法具有較好的回收率以及良好的穩定性。29種芳香胺化合物的基質加標曲線回歸係數均達到0.996以上（如圖2所示），表明線性良好。該實驗方法足以滿足標準GB 31604.52-2021的定量檢測要求。

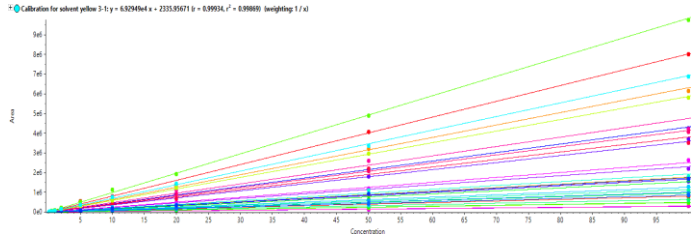


圖**2.** 29種芳香伯胺的標準曲線

3. 小結

本文採用SCIEX ExionLC™ 2.0+和SCIEX Triple Quad™ system建立了食品遷移物種芳香伯胺的快速檢測方法，該方法詳細優化了色譜質譜條件，具有覆蓋範圍廣，靈敏度高，分析速度快，重現性好等特點。該方法足以滿足標準GB 31604.52-2021的定量檢測要求，對於食品包材中芳香胺類化合物的分析檢測具有重要的參考意義。

參考文獻：

1. 食品安全國家標準 食品接觸材料及製品遷移試驗通則，GB 31604.1-2015
2. 食品安全國家標準 食品接觸材料及製品遷移試驗預處理方法通則，GB 5009.156-2016
3. 食品安全國家標準 食品接觸材料及製品 芳香族伯胺遷移量的測定，GB31604.52-2021

附表**.** 29種芳香伯胺的質譜離子對列表

化合物名稱	Q1(m/z)	Q3(m/z)	DP(V)	CE(eV)
4-氨基聯苯	170.2	152.1	80	23
	170.2	153.1	80	36
聯本胺	185.2	168.2	80	28
	185.2	167.1	80	38
4-氯-2-甲基苯胺	142.1	125.0	80	31
	142.1	89.1	80	48
2-萘胺	144.1	127.1	80	30
	144.1	77.1	80	29

附表. 29種芳香伯胺的質譜離子對列表（續）

化合物名稱	Q1(m/z)	Q3(m/z)	DP(V)	CE(eV)
鄰氨基偶氮甲苯	226	91.1	70	23
	226	121.1	70	47
2-氨基-4-硝基甲苯	153.1	107.1	80	24
	153.1	89.1	80	29
對氯苯胺	128.1	93.1	80	23
	128.1	111.1	80	23
4-甲氧基間苯二胺	139.1	124.1	70	31
	139.1	108.1	70	63
4,4'-二氨基二苯甲烷	199.2	106.1	80	30
	199.2	77	80	40
3,3-二氯聯苯胺	253.1	217	100	46
	253.1	182.1	100	27
3,3'-二甲基聯苯胺	213.2	180.1	100	33
	213.2	196.1	100	34
4,4'-二氨基-3,3'-二甲基聯苯基甲烷	227.2	120.2	100	22
	227.2	178.1	100	31
3-氨基對甲苯甲醚	138.2	123.1	70	27
	138.2	106.1	70	44
4,4'-二氨基二苯醚	201.2	108.1	90	30
	201.2	80	90	26
4,4-三氨基三苯硫醚	217.2	124.1	90	25
	217.2	200	90	23
鄰甲苯胺	108.1	91	75	21
	108.1	93	75	38
2,4-二氨基甲苯	123.1	106.1	100	31
	123.1	77.1	100	23
2,4,5-三甲基苯胺	136.2	91.1	80	23
	136.2	121.1	80	39
鄰甲氧基苯胺	124.1	109.1	70	27
	124.1	80.2	70	33
對氨基偶氮苯	198.2	77.1	70	34
	198.2	93.2	70	34
2,4-二甲基苯胺	122.2	77.1	80	22
	122.2	105.1	80	25
2,6-二甲基苯胺	122.2	105.1	80	25
	122.2	77	80	22
苯胺	94	77.1	70	22
	94	51.1	70	31
對苯二胺	109.2	92.1	70	42
	109.2	65.2	70	35
4,4'-二氨基-3,3'-二氯二苯甲烷	267.2	195.2	90	43
	267.2	140.1	90	26
3,3'-二甲氧基聯苯胺	245.2	187.2	90	26
	245.2	230.1	90	42
2-[(4-氨基苯)甲基]苯胺	199.2	106.1	80	30
	199.2	77	80	40
2,2'-亞甲基二苯胺	199.2	106.1	80	30
	199.2	77	80	40
間苯二胺	109.2	92.1	70	42
	109.2	65.2	70	35

SCIEX

The Power of Precision

台灣塞爾克斯應用生技有限公司 | 11492 台北市內湖區瑞光路631號4F
SCIEXNow Toll Free : 0800-818-978 或 service.taiwan@sciex.com