2022 迷你食品應用文集

第一期 --- 食品添加物

SCIEX
The Power of Precision

您的實驗室準備好進行測試了嗎？

全球營養補充品市場正在增長

請不要依據包裝上的標籤來判斷營養補充品，因為政府通常會在產品進入消費市場後才開始對成分進行監控[1]。同時，標籤上可能沒有提及其它用以解決或改善補充品副作用的添加物。在美國就是這種情況，儘管美國聯邦法律要求補充品需有膳食補充品標籤或替代術語，但一旦補充品上市才會開始監控。與此同時，中國國家食品藥品監督管理總局（CFDA）的保健品潛在非法添加物清單中，明確規定了減肥、降血糖、降血壓、抗疲勞、改善睡眠、改善和增強免疫功能等六大類營養補充品中添加物的監控流程。

對於負責分析的實驗室來說，跟上添加物的步伐並不是一件容易的事。 Grand View Research 的一份研究報告指出，運動營養品在美國和中國的銷量上升以及新產品的推出可能會對行業產生重大影響。預計到 2024 年[2]，該市場將產生價值 371.6 億美元的收入。

然而，由於藥物相互作用可能不清楚，因此它們仍然值得澄清。想為您的實驗室找到跟上營養補充品篩查的步伐嗎？第一期的迷你食品應用文集將帶您了解如何克服複雜基質中的基質干擾，以準確測試這些添加物。

1. https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm050803.htm
2. https://globenewswire.com/news-release/2016/07/18/856668/0/en/Dietary-Supplements-Market-Size-Is-Projected-To-Reach-278-02-Billion-By-2024-Demand-In-Food-Beverage-Sector-Grand-View-Research-Inc.html

目錄

SCIEX QTRAP®質譜對食品中63種添加物的檢測方案

SCIEX QTRAP® Mass Spectrometry for Rapid Detection of 63 Additives in Food

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關鍵字： QTRAP；食品添加物

Key words: QTRAP; Food Additives

引言

食品添加物是為改善食品色、香、味等品質，以及為防腐和加工工藝需要加入食品中的人工合成或者天然物質。目前中國食品添加物有23個類別，2000多個品種，包括酸度調節劑、抗結劑、消泡劑、抗氧化劑、漂白劑、膨松劑、著色劑、護色劑、酶製劑、增味劑、營養強化劑、防腐劑、甜味劑、增稠劑、香料等。食品添加物作為食品工藝中不可或缺的一部分，其安全性也越來越受到消費者的關注。近些年，新聞多次報導過一些不良商家超範圍、超量使用添加物事件，對消費者的身體健康造成嚴重的影響。因此，食品添加物生產和使用者必須嚴格把握、正確理解食品添加物的使用原則，深入瞭解被允許使用的食品添加物特性，結合自身產品的工藝需要，拒絕使用不必要的食品添加物。

SCIEX基於QTRAP®質譜系統針對食品中建立起63種常見食品添加物LC-MS/MS檢測方法，實現快速鑒定和定量檢測，為消費者的食品安全提供強大的後盾和保障。

SCIEX QTRAP® 質譜系統是將三重四極杆質譜技術與線性離子阱技術相結合；不僅具有這兩類質譜的所有掃描模式，還提供多種獨特的複合功能。其品質分析器可在兩類質譜的工作模式之間進行暫態切換，可智慧化的實現一針進樣，同時獲得不同掃描模式下的資料。本方案採用QTRAP®質譜系統的MRM-IDA-EPI 的掃描模式，實現一針進樣，同時進行定性或定量分析。

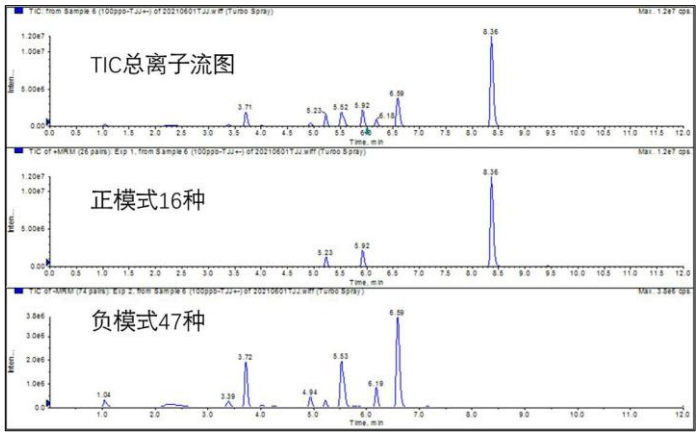

圖**1.** MRM掃描模式下的63種食品添加物色譜圖

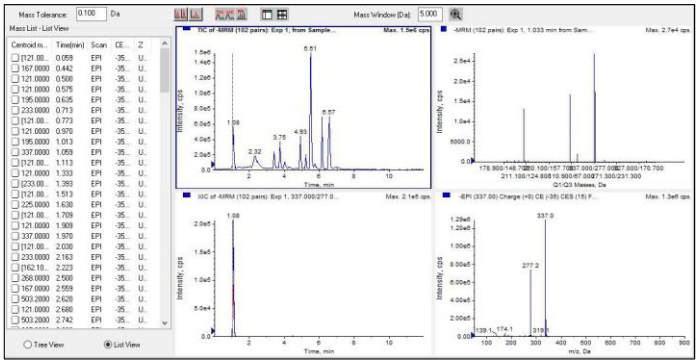

圖**2.** 63種食品添加物的EPI二級增強質譜圖

實驗方法特點：

1. 前處理簡便快速、操作簡單省時省力，可以快速篩查並定量食品中63種食品添加物。

2. 檢測方法簡單高效，11 min梯度洗脫，一針進樣，應用QTRAP®質譜的MRM-IDA-EPI複合掃描模式同時獲得MRM資料以及高靈敏度的二級碎片全譜資料（EPI）（見圖2）。

3. 強大的定性功能：QTRAP®質譜的EPI模式，即增強型子離子掃描模式，可得到靈敏度更高（與三重四極杆相比可提高兩個數量級以上），且不同能量碎裂的全品質範圍的二級碎片全譜。軟體通過與標準品的譜庫自動比對，幫助更準確的篩查和定性。

4. 卓越的定量功能：QTRAP®質譜具有與三重四極杆質譜完全一致的定量性能，同時，擁有出色的系統重現性和穩定性。線性各濃度點準確度均在80-120 %之間，且r均大於0.995，線性相關性良好，定量準確。

實驗方法

1. 色譜條件

a）色譜柱：Phenomenex Kinetex C18（100×2.1 mm, 2.6 µm），或性能相當者。

b）流動相：A為H_2O (10 mM乙酸銨)，B為ACN，梯度洗脫程式見表1。

c）流 速： 300 µL/min。

d）柱 溫：40 ℃。

e）進樣量：10 µL。

2. 表**1** 梯度洗脫

時間/min	流速/（mL/min）	A/%	B/%
0	0.3	95	50
1	0.3	95	5
6	0.3	35	65
7	0.3	5	95
9	0.3	5	95
9.1	0.3	95	5
11	0.3	95	5

2. 質譜方法

掃描方式： MRM-IDA-EPI

離 子 源： ESI 源

離子源參數：

電 壓 IS : 5500V / - 4500 V

氣簾氣 CUR : 30 psi

霧化氣 GS1 : 50 psi

源溫度 TEM :550 ℃

碰撞氣CAD : Medium

輔助氣GS2 : 55 psi

表**2** 63種食品添加物的MRM參數

化合物	Q1	Q3	DP（V）	CE（V）	RT（min）
檸檬黃	470	349	25	52	9.21
	470	454	25	58	9.21
赤蘚紅	837.5	583.9	3	64	5.92
	837.5	330	3	80	5.92
鹼性橙2	213.1	121.1	44	27	7.97
	213.1	196.1	44	27	7.97
鹼性橙21	315.2	270	250	48	7.5
	315.2	300.2	250	31	7.5
鹼性橙22	391.1	376.1	250	37	8.55
	391.1	220.1	250	34	8.54
結晶紫	372	356	220	56	8.93
	372	251	220	49	8.92
隱性結晶紫	374.3	358.2	200	45	9.96
	374.3	239.2	200	45	9.96
羅丹明B（鹼性玫瑰精）	443.2	297.1	227	98	8.06
	443.2	399	227	56	8.06
蘇丹紅I	249.1	92.9	65	35	9.6
	249.1	156	65	21	9.6
蘇丹紅Ⅱ	277.1	121	70	30	10.6
	277.1	156	70	18	10.6
蘇丹紅Ⅲ	353.1	156.1	90	30	8.99
	353.1	77	90	60	8.99
蘇丹紅Ⅳ	381.2	224.2	100	29	8.82
	381.2	90.9	100	52	8.81
隱性孔雀石綠	331.2	316.1	10	27	9.85
	331.2	239.2	10	42	9.86
阿力甜	332	129	70	22	5.29
	332	159	70	28	5.29

化合物	Q1	Q3	DP（V）	CE（V）	RT（min）
偶氮玉紅（酸性紅）	459	233.1	115	34	5.24
	459	442	115	27	5.24
2,6-二叔丁基對甲酚BHT	221	161	40	10	10.6
	219	219	-120	-10	10.6
叔丁基羥基茴香醚BHA	178.9	148.7	-74	-34	8.38
	178.9	164	-75	-21	8.38
特丁基對苯二酚TBHQ	164.8	149	-114	-33	8.39
	164.8	108	-114	-25	8.37
對羥基苯甲酸苄酯	227	92	-91	-32	8.04
	227	135.9	-91	-20	8.04
對羥基苯甲酸甲酯	151	92	-76	-26	6.31
	151	136.1	-76	-17	6.32
對羥基苯甲酸丙酯	179	93	-85	-26	7.56
	179	136.8	-85	-20	7.56
對羥基苯甲酸丁酯	193	136	-97	-22	8.07
	193	92	-97	-29	8.07
2,4,5-三羥基苯丁酮（THBP）	195	125	-90	-24	6.57
	195	151	-90	-27	6.57
沒食子酸丙酯（PG）	211.1	169	-90	-22	6.13
	211.1	124.8	-90	-31	6.13
沒食子酸辛酯（OG）	281.1	169	-113	-29	8.52
	281.1	123.8	-113	-37	8.51
去甲二氫愈創木酚（NDGA）	301.2	122	-134	-35	7.49
	301.2	273.2	-134	-33	7.49
偶氮玉紅（酸性紅）	478.9	243.6	-101	-35	5.25
	478.9	397	-101	-31	5.26
橙黃1	326.7	170.9	-64	-32	6.4
	326.7	155.8	-64	-37	6.4
酸性紅G（別名紅2G）	231.5	179	-73	-15	4.89
	231.5	158	-73	-20	4.9
酸性紅87	646.7	520.7	-128	-35	5.46
	646.7	522.8	-128	-36	5.46

化合物	Q1	Q3	DP（V）	CE（V）	RT（min）
酸性藍3鈣鹽	559	479.2	-150	-42	6.43
	559	435	-150	-58	6.43
酸性紫7	260.1	157.7	-68	-24	4.46
	260.1	171.5	-68	-26	4.46
螢光桃紅	784.5	658.6	-155	-41	6.35
	784.5	704.6	-155	-39	6.35
孟加拉紅	972.5	674.3	-175	-52	6.7
	972.5	892.7	-175	-39	6.7
麗春紅3R	449	369.1	-148	-35	5.58
	449	301.6	-148	-38	5.58
橙黃G	407	302	-76	-26	4.66
	407	238	-76	-41	4.66
酸性綠50	553.1	496	-197	-54	5.48
	553.1	511	-197	-41	5.48
螢光素鈉	331.1	287.1	-95	-23	5.6
	331.1	243.1	-95	-37	5.6
苯甲酸	121	77	-36	-18	3.09
	121	93	-36	-20	3.09
山梨酸	110.9	67	-45	-12	3.72
	110.9	69	-45	-15	3.72
糖精鈉	182	42	-80	-50	3.98
	182	105.9	-80	-24	3.98
脫氫乙酸	167	82.9	-47	-17	3.89
	167	123	-47	-10	3.9
安賽蜜	162.1	77.9	-80	-40	2.32
	162.1	82	-80	-17	3
甜蜜素	178	80	-80	-37	4.33
	178	178	-80	-5	4.32
三氯蔗糖	395.1	359.1	-80	-10	5.03
	395.1	34.9	-80	-25	5.02
阿斯巴甜	293	200	-80	-23	4.98
	293	261	-80	-14	4.97
紐甜	377.2	200.2	-80	-25	6.58
	377.2	301.2	-80	-25	6.58

化合物	Q1	Q3	DP（V）	CE（V）	RT（min）
抗壞血酸葡糖苷	337	277	-99	-25	1.22
	337	174	-99	-29	1.21
異麥芽糖	341	178.8	-69	-11	1.28
	341	220.9	-69	-18	1.25
異麥芽三糖	503.2	178.9	-100	-27	1.25
	503.2	341.1	-100	-17	1.24
異麥芽四糖	665.2	503.2	-125	-20	1.24
	665.2	545.2	-125	-29	1.23
莧菜紅	537	236.9	-175	-56	3.76
	537	316.8	-175	-43	3.76
亮藍	747.2	561.3	-175	-62	5.36
	747.2	667.3	-175	-50	5.36
新紅	271.3	155.8	-61	-19	3.7
	271.3	171.9	-61	-22	3.7
胭脂紅	268	220.8	-72	-24	4.21
	268	205.9	-72	-15	4.21
日落黃	203	170.9	-50	-19	4.41
	203	155.8	-50	-25	4.4
誘惑紅	225	214	-52	-21	4.66
	225	206.9	-52	-21	4.66
喹啉黃	215.4	79.7	-73	-46	4.2
	215.4	161.5	-73	-27	4.2
酸性橙7	327	107.1	-70	-54	6.39
	327	156	-70	-42	6.39
靛藍	210	79.9	-69	-44	3.88
	210	155.9	-69	-29	3.88
檸檬黃	233	171.1	-38	-16	1.37
	233	198	-38	-19	1.37
赤蘚紅	834.6	662.7	-160	-54	5.9
	834.6	507.8	-160	-82	5.9
阿力甜	330	167.2	-80	-30	5.28
	330	215.2	-80	-28	5.28

3. 樣品前處理

準確稱量1.00 g（精確0.01 g）樣品置於50 mL容量瓶中, 然後加入10 mL 50 %甲醇水, 渦旋，超聲提取5 min, 冷卻至室溫, 用50 %甲醇定容。4000 r/min離心5 min，取上清液過膜，根據實際濃度適當稀釋至線性範圍內，上機LC-MS/MS測試。

結果與討論

1. 定性實驗結果

63種食品添加物的標準譜庫建立

QTRAP®質譜的EPI掃描模式，利用碰撞池的多能量碎裂功能以及離子阱品質分析器的富集功能，可得到更全品質範圍的二級碎片譜，根據其二級碎片全譜資訊，建立63種食品添加物的標準譜庫。樣本實測二級全譜與標準品譜庫的自動比對，可幫助更好的排判別假陽性和假陰性，保證定性結果的準確無誤；可用於食品添加物的快速篩查以及定性確證。

2. 定量實驗結果

將空白樣品經過前處理提取，得到空白基質，應用空白基質配標。用空白基質配置各物質在2-100 ng/ml的標準曲線，結果表明，線性關係良好，r值均大於0.995，（見圖4），且各濃度點準確度均在要求範圍之間，可保證不同濃度水準樣品的準確定量。

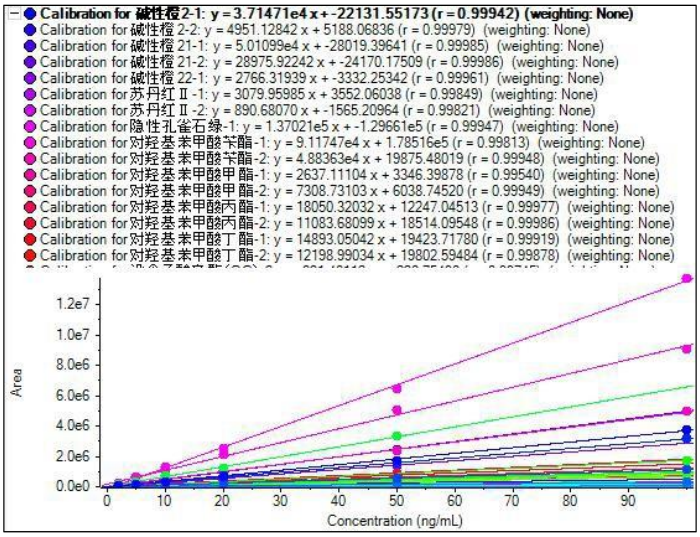

圖**4.** 63種食品添加物在基質樣本中的線性曲線

實際樣品的檢測：

某市食品藥品檢驗所對某地區內食品、飲料進行抽查檢測，在某樣品中檢測出三氯蔗糖超標。其色譜圖見圖5。經測試含量77.6 mg/kg。同時，應用QTRAP進行定性確證，二級譜庫搜庫對比評分為100分，進一步陽性確證。

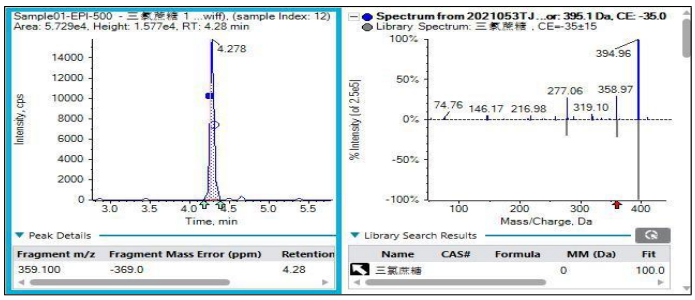

圖**5.** 某樣本中三氯蔗糖檢出圖譜

總結

1. 該方法基於SCIEX QTRAP® 系統的MRM-IDA-EPI複合掃描模式建立了食品中常見63種食品添加物的定量和定性篩查方法。一針進樣，同時得到準確高品質的MRM資料和EPI資料，使得定量 和定性篩查可以一針完成，省時省力。
2. SCIEX Turbo V™ 離子源的獨特設計和主動排廢的功能帶來高離子化效率和卓越的抗污染能力。在日常工作中，大批量樣本檢測過程，仍可以保證穩定的高靈敏度和重現性。
3. QTRAP®質譜的EPI掃描模式，可得到更全品質範圍的二級碎片譜，根據其二級碎片 全譜資訊，建立食品添加物標準譜庫，可用於日常快速篩查以及定性確證。
4. 此方法和實驗思路同樣適用於SCIEX 其它型號QTRAP®系統，為食品添加物快速篩查定量提供一種有效的方法。

Triple Quad™ 3500在食品中Sodium Picosulfate定量檢測中的應用

A Sensitive and Robust New Method for Quantification of Sodium Picosulfate in Food by Triple Quad™ 3500

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Key words: Triple Quad™ 3500; Quantification; Food; Sodium Picosulfate

引言

Sodium Picosulfate又名4,4'-(吡啶-2-基亞甲基)雙苯酚基雙硫酸酯鈉鹽，是一種特殊緩瀉劑，它對大腸黏膜直接作用產生溫和的緩瀉效果，臨床上主要用於便秘和手術後、鋇餐後排便。部分國家有檢測到Sodium Picosulfate添加製食品中

本測定方法，利用Triple Quad™ 3500液質聯用系統建立了一個快速、靈敏的MRM定量方法，並對方法的線性、回收率和重複性進行了驗證，為不同儀器型號的使用者提供了通用的解決方案。

該方法優勢如下：

1、 快速、靈敏

本方法中液相洗脫時間只需要4 min，Sodium Picosulfate定量限低至0.4 ng/mL（圖2），遠高於標準要求，本公司不同型號三重四級杆質譜的用戶均可使用。

2、 線性範圍寬

Sodium Picosulfate在0.4~1000 ng/mL 的超寬濃度範圍內擁有良好的線性關係，相關性係數r＞0.999（圖3），可有效減少用戶在檢測不同食品過程中的稀釋步驟。

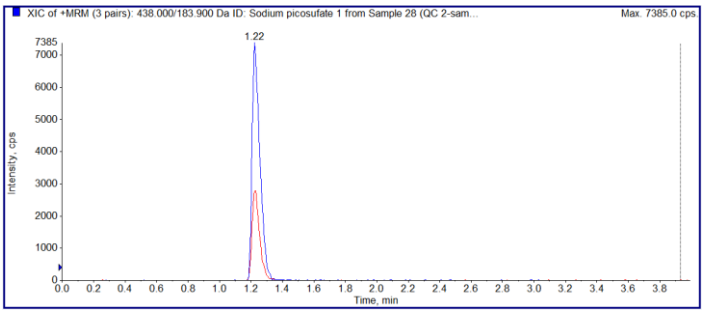

圖1. Sodium Picosulfate的典型色譜圖（話梅基質，60 ng/mL）。

3、 適用範圍廣

本方法對標準中提到的5種基質均作了加標回收率驗證，實驗表明，5中食品基質均不干擾化合物的測定（表1）。

表1. 五種不同基質中Sodium Picosulfate的加樣回收率。

	濃度（ng/mL）		
化合物名稱	6	60	600
保健品	109.0	94.8	95.0
糖果	101.0	96.7	96.1
固體飲料	108.5	99.7	90.9
話梅	102.8	106.3	91.5
果凍	105.7	97.2	93.2

實驗方法

1. 液相條件：

色譜柱：Phenomenex Kinetex C18, 2.6 µm, 2.1 mm×50 mm;

流動相：A相： 5 mM甲酸銨水溶液
B相：乙腈

流速：0.6 mL/min；

色譜柱溫度：40 ℃；

進樣量：5 μL；

進樣器溫度：15 ℃

洗脫程式：

Time(min)	A (%)	B (%)
0.0	95	5
1.5	50	50
1.6	10	90
2.5	10	90
2.6	95	5
4	95	5

2. 質譜方法：

離子源：ESI源，正離子模式

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　　源溫度 TEM: 500 ℃

化合物名稱	母離子	子離子	DP	CE
Sodium Picosulfate	438.0	183.9	100	44
		277.9	100	30

3. 樣品製備

按照《食品中Sodium Picosulfate的測定》食品補充檢驗方法中樣品製備流程對某片劑保健品、糖果、固體飲料、話梅以及果凍分別進行前處理[1]。

標準曲線樣品用純水稀釋，分別製成濃度為0.4、1、2、6、20、60、200、600、1000 ng/mL的溶液，共9個濃度點。

結果與討論

1. 靈敏度及線性範圍

本方法檢測濃度低至0.4 ng/mL（圖2），在0.4~1000 ng/mL 的超寬濃度範圍內擁有良好的線性關係，相關性係數r＞0.999（圖3）。進樣1000 ng/mL高濃度點後空白無殘留。

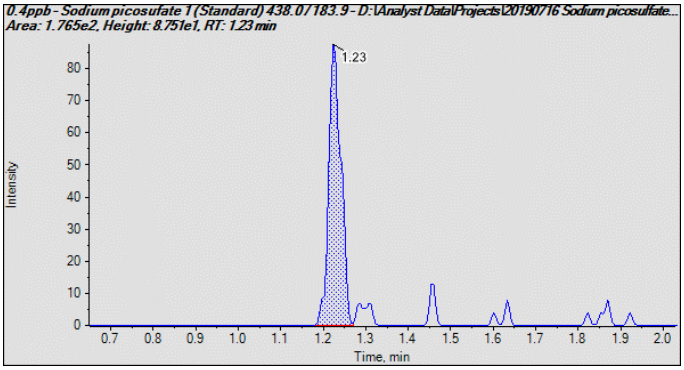

圖2. 定量下限濃度（0.4 ng/mL）處化合物色譜圖。

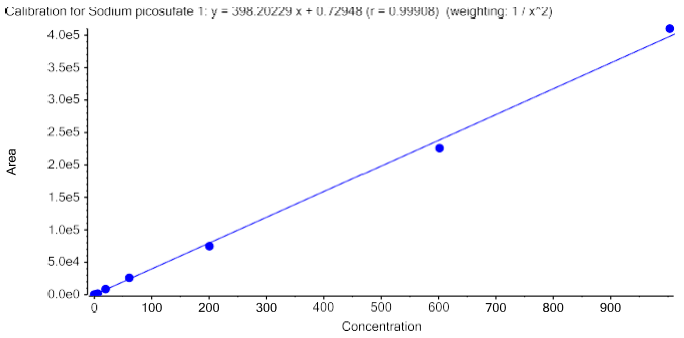

圖3. Sodium Picosulfate標準曲線（0.4~1000 ng/mL）。

2. 加樣回收率

以5種空白樣品前處理溶液為溶劑，分別配製6 ng/mL、60 ng/mL和600 ng/mL三個濃度樣品，5種基質的加標回收率均在90.9 %~109.0 %之間（表1），表明該方法不受樣品基質干擾。

3. 重複性

以話梅基質為例，對化合物在6 ng/mL、60 ng/mL和600 ng/mL三個不同濃度點的重複性進行考察，結果見表3。三個不同濃度加標樣品，連續進樣6次，峰面積RSD除6 ng/mL的低濃度點為4.78%外，其餘濃度均在2%以內。

表**3.** 話梅基質中Sodium Picosulfate進樣重複性。

	回收率%		
濃度(ng/mL)	6	60	600
1	101.44	97.57	97.06
2	102.87	97.47	97.10
3	98.53	96.97	95.57
4	97.73	94.99	93.82
5	89.92	95.6	95.42
6	95.08	94.78	92.51
RSD%	4.78	1.31	1.90

4. 樣品測定

五種樣品基質中均未檢出Sodium Picosulfate，相關色譜圖見圖4.

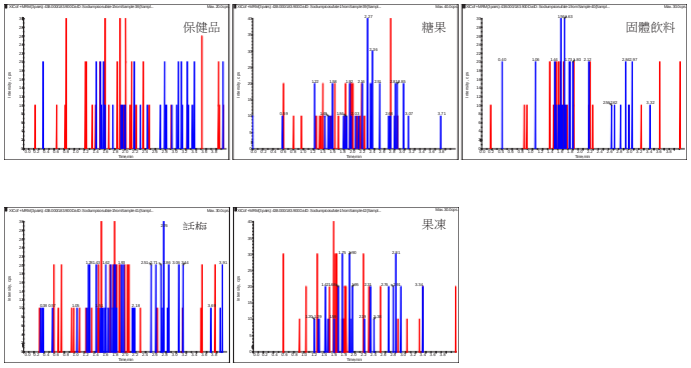

圖**4.** 五種樣品提取液色譜圖。

總結

本文利用Triple Quad™ 3500液質聯用系統建立了一個食品中Sodium Picosulfate的定量方法，該方法靈敏度高，重複性好，在0.4~1000 ng/mL的超寬範圍內有良好的線性關係，且待測物在5種不同食品基質中均無明顯基質效應。方法洗脫時間僅4 min，非常適用於大批量樣品檢測。

白酒類飲料中9種甜菊糖苷的液質快速檢測方法

Simultaneous Determination of 9 kinds of Steviol Glycosides in Chinese Liquor and Beverages by QTRAP® System

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Key Words : QTRAP; Chinese liquor; Steviol glycosides

引言

甜菊糖苷是一類以甜葉菊幹葉為原料，經提取、精製而得的食品添加物。其主要成分為四環二萜類化合物，包括甜菊苷、瑞鮑迪苷A 、瑞鮑迪苷B、瑞鮑迪苷C、瑞鮑迪苷D、瑞鮑迪苷F、甜茶苷、杜克苷、甜菊雙糖苷等。它們具有相同的苷元：甜菊醇 （見圖1），在C19和C13位上連接不同數量的葡萄糖基，鼠李糖基或木糖基，從而形成味質、理化性能各異的甜菊糖苷。甜菊糖苷可在飲料中可按生產需要適量使用。在蒸餾酒中，中國依據《食品添加物使用標準》（GB 2760-2014）中規定不允許添加甜味劑，配製酒和飲料中的甜味劑的使用也不得超過限量。

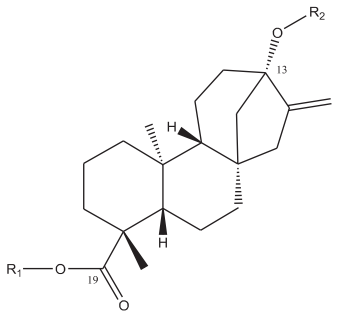


圖**1.** 甜菊糖苷的結構式

目前，針對食品中甜味劑的檢測，研究較多的主要為人工合成甜味劑，甜菊糖苷等天然甜味劑報導較少。中國的甜菊糖苷食品添加物安全標準（GB8270-2014）中採用的是容量法和HPLC法，由於此類甜味劑結構類似，故採用HPLC不能得到很好地分離。

我們通過QTRAP® 系統建立了白酒及飲料中常見9種甜菊苷的精確定量方法，並通過QTRAP® 系統的線性離子阱功能建立了其二級碎片譜庫，可用於後期的篩查分析。該方法具有以下特點：

1. 本方法覆蓋目前食品添加物安全標準（GB8270-2014）中9種甜菊糖苷的檢測。
2. 抗基質干擾，特異性強，保證結果可靠性。
3. 10 min內完成9種甜菊糖苷的檢測，快速方便。
4. 結合QTRAP®系統的EPI功能，通過SCIEX OS軟體建立其二級譜庫，便於後期篩查和鑒定。

儀器設備

SCIEX ExionLC™ 系統 + QTRAP® 系統

SCIEX ExionLC™ 系統 + QTRAP® 系統

樣品處理：

取樣約1 g左右，使用90% 乙腈水溶液稀釋5-10倍，離心5min，過濾，上機分析。

色譜方法：

色譜柱：BEH Amide Column, 1.7µm, 2.1 mm X 100 mm

流動相：A：10 mmol/L 甲酸銨水溶液

B：乙腈

梯度洗脫：

時間 [min]	流速 [mL/min]	B[%]
0.00	0.4000	90
0.50	0.4000	90
5.00	0.4000	50
6.00	0.4000	50
6.10	0.4000	90

流速：0.4 mL/min；

柱溫：40℃；

進樣量：5 µL

質譜方法：

掃描方式：負模式

離子源：ESI

離子對列表見附表

實驗結果

化合物提取離子流色譜圖（圖2）

1. 色譜質譜條件優化

分別使用C18，氟苯基柱，及氨基柱優化條件，發現當時用氨基柱正相色譜模式分析時，各甜菊糖苷可以得到較好分離，但此模式色譜峰型受樣品溶劑影響較大，使用90% 乙腈水溶液作為樣品溶劑，峰型較好。另外分別使用0.1% 甲酸水，2 mmol/L、5 mmol/L、10 mmol/L甲酸銨水溶液作為流動相測試，發現當使用10 mmol/L甲酸銨水溶液作為流動相時，靈敏度最好。

本實驗在QTRAP® 系統上進行，使用正負模式分別測試，發現甜菊糖甘類均有均有回應，但負模式減氫峰回應更好，故採用負模式進行分析。

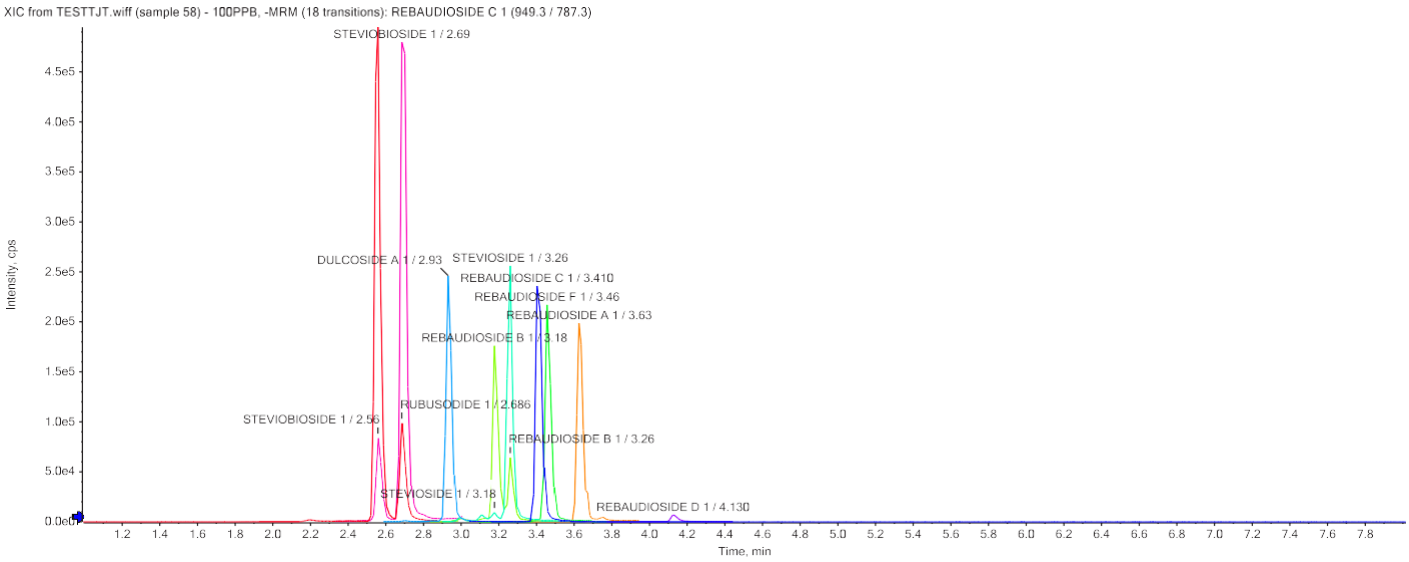


圖**2.** 9種甜菊糖苷的提取離子流色譜圖

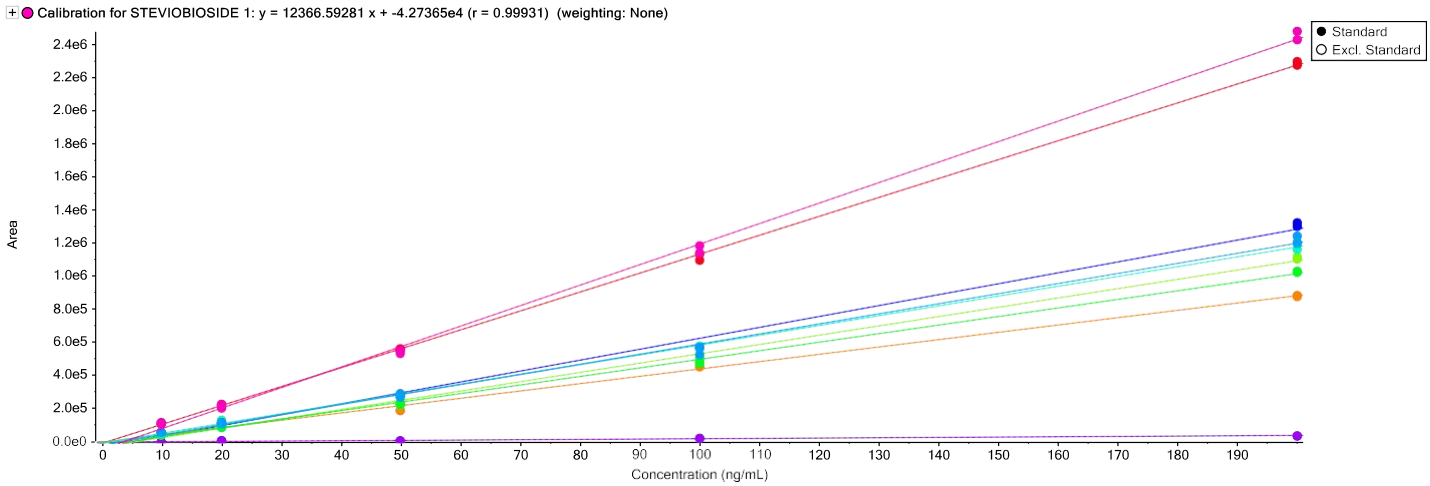


圖**3.** 9種甜菊糖苷的校準曲線

2. 方法學驗證結果

線性範圍

使用空白白酒基質，在1 ng/mL-200 ng/mL範圍內進行線性實驗，發現所有甜菊糖苷類均有很好的的線性，線性相關係數r在0.99543-0.99575之間。

3. 重現性實驗

使用空白基質考察實驗的重現性，添加濃度為5 ng/mL，連續進樣6針，所有化合物RSD值均小於3.5 %。

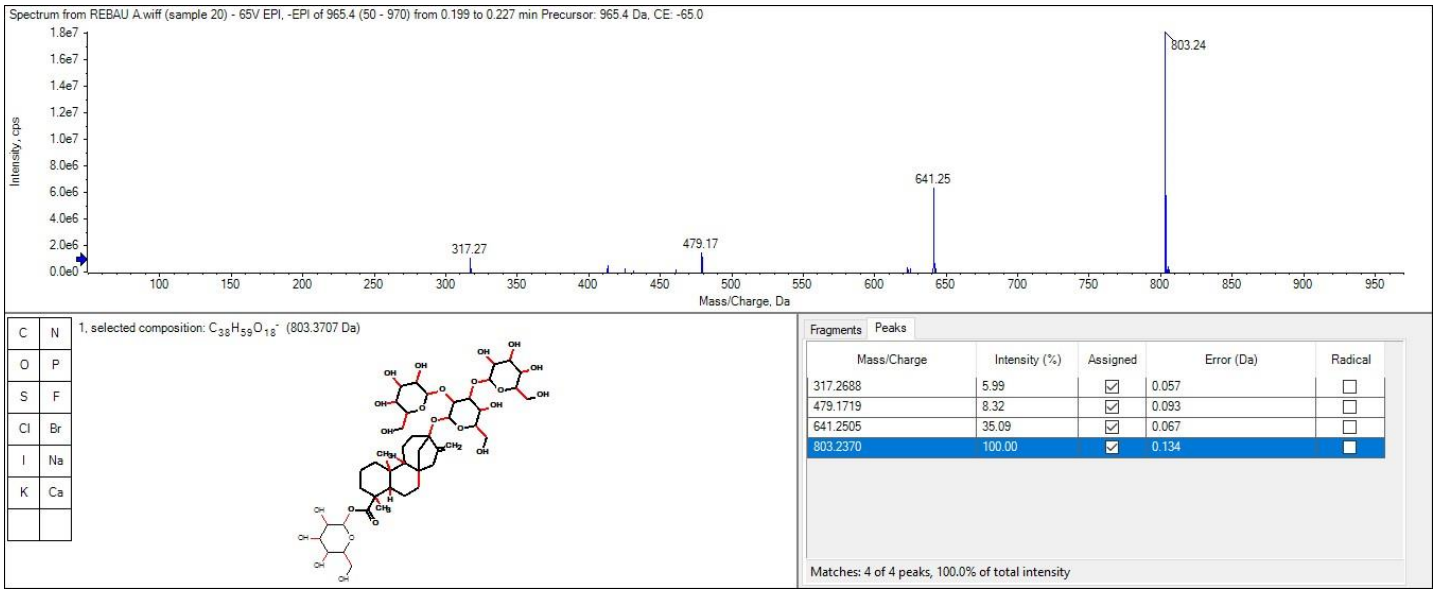
化合物名稱	RSD%（n=6）	化合物名稱	RSD%（n=6）
瑞鮑迪苷A	2.36	甜菊苷	2.51
瑞鮑迪苷B	2.18	杜克甙	2.79
瑞鮑迪苷C	1.59	甜茶苷	1.93
瑞鮑迪苷D	3.22	甜菊雙糖苷	-
瑞鮑迪苷F	2.94	-	-

4. 回收率

使用空白白酒基質進行回收率考察，取樣1 g配製成20 ng/mL的基質試劑，上機測試，各種甜菊糖苷回收率在91%-97%之間，可直接白酒類飲料中甜菊糖苷類的定量或篩查。

5. 定性分析

在QTRAP®系統上，使用MRM-IDA-EPI模式採集資料，可同時得 到用於定量的MRM資料及化合物的二級碎片資料，從而同時進行 定性及定量分析。以瑞鮑迪苷A為例，對其採集到的EPI譜圖，利 用SCIEX OS軟體進行分析，發現m/z 317.3的碎片為甜菊糖苷類均具有的苷元甜菊醇，而m/z 479.2由甜菊醇在C19位結合一個葡萄糖得到，而m/z 641.3及m/z 803.2分別為C13位上分別結合兩個和三個葡萄糖單元得到。使用MRM-IDA-EPI結合SCIEX OS軟體可準確匹配其質譜碎片資訊對應的結構，為化合物鑒定提供依據。



總結

本實驗在 QTRAP® 系統上，建立了9種甜菊糖苷類的 LC-MS/MS 方法，該方法可用于白酒類飲料中的甜菊糖苷類化合物的快速篩查和定量。方法具有快速簡便重現性好的優點，使用MRM-IDA-EPI 模式得到的二級碎片圖譜可為定性鑒定提供可靠依據，從而實現同時定量與定性分析。

參考文獻：

1. 《食品安全國家標準》食品添加物甜菊糖苷，GB 8270-2014
2. 《食品安全國家標準》食品添加物使用標準，GB 2760-2014

附錄：9種甜菊糖苷的質譜離子對參數

中文名	英文名	Q1	Q3	DP	CE
瑞鮑迪苷A	Rebaudioside A	965.4	803.4	-165	-44
			641.1	-165	-82
瑞鮑迪苷B	Rebaudioside B	803.4	641.4	-176	-65
			479.3	-176	-65
瑞鮑迪苷C	Rebaudioside C	949.323	787.3	-160	-34
			625.3	-160	-78
瑞鮑迪苷D	Rebaudioside D	1127.4	803.3	-245	-68
			641.4	-245	-86
瑞鮑迪苷F	Rebaudioside F	935.342	773.2	-155	-40
			479.3	-155	-90
甜菊苷	Stevioside	803.4	641.4	-142	-37
			479.3	-142	-80
杜克甙	Dulcoside A	787.4	625.4	-140	-32
			479.3	-140	-77
甜茶苷	Rubusoside	641.302	479.3	-135	-42
			317.2	-135	-62
甜菊雙糖苷	Steviolbioside	641.222	479.3	-175	-56
			317.2	-175	-62

SCIEX X500R QTOF SWATH®技術在白酒中7種甜味劑的篩查和定量應用

SWATH® research of High Resolution Mass Spectrometry SCIEX X500R QTOF system on confirmation and quantitative of seven sweeteners in liquor

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*SCIEX*亞太應用支持中心，上海

引言

白酒有幾千年的歷史，在白酒中加入甜味劑以增加白酒的甜味和回甜感，改善口感已成為白酒行業的潛規則。甜味劑是人工合成的非發酵物質，目前，廣泛使用的人工合成甜味劑主要包括甜蜜素、阿斯巴甜、糖精鈉、安賽蜜等。甜味劑含量超標會對肝臟和神經系統造成危害，中國國家規定各香型的白酒中不得添加甜味劑。

SCIEX X500R QTOF 高分辨質譜系統可以達到 100HZ 的快速掃描速度，結合 SCIEX 獨有的 SWATH® 技術將母離子的品質範圍分 成多個可變視窗，每個視窗內的所有離子一起碰撞碎裂，軟體強 大的去卷積功能可將母離子和子離子進行一一歸屬，從而保證得 到全部化合物的離子碎片資訊。SWATH® 技術二級碎片的靈敏度高， 可以實現同時定性和定量，因此可對白酒中的甜味劑添加一針進樣實現確證和定量。

本實驗使用 SWATH® 技術建立了白酒中甜蜜素、糖精鈉、安賽蜜、阿斯巴甜、妞甜、阿力甜和三氯蔗糖 7 種甜味劑的方法，可快速高效的監控和確證白酒中甜味劑的非法添加。

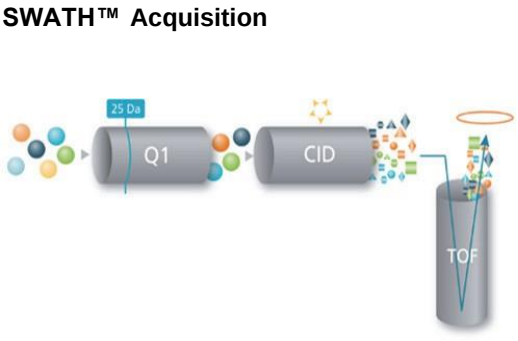


圖1. SWATH®示意圖。

實驗方法：

液相方法：

液相：SCIEX ExionLC™ AC；

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

流動相：A 相 : Mill-Q 水　B 相 : ACN

流速：0.25 mL/min；

柱溫：40 ℃；

進樣量：5 µL；

梯度洗脫程式：

Time（min）	A 相（%）	B 相（%）
1	90	10
6	10	90
8	10	90
8.1	90	10
10	90	10

質譜方法：

離子源參數如表 1：

Table 1. Ion Source Parameters. Electrospray Ionization (ESI) was conducted in Negative ion mode

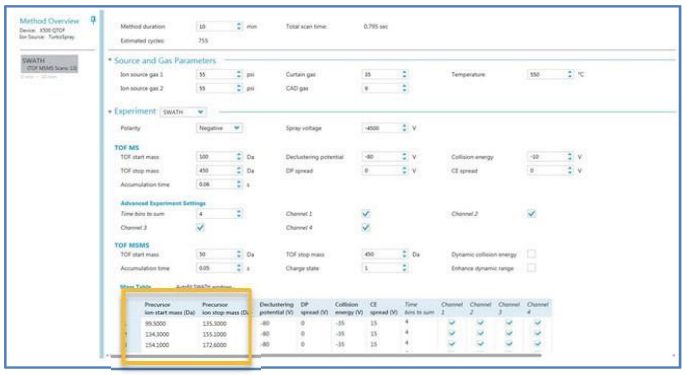
Parameter	Setting
Curtain Gas (CUR)	35
CAD gas	9
Spray voltage (IS)	-4500
Temperature (TEM)	550
Ion source gas 1	55
Ion source gas 2	55
Declustering potential（DP）	-80
Collision energy (CE)	-35
CE Spread	15

SWATH®方法編輯：

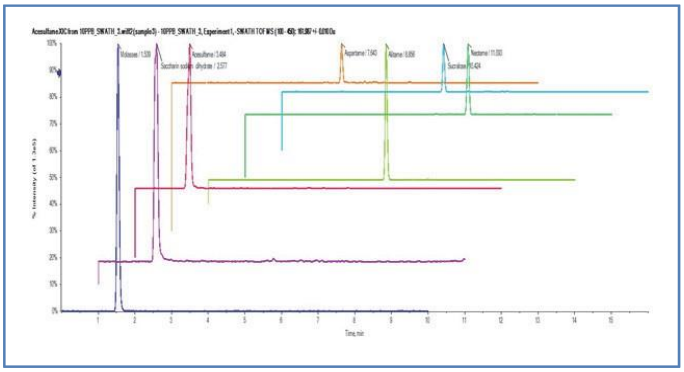
TOFMS: 100-450Da

TOFMSMS: 50-450Da

10 個 SWATH® 窗口

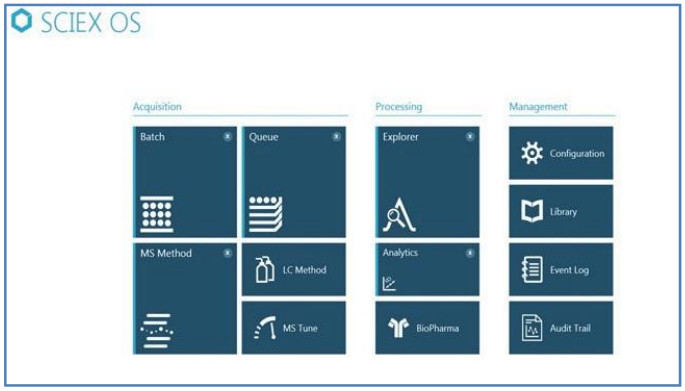


使用 SWATH® 方法採集資料，分別採集一系列標準品和測試樣品 1 和 2 的資料。7 種甜味劑保留時間和色譜峰型如下圖：

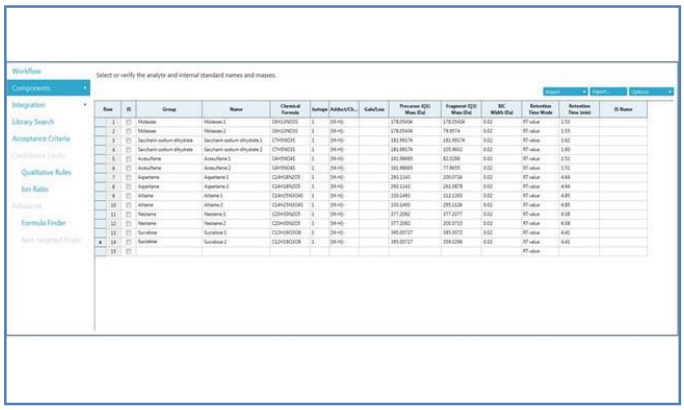


資料分析：

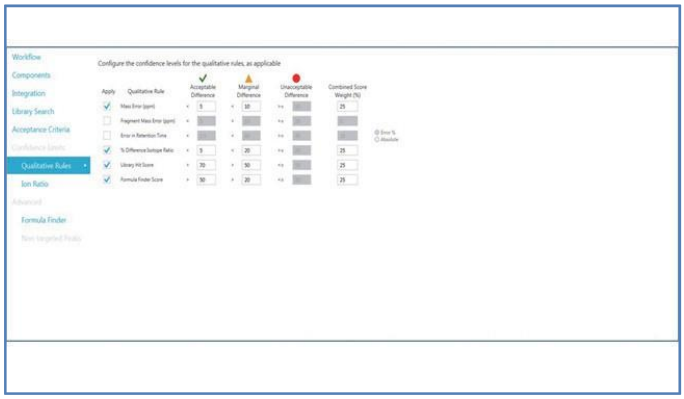
使用 SCIEX OS 軟體進行資料處理，智慧化的 SCIEX OS 套裝軟體含了方法編輯、資料獲取、資料查看和資料處理所有相關工具，介面簡單易學。



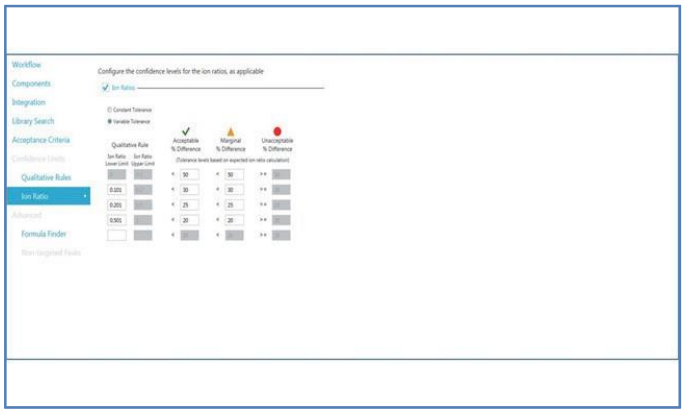
1. 導入7種甜味劑的名字和分子式，分組；設置每種甜味劑的定性和定量離子通道。



2. 設置篩查確證置信條件，根據一級品質準確度、同位素豐度比、二級譜庫確認和分子式，“四大關”鎖定化合物。信號指示燈指示判定結果，結果確證一目了然。

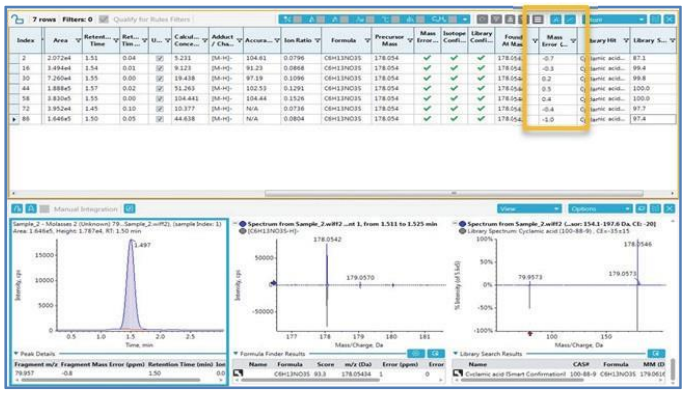


3. 設置MRM Ratio離子比率，SCIEX OS軟體具有符合歐盟規定的MRM Ratio離子比率編輯模組，可以設置不同離子比率的相對偏差，更加先進。

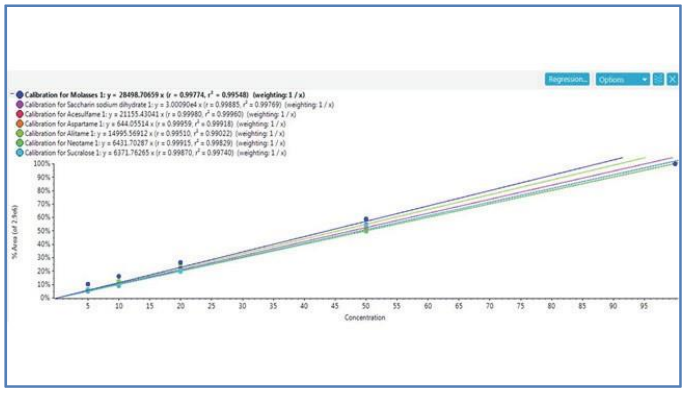


實驗結果：

SCEIX X500R 高分辨質譜獨特的 N 型離子路徑技術，確保了 TOF 管離子傳輸準確穩定，質核比漂移小，7 種甜味劑的品質準確度都在 1 ppm 以內。

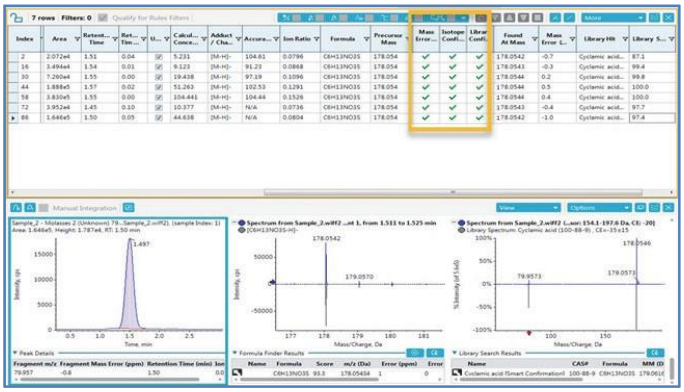


SWATH® 採集方法一針進樣，定性確證同時定量，7 種甜味劑 5 ng/mL-100 ng/mL 濃度範圍內的線性均能達到 0.99。



根據一級準確質量數、同位素比、二級碎片和離子比率，層層確證，確證樣品 1 和樣品 2 種檢測出甜味劑，含量分別為：

名稱	樣品 1	樣品 2
甜蜜素（ng/mL）	10.3	44.6



總結：

本實驗使用SCIEX X500R QTOF 建立了白酒中 7 種甜味劑的測試方法，使用 SCIEX 獨有的 SWATH® 技術，一針樣品 10 min。高靈敏度的 SWATH® 二級碎片即可完成化合物的確證，同時也可完成定量和計算離子比率，對於白酒中甜味劑的篩查確證非常方便。

參考文獻

1. SN/T 2360.20-2009中華人民共和國出入境檢驗檢疫行業標準。
2. SN/T 3538-2013中華人民共和國出入境檢驗檢疫行業標準。

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RUO-MKT-02-8439-ZH-A

Development of a targeted LC-MS/MS method for the detection of microbial Transglutaminase from *Streptomyces mobaraensis*

Peptide quantification using a QTRAP® 6500+ LC-MC/MS System coupled to an ExionLC™ System

Rebekah Sayers and Jianru Stahl-Zeng
SCIEX, UK and SCIEX, Germany

The so-called “meat glue enzyme” transglutaminase is used by the meat industry to add value to meat by gluing together smaller scraps into a larger piece. Transglutaminase can be used to cross-link smaller pieces of any type of meat, fish, or meat product, to produce large, virtually intact sections. This has raised several food safety concerns. Specifically, the transglutaminase enzyme is the target antigen of antibodies found in the serum of patients suffering from coeliac disease. A rapid and robust analytical method is necessary to ensure the protein content is correctly determined and consumers can be confident in the origins of their food.

The detected enzyme, microbial transglutaminase (MTG) from *Streptomyces mobaraensis*, consists of 331 amino acids. The following tryptic peptides are characteristic markers of MTG: VTPPAEPLDR (TG1), SPFYSALR (TG2), LAFASFDEDR (TG3) and GAYVITFIPK (TG4).[1,2] The detection of these marker peptides was carried out after chromatographic separation by HPLC using a RP-C18 column and subsequent detection using MS/MS. In each case three MRM transitions were measured for each marker peptide. Peptide detection was confirmed if all three mass transitions had a signal-to-noise ratio of at least 3:1. The detection of MTG protein would then be considered positive if at least three of the four peptide markers were detected.

A rapid and robust analytical method is required to ensure the protein content is correctly determined and consumers can be confident in the origin of their food. In this work, an improved analytical LC-MS/MS method is presented which utilizes multiple reaction monitoring (MRM) to detect 4 target peptides for microbial transglutaminase from *Streptomyces mobaraensis* (Figure 1).

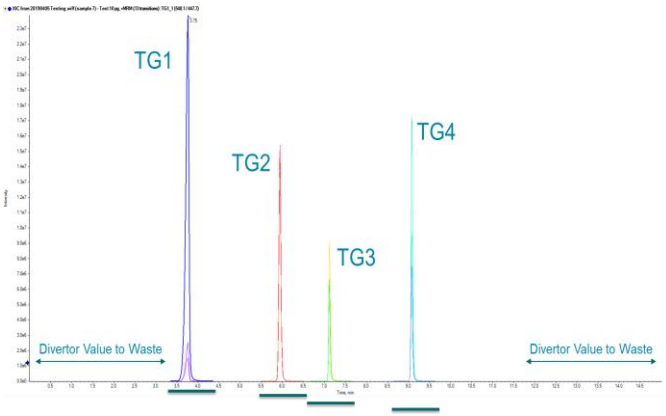

Figure 1. Chromatographic Separation of Peptides. Extracted Ion Chromatograms (XICs) for the three MRM transitions for each of the four peptides from transglutaminase by LC-MS/MS.

Key features of the targeted LC-MRM method

- With an increasingly health conscious consumer base, the demand to test for adulteration and authenticity of food products is growing
- An existing method was optimized to a more commercially viable one. This was aided by using DiscoveryQuant™ Software for automated optimization of method parameters and the *Scheduled* MRM™ Algorithm for time scheduled MRM transitions to maximize dwell times.
- The reproducibility and robustness of the resulting measurements was confirmed with a %CV < 6.55
- The assay showed sensitivity levels in pg/µL.

Method

Sample Preparation: Peptide standards synthesized by JPT peptide technologies were reconstituted in 0.1% formic acid in 90% water 10% acetonitrile to a stock concentration of 5 mg/mL. The stock was then diluted in chromatographic buffer A to prepare a calibration series in the range 0.01-100 pg/µL.

Chromatography: An ExionLC™ system with a Phenomenex Aeris peptide 1.7 µ XB C-18 150 mm column at 40 °C with a gradient of 2 to 70% acetonitrile over 10 min in 0.1% formic acid was used at a flow rate of 300 µL/min. The injection volume was set to 2 µL. Total run time was 15 minutes.

Mass Spectrometry: A SCIEX QTRAP® 6500+ LC-MS/MS System operated in low mass mode equipped with Turbo V™ Ion Source was used. Analyst® Software 1.7.1 was used for data acquisition. The enzyme was detected initially using 3 MRM transitions per peptide to allow quantification and identification based on the ratio of quantifier and qualifier transitions as defined by regulation 2002/657/EC. Every sample was analyzed in triplicate.

Data Processing: Samples were analyzed in triplicate and data processed using SCIEX OS-MQ Software to perform quantification and statistical analysis. Peak picking and integration were performed using the AutoPeak integration algorithm.

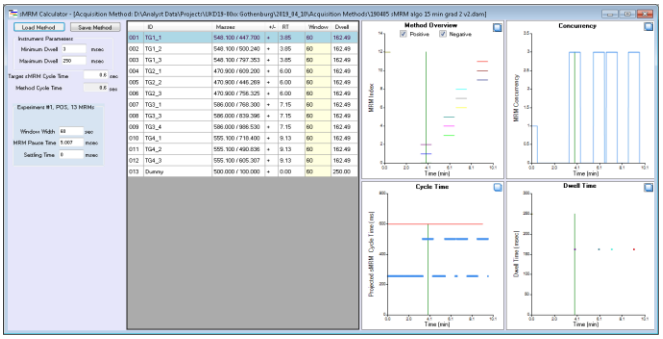

Figure 2. sMRM calculator for easy time scheduling MRM transitions. User inputs the peptide retention time, the target cycle time for the method and the desired MRM detection window width and the tool computes the concurrency and probable dwell times that will be used in the method. This allows the user to optimize the inputs before building the final method with *Scheduled* MRM Algorithm.

Optimization of the targeted peptide method

First, the MRM transitions for each of the target peptides were optimized using DiscoveryQuant Software using peptide standards. DiscoveryQuant Software significantly increases productivity by simplifying and expediting LC–MS method development cycle time for sensitive and selective assays, without compromising data integrity. The three most intense MRMs for each peptide were selected and the compound dependent parameters (CE and DP) were optimized.

Next using the optimized chromatography (Figure 1), the retention times for each peptide were determined such that MRM acquisition could be scheduled in time using the Scheduled MRM Algorithm (Figure 2). The Scheduled MRM Algorithm detects MRM transitions during a short retention time window only, this allows many more MRM transitions to be monitored in a single LC run, while still maintaining maximized dwell time and optimized cycle time for best accuracy and reproducibility.[3]

To build a method using the Scheduled MRM Algorithm, the sMRM calculator tool in Analyst Software can be used for optimizing inputs. The user inputs the MRM retention times, the MRM detection window, and the targeted scan time and the tool computes the projected concurrency and dwell times. This then allows the user to adjust the method inputs for best results before building the final method with the *Scheduled* MRM algorithm.

This two-step process produced a fully optimized LC-MS/MS method for the quantification of transglutaminase peptides from *Streptomyces mobaraensis*.

Determination of assay sensitivities

Calibration curves were constructed from a standard mix of the peptide markers (TG1-4) spiked into chromatographic buffer A to give final concentrations of 0.01-100 pg/µL. Assay sensitivities were determined using a minimum of two transitions per peptide with a signal-to-noise ratio >3:1. Calibration plots for quantifier and qualifier marker peptides are shown in Figure 3. Assay sensitivities, accuracies and %CV are shown in Table 1. Good performance of the peptides was observed, next steps will be to test in matrix to determine sensitivity and specificity in matrix.

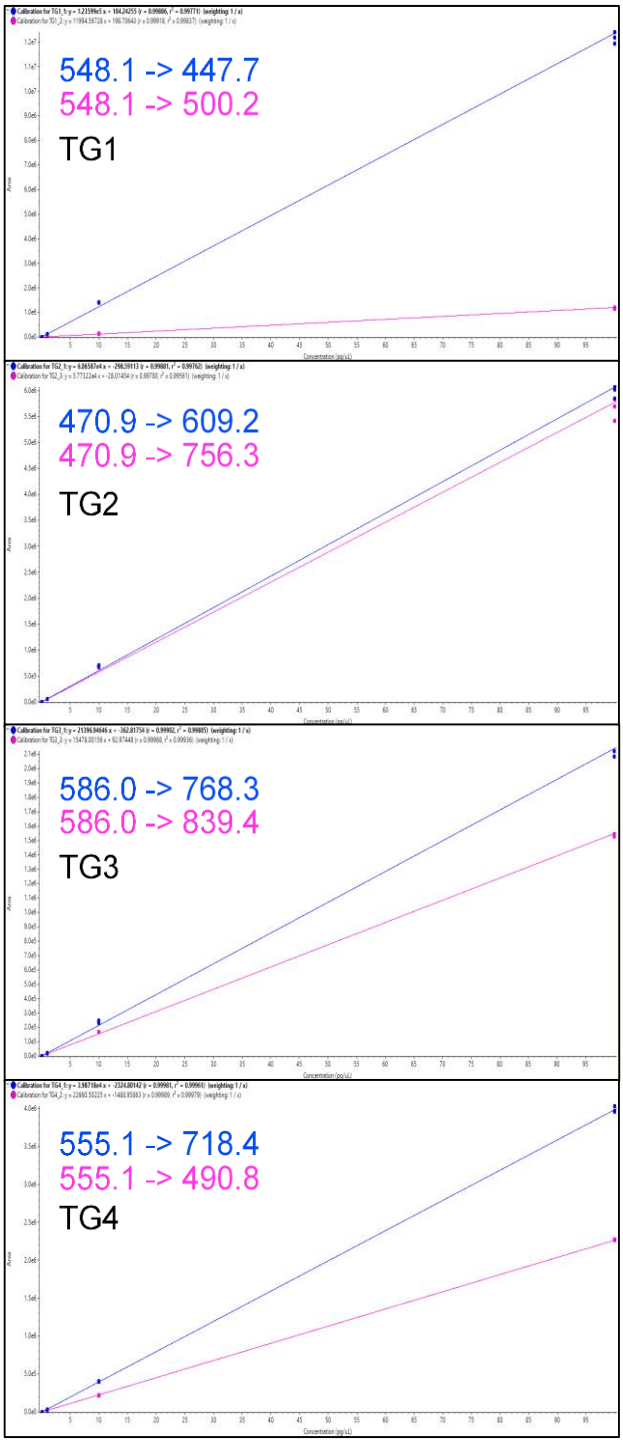

Figure 3. Calibration plots for the four marker peptides. Here the calibration curves from 0.01-100 pg/µL are shown for the quantifier (blue) and the qualifier (pink), with a 1/x weighting and R2 > 0.99.

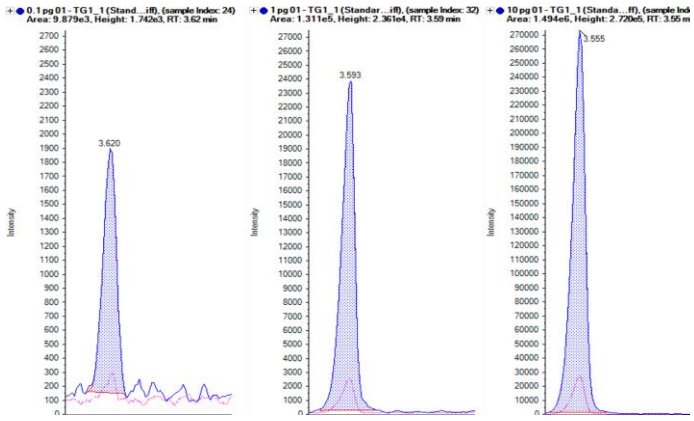

Figure 4. Example MRM transitions. Extracted ion chromatograms (XICs) of low, middle and high concentrations, quantifier and qualifier for TG1 peptide.

Conclusions

This work highlights a streamlined workflow for initial development of a targeted peptide method. The resulting analytical method provided reproduceable measurement of peptide targets found in MTG from Streptomyces mobaraensis in simple matrix. Multiple reaction monitoring (MRM) was used because of its high selectivity and sensitivity.

- The method parameters were first optimized using DiscoveryQuant Software from peptide standards
- Then, retention times were determined such that *Scheduled* MRM Algorithm could for best sensitivity and reproducibility
- The total HPLC run time was 15 minutes for higher throughput analysis
- Multiple peptides for the protein were monitored and two transitions per peptide are used for quantification
  - Peptide detection was confirmed if both mass transitions had a signal-to-noise ratio of at least 3:1
- Preliminary sensitivity of the MRM method was confirmed for the measurement of MTG in simple matrix using a dilution series of peptide standards, and good linearity from 0.01-100 pg/µL
- Next steps are to test this method in meat products to determine whether assay meets the required sensitivity limits

Table 1. Quantifier and Qualifier marker peptide transitions and assay sensitivities. The lower limits of detection (LLOD S/N > 3:1) and quantification (LLOQ S/N > 10:1) are shown for the calibration curves run in buffer. The reproducibility at both the LLOD and the LLOQ are reported as mean % CV. All assay sensitivities are shown in pg/µL.

Marker peptide	Analyte type	Transition	LLOD	LLOQ	Linear range	R2	%CV at LLOD	% CV at LLOQ
TG1-1	*Quantifier*	*548.1 / 447.7*	*0.01*	*0.1*	*0.01-100*	*0.99*	*16.6*	*3.7*
TG1-2	*Qualifier*	*548.1 / 500.2*	*0.1*	*0.1*	*0.1-100*	*0.99*	*8.5*	*8.5*
TG2-1	*Quantifier*	*470.9 / 609.2*	*0.01*	*0.1*	*0.01-100*	*0.99*	*12.6*	*6.6*
TG2-2	*Qualifier*	*470.9 / 756.3*	*0.01*	*0.1*	*0.1-100*	*0.99*	*8.8*	*7.3*
TG3-1	*Quantifier*	*586.0 / 768.3*	*0.1*	*0.1*	*0.1-100*	*0.99*	*5*	*5*
TG3-2	*Qualifier*	*586.0 / 839.4*	*0.1*	*0.1*	*0.1-100*	*0.99*	*10.7*	*5.7*
TG4-1	*Quantifier*	*555.1 / 718.4*	*0.1*	*0.1*	*0.1-100*	*0.99*	*2.9*	*2.9*
TG4-2	*Qualifier*	*555.1 / 490.8*	*0.1*	*0.1*	*0.1-100*	*0.99*	*12.2*	*12.2*

References

1. Jira, Wolfgang, and Fredi Schwägele. "A sensitive high performance liquid chromatography–tandem mass spectrometry method for the detection of microbial transglutaminase in different types of restructured meat." *Food chemistry* (2017) **221**, 1970-1978.
2. Jira, Wolfgang, and Siegfried Münch. "A sensitive HPLC-MS/MS screening method for the simultaneous detection of barley, maize, oats, rice, rye and wheat proteins in meat products." *Food chemistry* (2019) **275**, 214-223.
3. The *Scheduled* MRM™ Algorithm Pro - Automated Intelligent Design of High Throughput, High Quality Quantitation Assays. SCIEX Technical Note RUO-MKT-02-8539-A.

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SWATH® Acquisition LC-QTOF-MS/MS analysis of food colors and illegal dyes in spices

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[1]*Official Food Control Authority of the Canton de Vaud, Epalinges, Switzerland*
[2]*SCIEX, Baden, Switzerland*

Overview

In this study, a sensitive, robust, and fast method based on SWATH® Acquisition and data analysis was developed to determine and identify ninety-eight food colors and dyes in spices. High resolution MS and MS/MS data were collected using a SCIEX X500R QTOF System in both positive and negative modes in order to take into account the differences in ionization between the lipophilic illegal dyes and the hydrophilic artificial colors.

Introduction

Natural or artificial colors are added to many foods to enhance their attractiveness and compensate for either alterations or losses that could occur during processing or storage. Due to their low cost, effectiveness and excellent stability, artificial colors are usually preferred by the food industry over natural ones [1, 2]. Sudan dyes are a class of lipophilic azo dyes that are widely used for different industrial and scientific applications (coloring of fuels, waxes or oil, staining for microscopy, etc.) because of their colorfastness and low price. Since they are cheap and widely available, Sudan dyes are also attractive as food dyes as they can improve their appearance. However, due to the carcinogenicity of their metabolites, they are banned for food usage in most countries, including in the EU. Nevertheless, over the last years, these dyes have been found in various foodstuffs whether spices, tomato sauces or else [3]. In the case of spices, the Swiss legislation does not allow for the addition of food colours except for quinoline yellow (E104) that can be added to curry and tandoori preparations.

A suitable screening analytical method, amenable for both the lipophilic Sudan-type illegal dyes and the hydrophilic artificial ones, is required for their fast detection and identification. The SWATH Acquisition mode for MS/MS collection used by the X500R QTOF System allows for the simultaneous targeted and non-targeted screening of samples. The exact mass and MS/MS data provided produces sufficient data to confidently identify the analytes of interest but also identify unknown chemicals that may also be present in the sample.

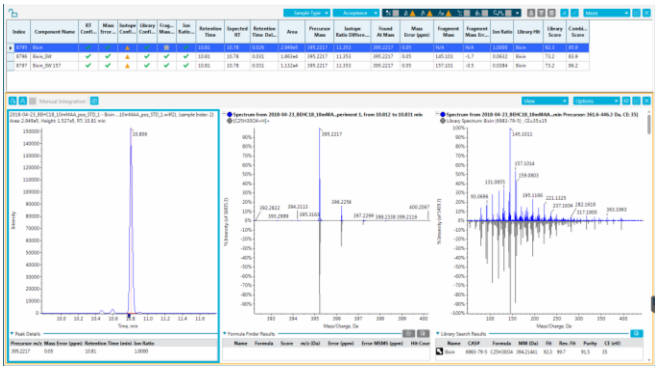


Figure 1. Results for a couscous sample adulterated with bixin (precursor mass, isotope distribution and MS/MS spectra).

Key advantages of SWATH Acquisition for dyes analysis

- Ensure collection of MS/MS data for every precursor in a complex spice sample, without missing any precursors due to low-level residue
- Confidently identify target dyes, food colours of importance based on collected MS/MS information and known formulae
- Create a custom spectral library using standards that can be employed to identify unknown component peaks in a sample
- Achieve extremely low false positive and false negative reporting rates using accurate mass information attained by the QTOF high resolution accurate mass platform

Methods

Samples: Approximately 1 g of spice was weighed and extracted for 30 minutes with a quaternary solvent mixture of water/methanol/acetonitrile/tetrahydrofuran (9:1:5:5 v/v/v/v).

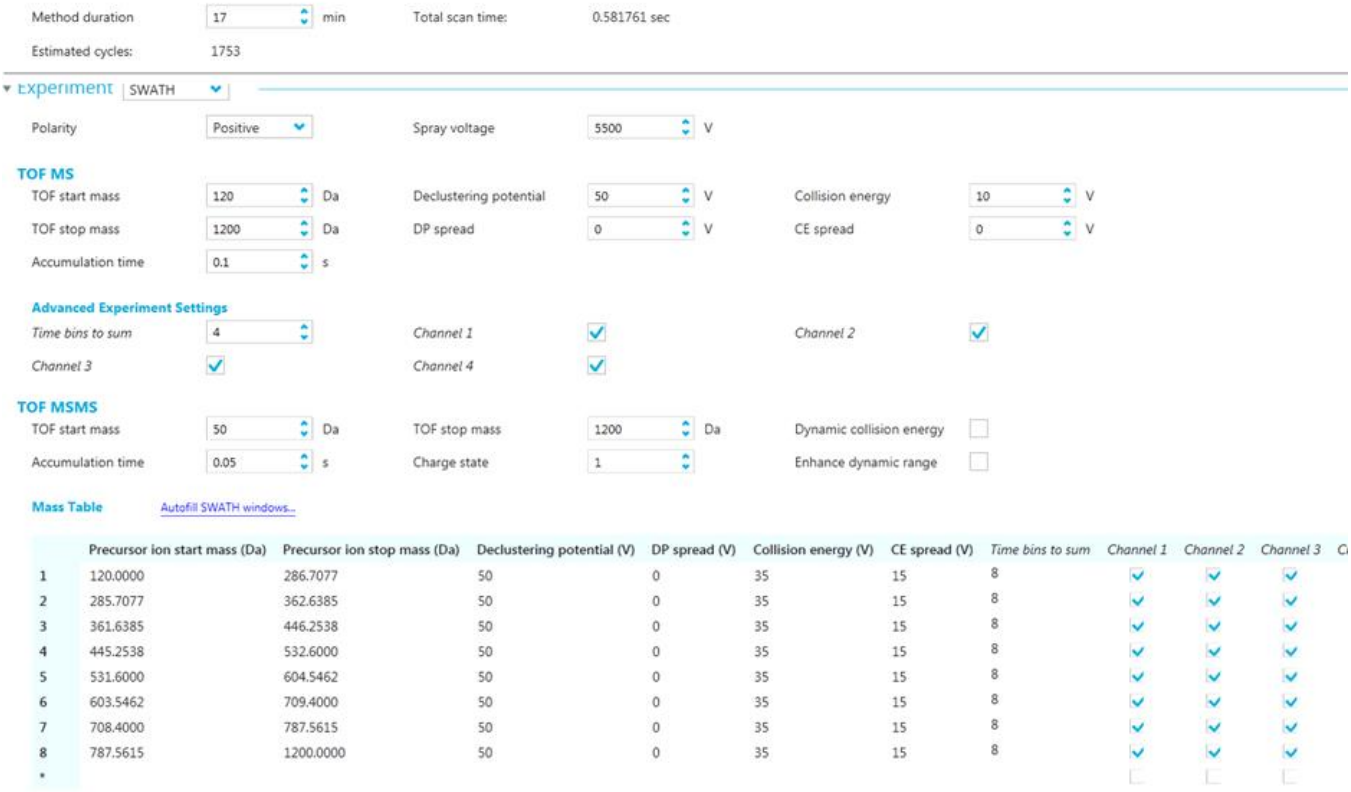
The solution was centrifuged for 5 minutes at 2500 rpm and an aliquot of the supernatant was then filtered using a 0.2 µm PTFE filter into an amber LC-vial containing three internal standards (Sudan I-d5, Sudan III-d6, Congo Red-d8).

LC separation: 2 µL of the spice extracts were injected onto an ExionLC™ AD System coupled to an X500R QTOF System equipped with the Twin Sprayer probes. Separation was performed using a gradient on a Waters BEH UPLC column (1.7 µm, 2.1 x 100 mm) using a mobile phase consisting of an ammonium acetate 10mM buffer (A) with methanol (B) at a flow-rate of 0.5 mL/min and column temperature of 50 °C. Gradient conditions are listed in Table 1.

Table 1: Gradient conditions used for the LC separation and subsequent identification of the target dyes.

Step	Time (min)	A (%)	B (%)
0	0.0	98	2
1	1.0	98	2
2	11.0	5	95
3	13.0	1	99
4	13.5	1	99
5	13.6	98	2
6	17.0	98	2

SWATH Acquisition method: Analyses were performed using the Turbo V™ Source in both negative and positive modes. The source temperature was set at 500 °C, the ion source gases 1 and 2 at 45 [AU], the curtain gas at 35 and the CAD gas at 7 [AU]. For the positive mode, the spray voltage was set at 5.5 kV and for the negative mode at -4.5 kV.



	Precursor ion start mass (Da)	Precursor ion stop mass (Da)	Declustering potential (V)	DP spread (V)	Collision energy (V)	CE spread (V)	Time bins to sum	Channel 1	Channel 2	Channel 3
1	120.0000	286.7077	50	0	35	15	8	✓	✓	✓
2	285.7077	362.6385	50	0	35	15	8	✓	✓	✓
3	361.6385	446.2538	50	0	35	15	8	✓	✓	✓
4	445.2538	532.6000	50	0	35	15	8	✓	✓	✓
5	531.6000	604.5462	50	0	35	15	8	✓	✓	✓
6	603.5462	709.4000	50	0	35	15	8	✓	✓	✓
7	708.4000	787.5615	50	0	35	15	8	✓	✓	✓
8	787.5615	1200.0000	50	0	35	15	8	✓	✓	✓
*										

Figure 2. MS method setup in SCIEX OS Software. The SWATH Acquisition is defined by the precursor mass windows and the MS/MS acquisition parameters.

The TOF MS survey scan was performed from 120 to 1200 Da using these parameters for the positive mode: the declustering potential was set at 50, the accumulation time at 0.1 second and the collision energy at 10 V. For the negative mode, the declustering potential was set at -80, the accumulation time at 0.1 second and the collision energy at -10 V.

Analytes were detected by SWATH Acquisition using eight windows according to table presented in Figure 2. The following parameters were used in positive mode: the declustering potential was set at 50, the collision energy at 35 V with a spread of 15 V. For the negative mode, the declustering potential was set at -80 and the collision energy at -35 V with a spread of 15 V. The accumulation time for both modes was 0.05 seconds. The variable SWATH Acquisition windows were optimized by evaluating the ion density over the whole chromatographic range of twelve different spices (paprika, curcuma, sweet paprika, hot chili) and spice blends (curry, satay, tandoori, garam masala, couscous ras-el-hanout, Cajun and a “seven spices” mix).

Library: In order to create the in-house library, and since most dyes had purity levels below 95%, they were injected using the LC method described above in IDA mode. This had the advantage of ensuring that the MS spectra were of the highest purity available. The MS/MS libraries were built at six different energy collisions: 20, 30, 40, 50 V and 35 +/-15 V and 40 +/-20 V. In the case of compounds that could be ionised in both positive and negative modes, both were added to the library.

Data processing: Data was processed using the SCIEX OS Software. The set-up of the peak finding criteria was done using Analytics. The criteria for the traffic lights which allows for data review and filtering can be seen in Figure 3.

Results - validation

Forty-one compounds were selected for the validation based on their color and their relevance to the study (mostly Sudan-type dyes and artificial ones). Two spices were used for validation: ground paprika and curry. Both extracts were spiked in a manner that they contained between one and thirty-two compounds. In total, forty vials were thus prepared with each analyte added randomly in twenty of them.

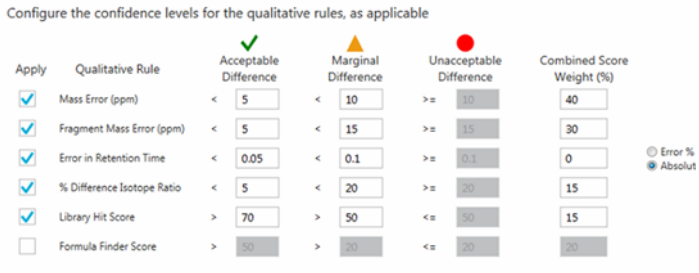


Figure 3. Qualitative rules applied for data processing. These are user-defined means of flagging results as confident matches within a set of acceptable tolerance limits for multiple parameters.

As established for screening methods, selectivity and specificity as well as the false positive and false negative rates were determined with these solutions. The vials were injected onto the LC-MS using, throughout the sequence, the integrated Calibrant Delivery System (CDS) with the Twin Sprayer probe to maintain the mass accuracy.

After reprocessing the false positive rate was determined as 0% for all compounds whereas the false negative rate was 0% except for Amaranth (E123) and Reactive Red 195 with rates of 10% and 5%, respectively. These results highlight the excellent identification capabilities of the instrument. The mass error of the precursor ion did not exceed +/- 2 ppm for 81% of measurements (out of 494 in total) in the negative mode and 63% in the positive mode (out of 646). Only 2% of the negative mode measurements (3% in the positive mode) were comprised between +/- 5 and +/- 10 ppm and none were above +/- 10ppm.

Intra-day repeatability was assessed using both curry and paprika extracts and a representative subset of compounds. It was determined by injecting ten times the same vial in each mode successively. The parameters monitored were the retention time (RT), the raw area, the mass error and the false negative rate at the detection level. The coefficients of variation (CV) of the RT were in average below 1% for all compounds, except for Tartrazine and Acid Yellow which were nonetheless lower than 5%. In terms of false negative results, Para Red did not meet the criteria for detection (n=10) in one instance as the mass error was higher than 5 ppm at -6.4 ppm in the negative mode. Similar results were obtained with the paprika extract.

Table 2: Intraday repeatability and summary of the results obtained in terms of raw area and number of false negatives for the curry extract.

Compound	Area (average)	CV (%)	Number of false negatives
Acid Yellow 9 (pos)	2640	21%	0
Erythrosine (neg)	9729	4%	0
Erythrosine (pos)	1004	9%	0
Para red (neg)	9248	7%	0
Para red (pos)	3120	11%	1
Ponceau 6R (neg)	34761	2%	0
Ponceau 6R (pos)	30864	4%	0
Sudan IV (pos)	18413	18%	0
Sunset Yellow (neg)	1535	7%	0
Sunset Yellow (pos)	547	10%	0
Tartrazine (neg)	1995	8%	0

Results with samples

More than 80 spice samples were purchased by local authorities from various markets and supermarkets. The traffic lights system was used to filter the data for a quick and efficient review (see Figure 3).

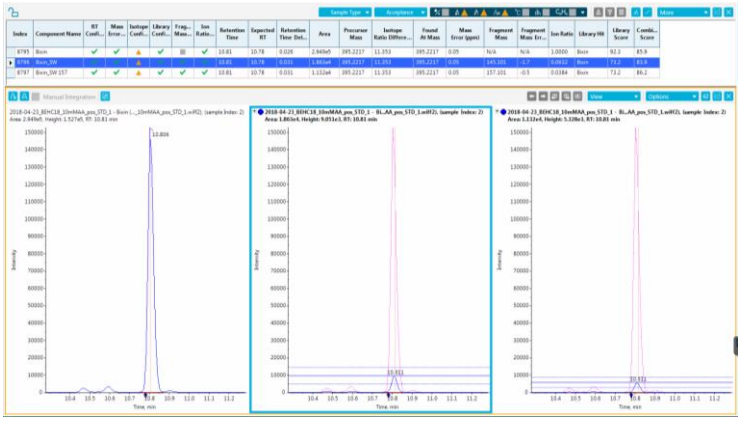


Figure 4. Ion ratios obtained for bixin with two SWATH Acquisition fragments.

Conclusions

A new screening analytical method was developed to detect and identify both lipophilic Sudan-type illegal dyes and the hydrophilic artificial ones in spices. A simple solvent extraction was used and a common LC method was optimized for the analysis of 98 dyes that were added to the custom library. Forty-one colours and dyes were selected for the validation. A high degree of mass accuracy was obtained with the X500R LC-QTOF System at sufficient mass resolution regardless of the matrix type. The screening method was applied to spice samples. Sudan IV, an illegal dye, was identified with a high confidence level in a paprika sample whereas a natural food colour, bixin, was detected in a couscous spice blend. The concurrent SWATH Acquisition and TOF-MS data provided excellent means of identification thanks to the accurate mass of the fragments and their ion ratios in addition to the accurate mass of the precursor ion and its' isotopic distribution. Furthermore, this type of acquisition would also allow for the retrospective analysis of suspect samples should a new "emerging" dye appear.

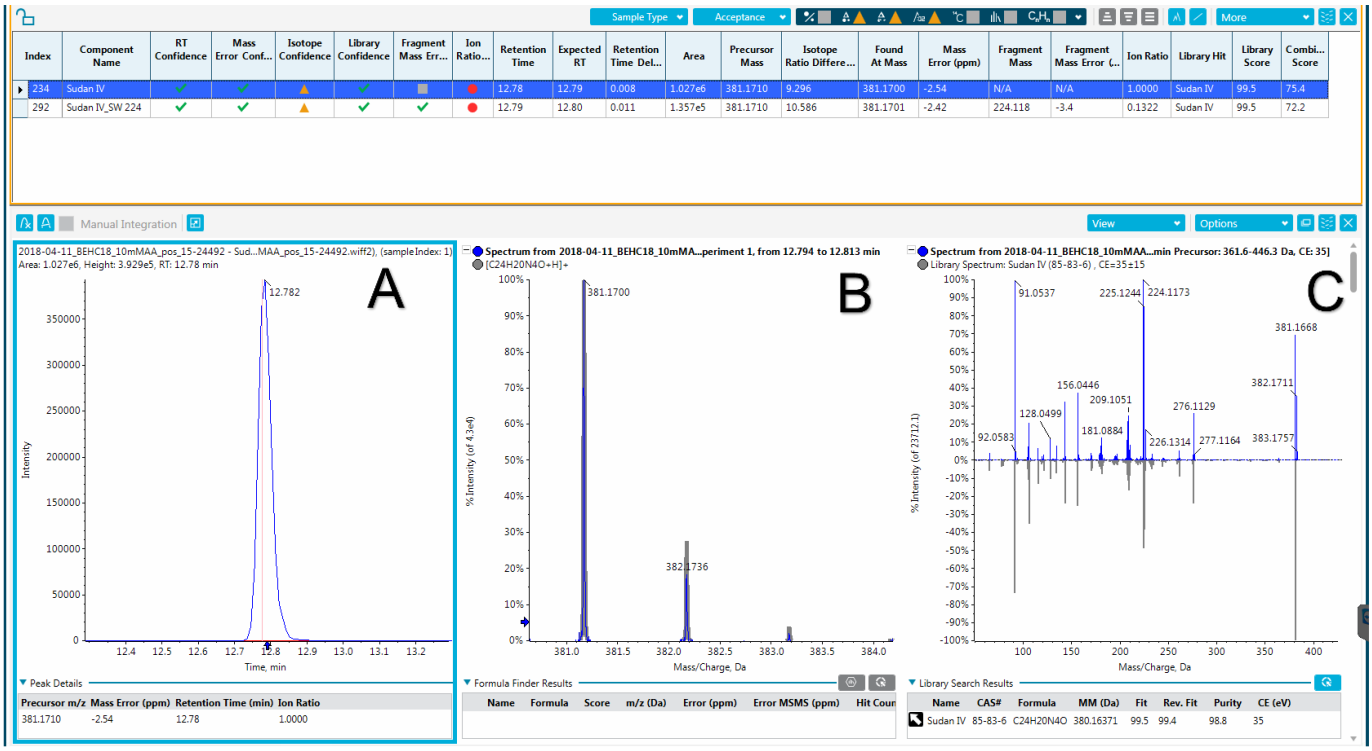


Figure 5: Qualitative results for a sample contaminated by Sudan IV. A) Chromatographic peak showing precursor and retention time. B) High resolution MS data including matching the observed isotope pattern to that of the target analyte. C) Matching of the MS/MS spectrum collected by SWATH Acquisition to that of the target analyte in the spectral database.

References

1. Oplatowska-Stachowiak M, Elliot C., (2017) Food colors: Existing and emerging food safety concerns. *Critical reviews in Food Science and Nutrition*, **57** (3), 524-548
2. Colours Additives for Foods and Beverages, Ed. by M. J. Scotter, Woodhead Publishing, 2015
3. Review the toxicology of a number of dyes illegally present in food in the EU, (2005) *The EFSA Journal,* **263**, 1-71

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Total Solution of QTRAP® Mass Spectrometry on Screening of Additives in 68 Common Chinese Health Products

Total solution of QTRAP on drug screening of health products

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SCIEX, Asia Pacific Application Support Center, Shanghai, China

Introduction

With the increase in living standards, so has the health products market. With fierce market competition, in an effort to provide quick results to consumers, some manufacturers have added drugs to health products in order to accelerate results. For instance, additives like sibutramine and phenolphthalein are found in many weight loss products. Adulterated health products may provide consumers with quick results, but they may also encounter side effects such as drug dependence, liver damage, and tachycardia. The China National Food Safety Supervision and Sampling Implementation Guidelines (2017 version) have established monitoring parameters for health products. This guide covers monitoring of 68 drugs in 6 different categories of health products.

In traditional mass spectrometry, MRM mode often produces matrix effects in practice due to matrix complexity. This leads to retention time and ion ratio discrepancies and causes “false peaks” and “false positives” that interfere with determinations. To overcome the challenges of MRM mode in traditional mass spectrometry, the SCIEX complex mass spectrometry QTRAP system uses a uniquely integrated MRM-IDA-EPI scanning mode. This provides MRM spectral peaks and enhanced secondary fragments with just one sample injection. MRM ion channel chromatographic peak quantification and EPI different energy complex secondary spectra form a “fingerprint” spectrum. The library is searched and verified, effectively overcoming the traditional mass spectrometry challenges and increasing the accuracy of testing and analysis work.

The following method was developed on the QTRAP platform to detect the 68 health products in the 2017 National Food Safety Supervision and Sampling Implementation Guidelines. It also includes a secondary library to help users search, identify, and enhance the efficiency of monitoring and analysis. This monitoring protocol includes these advantages:

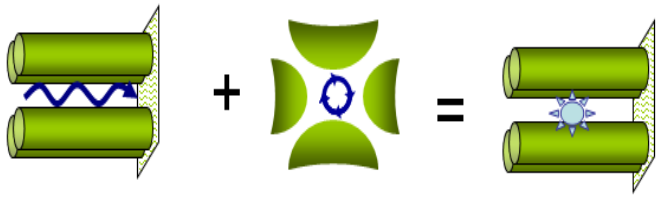
1. Covering all drug types, this method includes all drug additives to health products that must be detected per the 2017 National Food Safety Supervision and Sampling Implementation Guidelines.
2. With one sample injection and simultaneous positive and negative mode scanning, it is quick and easy.
3. This method includes sample preprocessing, MRM ion pair data, instrumentation methods, and secondary search libraries. The QTRAP comprehensive solution includes the advantage of simultaneous quantitative and qualitative validation with a single sample injection.
4. The QTRAP comprehensive solution satisfies multiple user needs; in use, it improves work efficiency and saves time.
5. Secondary search databases have high, medium, low, and combined energy fragment spectra. They contain a large amount of fragment information and effectively exclude false positives.

Experimental process

1. Integrated mass spectrometry with QTRAP involves one sample injection for simultaneous quantification and qualification. When the “fingerprint” of an “illegal additive” is detected, one can also obtain information on the content, as well as quantitative and qualitative validation data. This provides a novel workflow for analysis work.

Complex mass spectrometry with QTRAP® involves one sample injection for simultaneous quantification and qualification.

QTRAP series



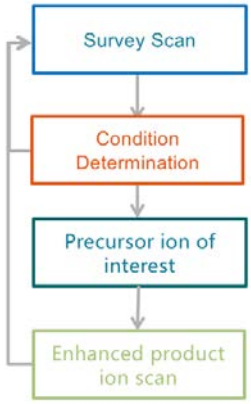
Triple Quadrupole
Satisfies the international legal requirement for Two pair MRM and MRM Ratio

Ion Trap
Can store ions, increase ion amounts
The enhanced spectrum satisfies the need for validation

QTRAP
Simultaneous qualification and quantification
Exclude false positives

2. QTRAP's unique MRM-IDA-EPI workflow is the solution required to identify drugs.

MRM triggered IDA principle



Procedure 1: Survey scan

Procedure 2: System automated determination: Intensity of survey scan signals exceeds expected values ("apparent chromatographic peaks")

Procedure 3: When Procedure 2 conditions are met, the system automatically and rapidly (<1 ms) switches to linear ion trap mode and performs Enhanced Product Ion (EPI) scanning to create high quality parent ion MS^2 spectra from survey scans. Return to Procedure 1.

3. Using library search, with positive and reverse matching, determine the overall degree of matching. Efficiently eliminate false positive results.

Library search flowchart

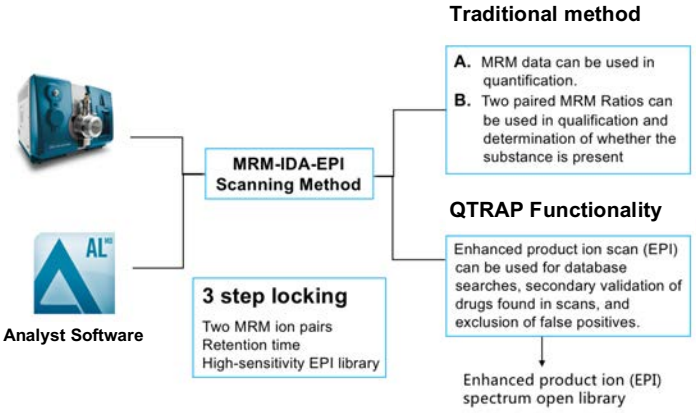
The ion pair of the substance to be measured is extracted from the MRM general spectrum to determine if the sample contains the substance.

If peaks occur in this substance, double-click the EPI spectrum of the corresponding retention time to obtain an enhanced secondary spectrum (EPI).

Enhanced (EPI) secondary spectra, right-click library search, positive sample validation, exclusion of false positives

Complex mass spectrometer QTRAP screening workflow

QTRAP screening workflow--- Analyst Software + database



Liquid phase conditions

Chromatographic Column:

Phenomenex Kinetex C18,2.6u,50×2.1mm

Mobile phase:

A: Acetonitrile

B: 5 mmol/L aqueous ammonium acetate solution

Gradient elution was performed as shown below:

Time（min）	A%	B%
0	90	10
10.0	10	90
12.0	10	90
12.1	90	10
15.0	90	10

Flow rate: 250 μL/min； Column temperature: 40℃；

Amount injected: 10 μL

Mass spectrometry method

SCIEX QTRAP 4500 triple quadrupole complex ion trap mass spectrometer

Scanning method: MRM full scanning mode

Positive, negative scheduled MRM mode simultaneous scan

Ion source: Turbo V™ ESI source

Mass spectrometry parameter establishment

ESI ion source parameters:

Air curtain gas CUR: 30psi; Collision gas CAD: High

IS voltage: 5500V/-4500V

Ion source temperature: 550°C

Atomizing gas GAS1: 55psi

Auxiliary gas GAS2: 55psi

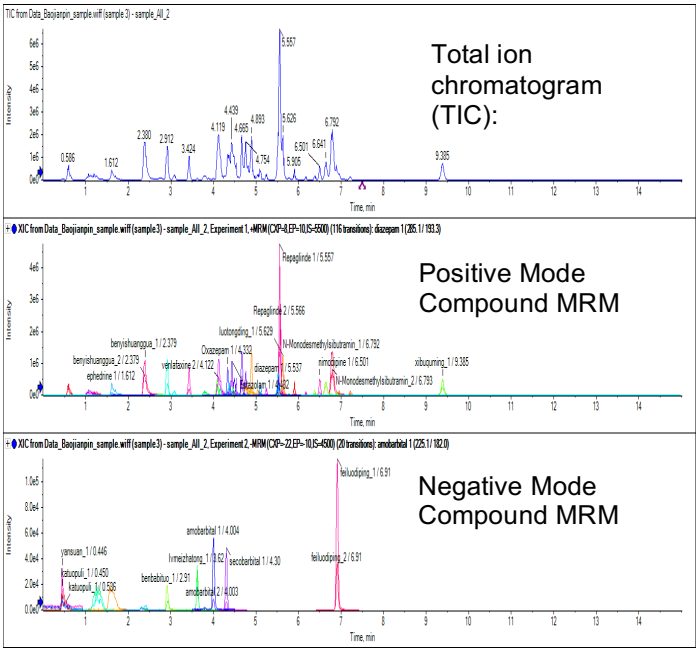
Collision energy: 35±15V

MRM detection window: 60 sec

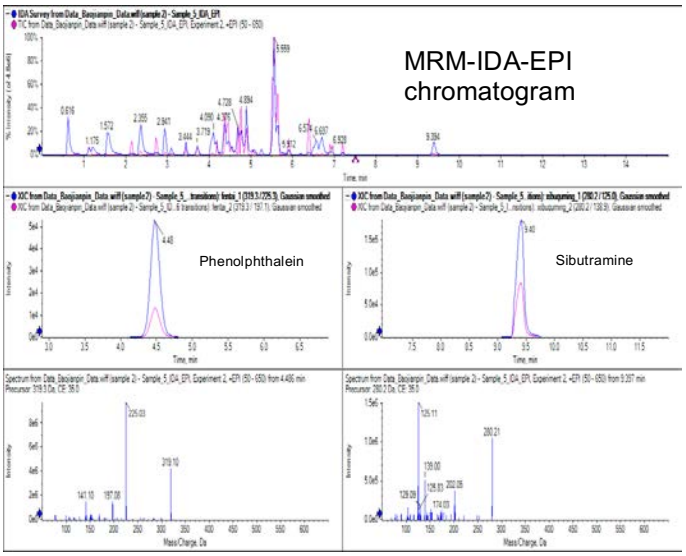
Target Scan Time: 0.25 sec

Experimental results

10 ppb single sample injection of drug mixture, simultaneous positive and negative mode scanning. Extract ion chromatograms as below:

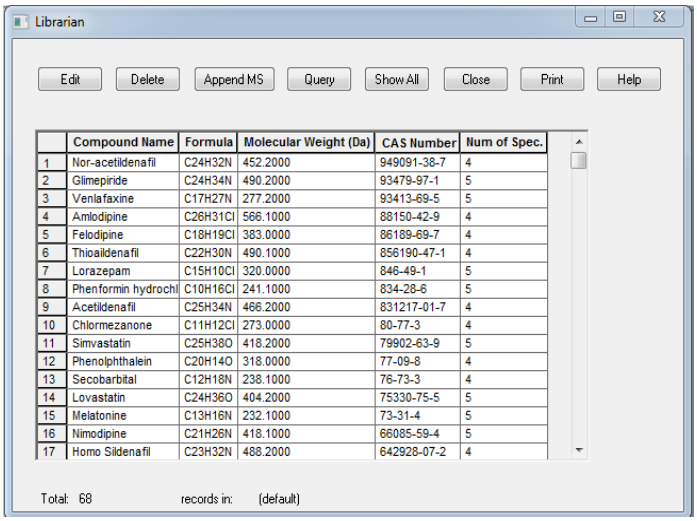


Typical compound MRM extracted ion chromatogram and EPI chromatogram are as follows:



Enhanced secondary fragment （EPI） database

This experimental protocol simultaneously established a database of enhanced secondary fragments (EPI) of 68 drugs. This database includes low, medium, high, and combined energy spectra with large amounts of fragment information and comprehensive validation.



	Compound Name	Formula	Molecular Weight (Da)	CAS Number	Num of Spec.
1	Nor-acetildenafil	C24H32N	452.2000	949091-38-7	4
2	Glimepiride	C24H34N	490.2000	93479-97-1	5
3	Venlafaxine	C17H27N	277.2000	93413-69-5	5
4	Amlodipine	C26H31Cl	566.1000	88150-42-9	4
5	Felodipine	C18H19Cl	383.0000	86189-69-7	4
6	Thioaildenafil	C22H30N	490.1000	856190-47-1	4
7	Lorazepam	C15H10Cl	320.0000	846-49-1	5
8	Phenformin hydrochl	C10H16Cl	241.1000	834-28-6	5
9	Acetildenafil	C25H34N	466.2000	831217-01-7	4
10	Chlormezanone	C11H12Cl	273.0000	80-77-3	4
11	Simvastatin	C25H38O	418.2000	79902-63-9	5
12	Phenolphthalein	C20H14O	318.0000	77-09-8	4
13	Secobarbital	C12H18N	238.1000	76-73-3	4
14	Lovastatin	C24H36O	404.2000	75330-75-5	5
15	Melatonine	C13H16N	232.1000	73-31-4	5
16	Nimodipine	C21H26N	418.1000	66085-59-4	5
17	Homo Sildenafil	C23H32N	488.2000	642928-07-2	4

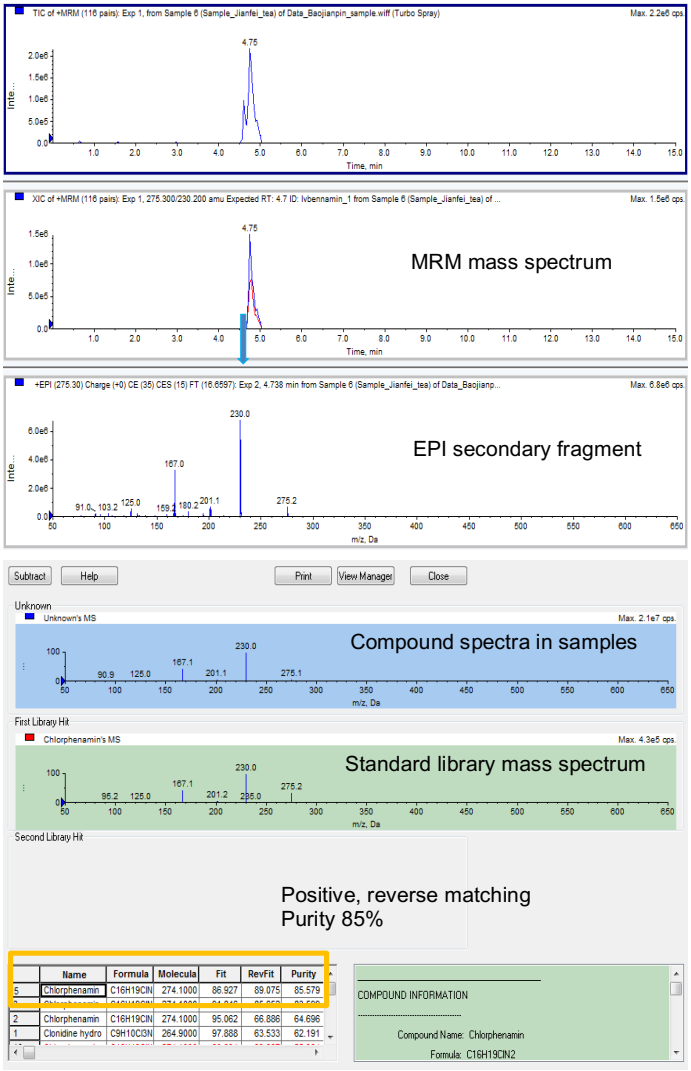
Sample screening results:

Preprocessing: Weight loss tea samples are taken directly after steeping.

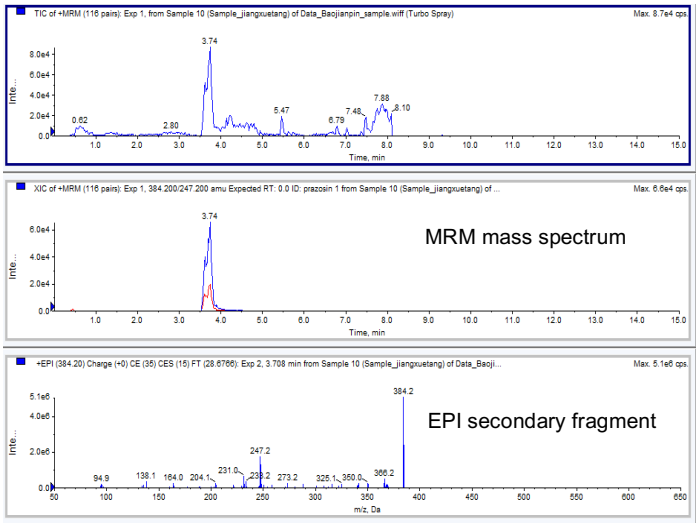
Capsules and tablet drugs: Remove 10mg of capsule contents (or grind tablets to a fine powder), ultrasonicate with 10mL methanol, centrifuge, remove supernatant directly as the sample.

Chlorphenamine has been found in some weight loss tea samples. Right-click library searches based on positive and reverse matching results have verified the presence of chlorphenamine. Chlorphenamine is a drug mainly used for rapid allergy relief.

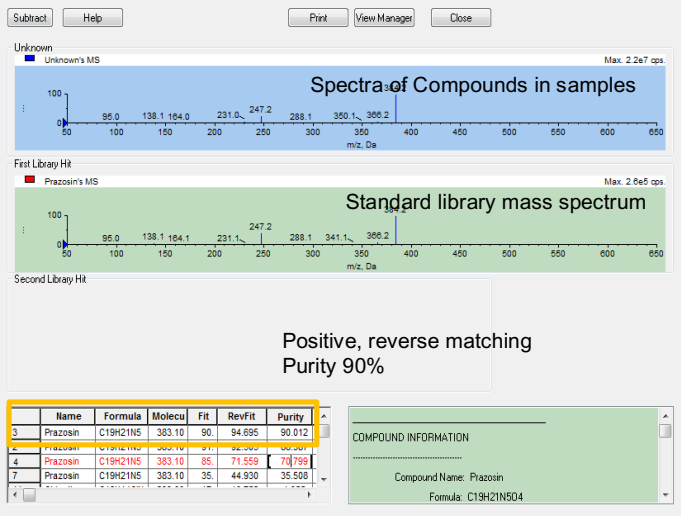
Chlorphenamine has been found in some weight loss tea samples as shown:



Prazosin has been detected in blood sugar-reducing capsules as shown:



Right-click library search based on positive and reverse matching results has verified the presence of prazosin. Prazosin is a prescription drug mainly used for high blood pressure and can cause fainting, dizziness, headache and other adverse reactions.



Conclusions

The SCIEX complex mass spectrometer QTRAP® System's triple quadrupole quantification function and linear ion trap qualification function use just one sample injection to complete both qualitative validation and quantification.

EPI secondary spectral sensitivity is at least 500 times higher than that of traditional MRM scanning. Low, medium, high, and combined energy high sensitivity spectra have more fragments and compensate for the traditional ion trap quality loss effect, so low-mass terminal fragments are abundant. EPI enhanced high-sensitivity secondary spectra effectively help to validate low concentration point detections from complex matrices, which are prone to false positives. This makes results more accurate and reliable. The triple quadrupole complex ion trap mass spectrometer QTRAP's unique MRM-IDA-EPI scanning mode is an effective and comprehensive method for resolving false positives and validating drug detections.

This method is a comprehensive solution based on the SCIEX QTRAP platform for monitoring 68 health products in the 2017 National Food Safety Supervision and Sampling Implementation Guidelines. This protocol includes sample preprocessing, liquid chromatography-mass spectrometry, and secondary validation libraries. It is convenient and quick to use.

Positive ion chemical compound MRM parameters:

No.	Q1	Q3	RT	ID	DP	EP	CE	CXP
1	285.1	193.3	5.55	Diazepam 1	80	10	45	8
	285.1	154.1	5.55	Diazepam 2	80	10	45	8
2	287.2	241.2	4.33	Oxazepam 1	50	10	31	8
	287.2	269.3	4.33	Oxazepam 2	50	10	31	8
3	321.1	275.1	4.48	Lorazepam 1	60	10	30	8
	321.1	303.1	4.48	Lorazepam 2	60	10	30	8
4	295.2	267.3	4.45	Estazolam 1	70	10	34	8
	295.2	205.2	4.45	Estazolam 2	70	10	34	8
5	309.1	281.1	4.66	Alprazolam 1	80	10	33	8
	309.1	274.2	4.66	Alprazolam 2	80	10	33	8
6	343.2	308.2	4.76	Triazolam 1	80	10	36	8
	343.2	315.2	4.76	Triazolam 2	80	10	36	8
7	316.2	270.2	4.54	Clonazepam 1	75	10	36	8
	316.2	214.1	4.54	Clonazepam 2	75	10	49	8
8	267.2	145.2	1.04	Atenolol 1	60	10	38	8
	267.2	190.3	1.04	Atenolol 2	60	10	26	8
9	278.3	58.1	4.04	Venlafaxine 1	40	10	40	8
	278.3	259.9	4.04	Venlafaxine 2	40	10	17	8
10	347.3	315.2	5.25	Nifedipine 1	60	10	12	8
	347.3	271.4	5.25	Nifedipine 2	60	10	16	8
11	361.3	315.1	6.17	Nitrendipine 1	80	10	13	8
	361.3	329.2	6.17	Nitrendipine 2	80	10	20	8
12	419	343.1	6.51	Nimodipine 1	60	10	13	8
	419	359.1	6.51	Nimodipine 2	60	10	22	8
13	232.2	159.3	4.33	Fenflutamine 1	20	10	32	8
	232.2	187.3	4.33	Fenflutamine 2	20	10	20	8
14	446.2	321.2	3.43	Glipizide 1	85	10	20	8
	446.2	103	3.43	Glipizide 2	85	10	62	8
15	453.3	230.2	5.56	Repaglinde 1	100	10	38	8
	453.3	162	5.56	Repaglinde 2	100	10	27	8
16	367.1	170.2	4.39	Glibornuride 1	82	10	24	8
	367.1	152.2	4.39	Glibornuride 2	82	10	27	8
17	206	60.2	2.36	Phenformin hydrochloride 1	80	10	31	8
	206	105	2.36	Phenformin hydrochloride 2	80	10	36	8
18	357.4	193	5.66	Pioglitazone hydrochloride 1	108	10	38	8
	357.4	165	5.66	Pioglitazone hydrochloride 2	108	10	34	8
19	266	125	6.7	N-monodesmethylsibutramin 1	62	10	32	8

	266	138.9	6.7	N-monodesmethylsibutramin 2	62	10	20	8
20	158.1	60.2	1.18	Butyl-biguanide hydrochloride 1	75	10	23	8
	158.1	116.1	1.18	Butyl-biguanide hydrochloride 2	75	10	23	8
21	130.3	60.2	0.58	Metformin hydrochloride 1	45	10	20	8
	130.3	71.2	0.58	Metformin hydrochloride 2	45	10	30	8
22	489.2	312.3	5.56	Vardenafil 1	130	10	53	8
	489.2	151	5.56	Vardenafil 2	130	10	53	8
23	489.2	72.3	5.93	Homo sildenafil 1	130	10	90	8
	489.2	113.3	5.93	Homo sildenafil 2	130	10	41	8
24	467.4	111.1	4.75	Acetildenafil 1	130	10	42	8
	467.4	127.1	4.75	Acetildenafil 2	130	10	42	8
25	505.3	113.3	7.2	Thioaildenafil 1	115	10	44	8
	505.3	327.1	7.2	Thioaildenafil 2	115	10	41	8
26	609.4	195.1	6.93	Reserpine 1	170	10	52	8
	609.4	397.2	6.93	Reserpine 2	170	10	38	8
27	355.9	192	5.64	Tetrahydropalmatine 1	115	10	39	8
	355.9	165	5.64	Tetrahydropalmatine 2	115	10	36	8
28	358.4	135.1	4.88	Rosiglitazone maleate 1	90	10	36	8
	358.4	107.1	4.88	Rosiglitazone maleate 2	90	10	51	8
29	275.3	230.2	4.68	Chlorphenamin 1	60	10	24	8
	275.3	167	4.68	Chlorphenamin 2	60	10	51	8
30	453.2	113.3	4.74	Noracetildenafil 1	130	10	44	8
	453.2	297.3	4.74	Noracetildenafil 2	130	10	53	8
31	460.3	283.1	6.9	Norneosildenafil 1	105	10	48	8
	460.3	299.3	6.9	Norneosildenafil 2	105	10	47	8
32	505.3	99.2	4.83	Hydroxyhomosildenafil 1	108	10	61	8
	505.3	299.2	4.83	Hydroxyhomosildenafil 2	108	10	56	8
33	330.2	181.1	2.72	Sinomenine 1	106	10	46	8
	330.2	239	2.72	Sinomenine 2	106	10	34	8
34	252.2	125	6.55	N,N-didesmethylsibutramin 1	50	10	30	8
	252.2	139	6.55	N,N-didesmethylsibutramin 2	50	10	16	8
35	460.3	283.3	6.39	Pseudovardenafil 1	105	10	49	8
	460.3	299.3	6.39	Pseudovardenafil 2	105	10	52	8
36	280.2	125	9.4	Sibutramine 1	50	10	33	8
	280.2	138.9	9.4	Sibutramine 2	50	10	22	8
37	475.2	100	5.47	Sildenafil 1	130	10	42	8
	475.2	283.1	5.47	Sildenafil 2	130	10	53	8
38	389.3	245	4.1	Zopiclone 1	62	10	23	8
	389.3	217	4.1	Zopiclone 2	62	10	44	8
39	390.1	268.2	4.72	Tadalafil 1	100	10	20	8

	390.1	169.2	4.72	Tadalafil 2	100	10	52	8
40	409.3	238	5.41	Amlodipine 1	116	10	16	8
	409.3	294.2	5.41	Amlodipine 2	116	10	15	8
41	319.3	225.3	4.46	Phenolphthalein 1	90	10	29	8
	319.3	197.1	4.46	Phenolphthalein 2	90	10	41	8
42	528.6	403.2	5.76	Gliquidone 1	98	10	19	8
	528.6	386.3	5.76	Gliquidone 2	98	10	31	8
43	324	110	3.72	Gliclazide 1	95	10	28	8
	324	127.1	3.72	Gliclazide 2	95	10	30	8
44	419.5	199.2	7.78	Simvastatin 1	90	10	18	8
	419.5	243.2	7.78	Simvastatin 2	90	10	19	8
45	306.2	236.2	4.15	Zaleplon 1	96	10	36	8
	306.2	264.2	4.15	Zaleplon 2	96	10	30	8
46	496.5	371.2	4.85	Glibenclamide 1	77	10	22	8
	496.5	171.2	4.85	Glibenclamide 2	77	10	38	8
47	405.5	199.3	7.32	Lovastatin 1	79	10	19	8
	405.5	285.3	7.32	Lovastatin 2	79	10	15	8
48	389.5	240	6.72	Nisoldipine 1	73	10	35	8
	389.5	194.9	6.72	Nisoldipine 2	73	10	30	8
49	230	160	2.1	Clonidine hydrochloride 1	80	10	47	8
	230	145	2.1	Clonidine hydrochloride 2	80	10	51	8
50	233.3	174.1	2.92	Melatonine 1	68	10	18	8
	233.3	158.9	2.92	Melatonine 2	68	10	34	8
51	271.3	155.2	3.07	Tolbutamide 1	71	10	25	8
	271.3	74.1	3.07	Tolbutamide 2	71	10	24	8
52	391.2	169	4.25	Amino tadalafil 1	104	10	45	8
	391.2	268.9	4.25	Amino tadalafil 2	104	10	21	8
53	166.1	148.1	1.55	Ephedrine 1	40	10	18	8
	166.1	133.1	1.55	Ephedrine 2	40	10	26	8
54	491.3	125.9	5.08	Glimepiride 1	50	10	35	8
	491.3	352.1	5.08	Glimepiride 2	50	10	35	8
55	300	283.1	4.6	Chlordiazepoxide 1	80	10	25	8
	300	227.1	4.6	Chlordiazepoxide 2	80	10	25	8
56	326.2	291.4	5.52	Midazolam maleate 1	65	10	37	8
	326.2	244.2	5.52	Midazolam maleate 2	65	10	35	8
57	282.2	236.2	4.38	Nitrazepam 1	70	10	32	8
	282.2	180.2	4.38	Nitrazepam 2	70	10	52	8
58	384.2	247.2	3.74	Prazosin 1	60	10	39	8
	384.2	138.2	3.74	Prazosin 2	60	10	43	8

Anion Compound Parameters

No.	Q1	Q3	RT	ID	DP	EP	CE	CXP
59	183.1	140	1.48	Barbital 1	-50	-10	-16	-22
	183.1	95.9	1.48	Barbital 2	-50	-10	-20	-22
60	122.2	77.8	0.43	Nicotinic acid 1	-45	-10	-16	-22
	122.2	122.2	0.43	Nicotinic acid 2	-45	-10	-10	-22
61	230.9	144.2	2.88	Phenobarbital 1	-57	-10	-22	-22
	230.9	85	2.88	Phenobarbital 2	-57	-10	-16	-22
62	225.1	182	4	Amobarbital 1	-30	-10	-17	-22
	225.1	85	4	Amobarbital 2	-30	-10	-19	-22
63	237.1	194	4.29	Secobarbital 1	-40	-10	-17	-22
	237.1	85	4.29	Secobarbital 2	-40	-10	-17	-22
64	295.9	268.9	1.16	Hydrochlorothiazide 1	-101	-10	-26	-22
	295.9	204.9	1.16	Hydrochlorothiazide 2	-101	-10	-32	-22
65	329	204.9	2.38	Furosemide 1	-109	-10	-26	-22
	329	284.9	2.38	Furosemide 2	-109	-10	-21	-22
66	382.1	144.8	6.92	Felodipine 1	-69	-10	-14	-22
	382.1	236	6.92	Felodipine 2	-69	-10	-20	-22
67	216	182	0.44	Captopril 1	-58	-10	-17	-22
	216	113.8	0.44	Captopril 2	-58	-10	-16	-22
68	271.9	179.8	3.62	Chlormezanone 1	-51	-10	-21	-22
	271.9	208	3.62	Chlormezanone 2	-51	-10	-16	-22

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Use of X500R QTOF for Monitoring Unexpected Additives in Nutritional Supplements

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Introduction

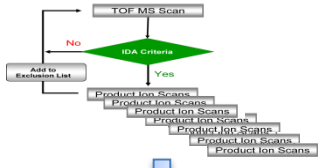
Nutritional supplements can supplement necessary nutrients and are believed to support recovery from illness. Generally, these products promote a particular effect or claimed function[1]; thus, in typical use, people often incorrectly believe they have a definite treatment efficacy. They are often linked to the alleviation of certain illnesses. In order to maximize these functions, manufacturers may add related drugs in order to increase their efficacy without including them as a listed ingredient. According to reports and discoveries from actual monitoring cases, unexpected additives to nutritional supplements are generally selected because they relate to the health product effects or address the additive side effects or functions; the additive usually takes the form of one or more drug additives, drug derivatives, etc.[5] Because these additives are generally high-dose, drug interactions can be unclear. Thus, a great potential hazard exists for human health [2-4]; the China Food and Drug Administration (CFDA) “Health product potential illegal additives list” clearly stipulates monitoring processes for additives in 6 different types of nutritional supplements: those with weight loss, blood sugar reduction, blood pressure reduction, anti-fatigue, sleep improvement, and immune strengthening functions. The purpose is to protect consumers’ health.

SCIEX’s X500R QTOF high resolution mass spectrometry system can be used for rapid monitoring of additives in nutritional supplements; after sample injection, a first order mass accuracy number and second order fragmentation spectrum are simultaneously obtained. Currently, over 50 additives can quickly be qualitatively confirmed in this way. Matrix interference in complex matrices can be overcome for specific screening of additives; preprocessing is even simpler and more convenient. The new SCIEX OS software fully integrates instrument control, data collection, data handling, and other processes. The workflow is more intuitive and smarter; this method provides an efficient means for rapid, high-throughput monitoring of nutritional supplements for additives.

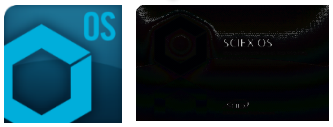
Experimental Process

1. Collect samples of 6 types of nutritional supplements currently on the market - those with weight loss, blood sugar reduction, blood pressure reduction, anti-fatigue, sleep improvement, and immune strengthening functions. Perform simple preprocessing.
2. Use TOF MS-IDA MS/MS mode for data collection; after sample injection, obtain first order ion and second order ion fragmentation spectrograms.
3. The mass accuracy number, isotope distribution, retention time, and standard library alignment are used for positive verification of samples and checking the accuracy of sample monitoring results.
4. Monitoring reports systematically summarize sample screening results; the report content can be tailored to specific requirements.

X500R high-resolution mass spectrometry screening workflow:



1. Both TOF-MS-IDA-MS/MS And TOF-MS/MS data gathered in the same injection



2. SCIEX OS is the integral software used to perform this analysis

3. Screening results and report generation

Preprocessing Method

1. Use tablets ground into a powder, granules from inside capsules, or liquid samples; weigh accurately a 1.0gsample, and place in a 10mLcentrifuge tube;
2. Add 5mL acetonitrile and agitate 2 min;
3. Vortex 2 min;
4. Centrifuge at 4℃ at 10000 Rpm for 15min;
5. Dissolve the supernatant 1-fold;
6. Pass through a 0.22μm filter and directly inject sample;

Liquid Phase Conditions

Chromatographic Column: Phenomenex Kinetex C18, 2.1*100mm, 2.6μm;

Elution gradient

Time (min)	A%	B%
0	*95*	*5*
5.0	*55*	*45*
15.0	*20*	*80*
20.0	*5*	*95*
25.0	*5*	*95*
25.1	*95*	*5*
30	*95*	*5*

Positive ion mode: A: 0.1% Formic acid Water; B: 0.1% Formic acid Acetonitrile;

Negative ion mode: A: Water; B: Acetonitrile;

Flow rate: 0.3mL/min;

Column temperature: 40℃;

Amount inserted: 10 μL;

Mass Spectrometry Method

Scanning method: TOF MS-IDA MS/MS

Ion source: ESI source

Scanning range: m/z 50-2000

CUR gas: 30 PSI

Collision gas CAD: 7

IS voltage: 5500V/-4500V

Source temperature: 600℃

Atomizing gas GAS1: 55 PSI

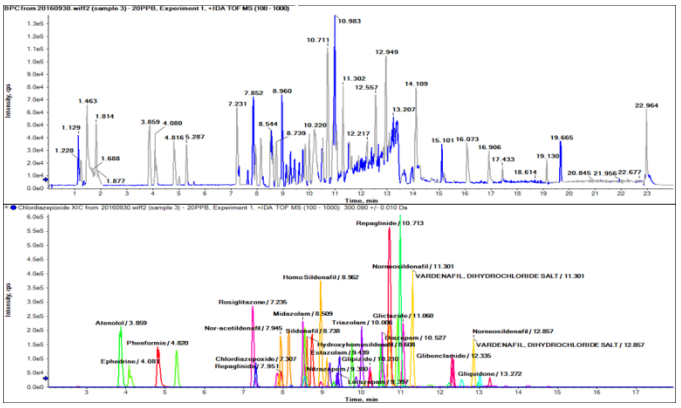
Auxiliary gas GAS2: 70 PSI

DP voltage: ± 60V

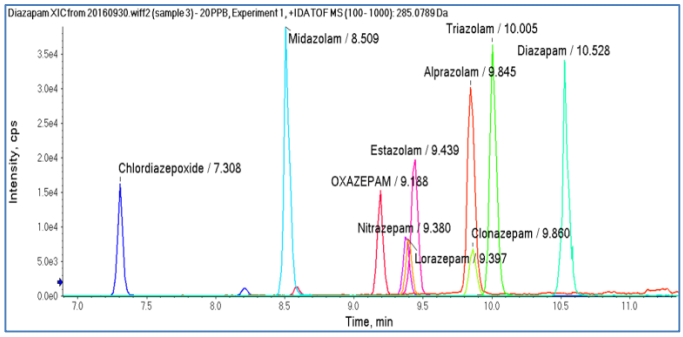
Collision energy: 35 ± 15V

Unexpected Additive Screening Method

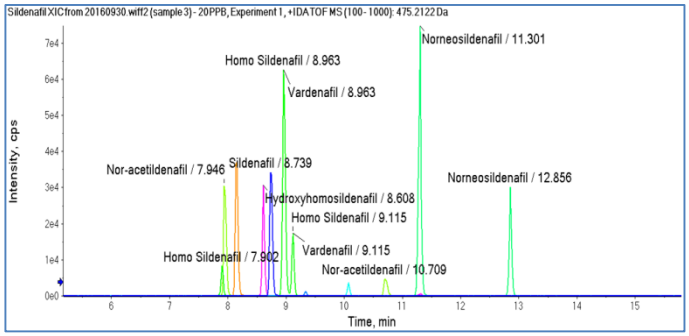
Injection of a single sample simultaneously monitors for over 50 unexpected additives:



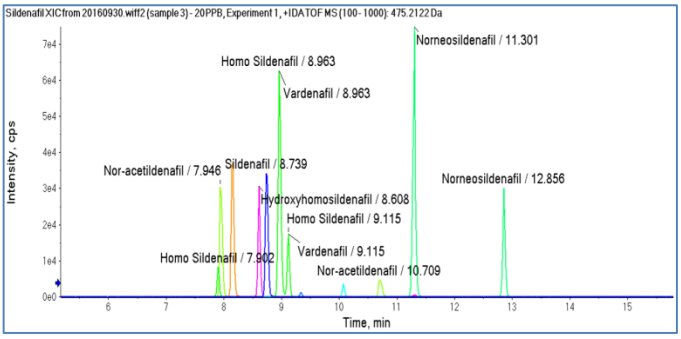
1. 10 sedative-hypnotic mixtures (20ppb), ion extraction flow diagram (XIC) appears below:



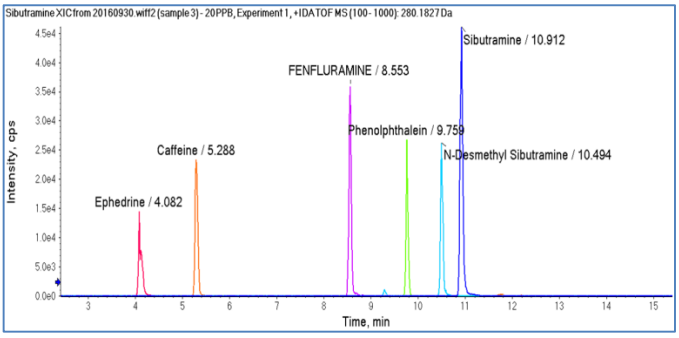
2. 7 blood glucose-lowering drugs (concentration 20ppb); ion extraction flow diagram (XIC) appears below:



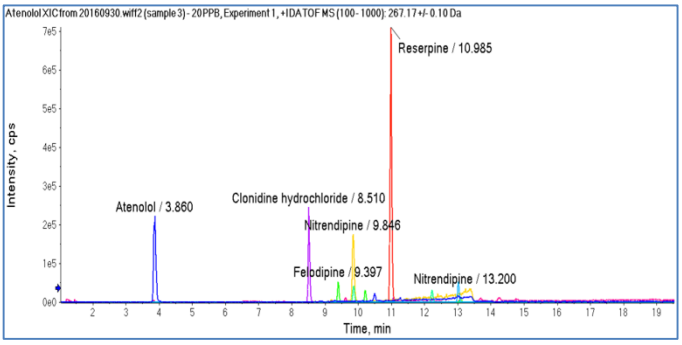
3. 8 impotence drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;



4. 6 weight loss drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;



5. 5 blood pressure-lowering drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;



Sample Information

Following the CFDA's "Health product potential illegal additives list" 6 different nutritional supplements were randomly selected, including those for weight loss, blood sugar reduction, blood pressure reduction, anti-fatigue, sleep improvement, and immune strengthening. Samples came from 19 different brands;

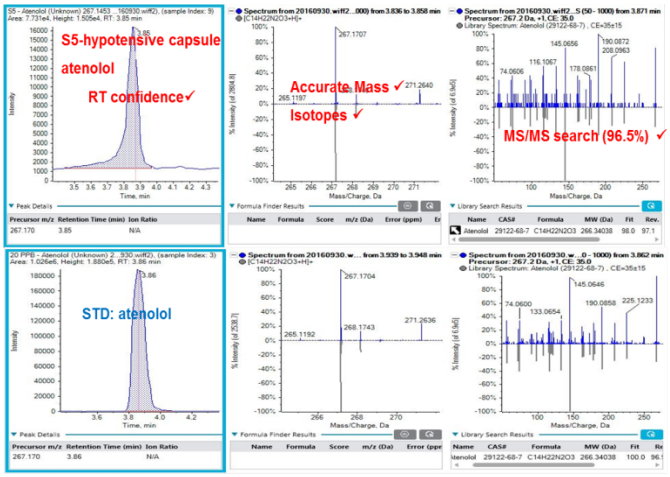
Sample No	sample type	name
Sample 1	sleeping	epiphysis pacify
Sample 2	hypoglycemic action	glycolipids safe
Sample 3	hypoglycemic action	hypoglycemic extract
Sample 4	anti-hangover	prime power
Sample 5	hypotensive	hypotensive capsule
Sample 6	sleeping	pacify syrup
Sample 7	hypoglycemic action	hypoglycemic TCM
Sample 8	slimming	slimming capsule
Sample 9	hypotensive	Hypotensive pill
Sample 19	.....	.....

Experimental Results

Blood Pressure-Lowering Drugs

1. Sample no. 5 - atenolol positive

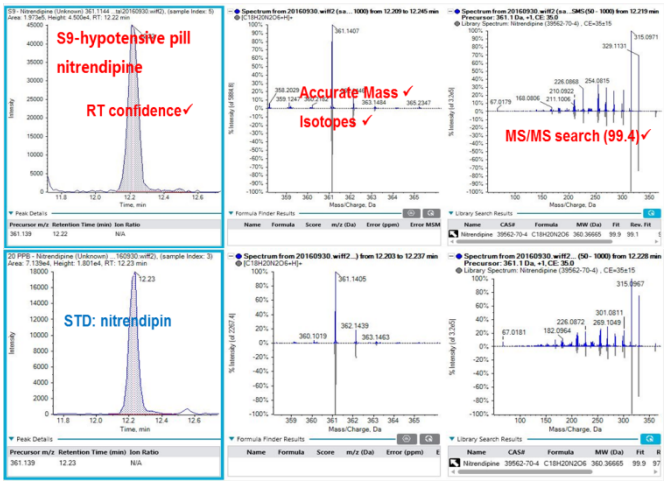
Sample no. 5 is a blood pressure-lowering capsule; it claims to have a rapid effect and prolonged use can control blood pressure.



Screening with the X500R QTOF system showed Sample no. 5 contains large amounts of the additive atenolol. Prolonged use of high-dose atenolol can lead to serious side effects including decreased vision, breathing difficulties, weakness, depression, unexplained rash and ankle swelling and other symptoms.

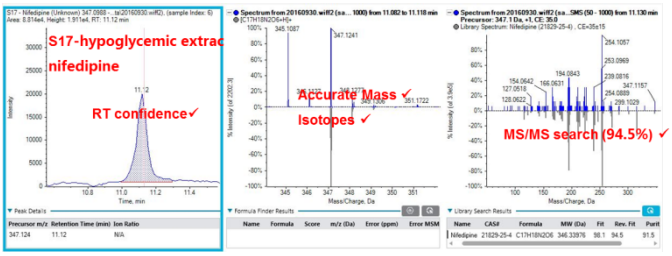
2. Sample no. 9 - nitrendipine positive

Sample no. 9 is from a brand of blood pressure-lowering tablet; screening shows a definite quantity of nitrendipine. The product claims to contain pure and natural extracts with no side effects, but prolonged oral nitrendipine can cause diseases like allergic hepatitis, rash, and even exfoliative dermatitis.



3. Sample no. 17 - nifedipine positive

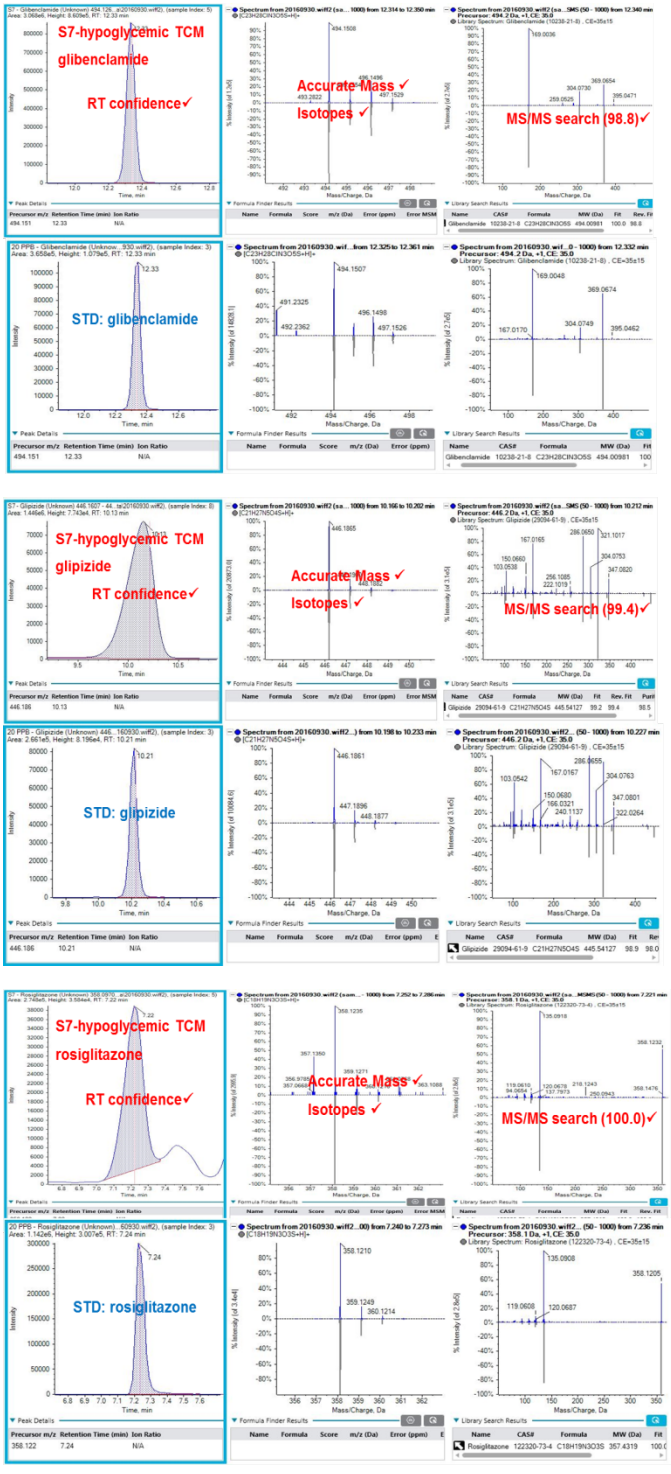
Sample no. 17 is from a brand of blood pressure-lowering Chinese medicine; screening shows a nifedipine additive. It claims to lower blood pressure with Chinese medicine, falsely advertising an anti-hypertensive effect.



Glucose-Lowering Drugs

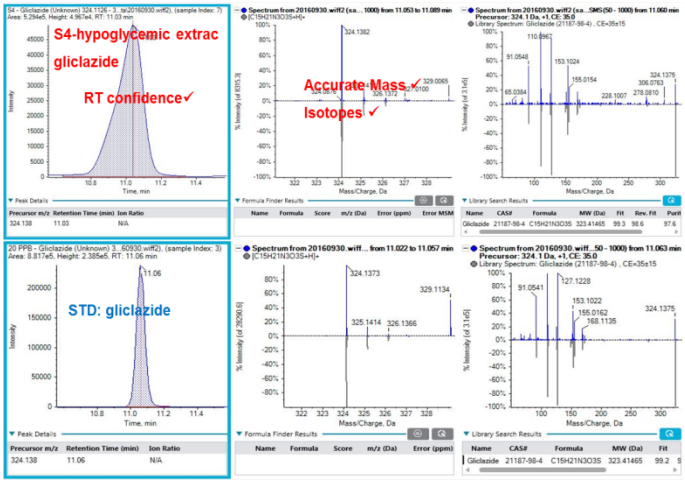
1. Sample no. 7 - glibenclamide, glipizide, rosiglitazone positive

Sample no. 7 is a brand of glucose- and lipid-lowering capsule; test results show sample no. 7 contains the 3 glucose-lowering drugs glibenclamide, glipizide, and rosiglitazone as additives. Improper use of sulfonylureas such as glibenclamide and glipizide can cause hypoglycemia; patients can rarely develop rash, erythema multiforme, edema, and liver and kidney damage. Thiazolidinediones like rosiglitazone can cause slight hypersensitivity and mild headache when used incorrectly or at improper doses.



2. Sample no. 4 - Gliclazide positive

Sample no. 4 is a brand of plant extract; it is mainly used to stabilize blood sugar. Screening results show an addition of glicazide, which produces a definitive glucose-lowering effect.

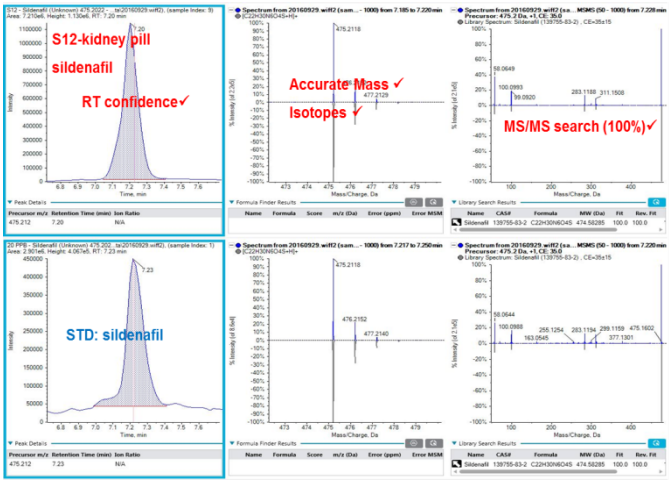


Glucose-lowering drugs are low-cost; they are common “functional components” added to nutritional supplements. These chemical drugs are often used to treat diabetes, as they have a clear hypoglycemic effect. However, their side effects are also quite evident; prolonged use can lead to hypoglycemia and kidney damage, even leading to death.

Anti-Fatigue/Impotence

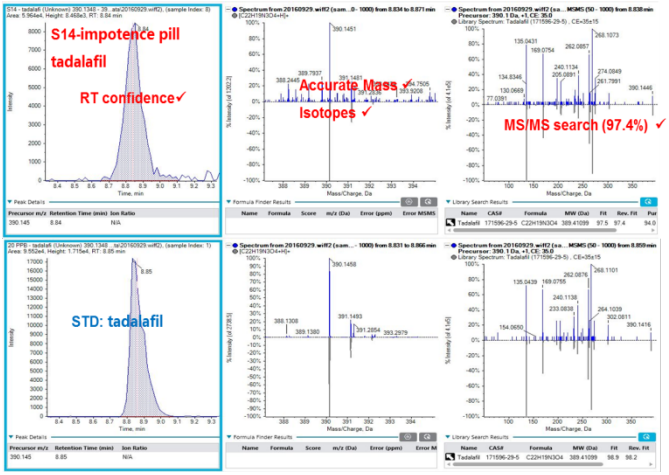
1. Sample no. 12 - sildenafil positive

Sample no. 12 is a kidney health product for the elderly; its description states it is pure Chinese medicine and contains several flavors of medicine. Screening shows an addition of large quantities of sildenafil in order to achieve its claimed kidney effects.



2. Sample no. 14 - tadalafil positive

Sample no. 14 is a brand of impotence health product. Impotence products are the most frequently found to contain additives. In order to accelerate the speed of onset, additives are generally used in large quantities; screening results showed sample no. 14 contained tadalafil.



When not used under the guidance of a specialized physician, prolonged use of nutritional supplements containing “impotence” additives can severely harm the body. Side effects can include dizziness, fainting, and even hearing loss.

Screening results appear in the table:

1. The problem of additives in nutritional supplements is widespread; additives appear in many samples;

2. Blood sugar- and pressure-reducing products contain many different additives; they generally take the form of multiple drugs, and use of Chinese medicine is especially serious.

3. Anti-fatigue and impotence health care products generally contain large amounts of additives;

Sample No	sample name	positvie results
Sample 1	epiphysis pacify	--------
Sample 2	glycolipids safe	--------
Sample 3	hypoglycemic action	--------
Sample 4	hypoglycemic extrac	gliclazide
Sample 5	hypotensive capsule	atenolol
Sample 6	pacify syrup	--------
Sample 7	hypoglycemic TCM	glipizide、rosiglitazone、glibenclamide
Sample 8	slimming capsule	--------
Sample 9	hypotensive pill	nitrendipine
Sample 12	kidney pill	sildenafil
Sample 14	impotence pill	tadalafil
Sample 17	hypoglycemic extrac	nifedipine

Summary

This study randomly selected 19 nutritional supplements commonly found on the market; these covered 7 glucose- and blood pressure-lowering products, 5 anti-fatigue, anti-impotence products, 4 sleep aids, and 3 weight loss products. Screening results showed that blood pressure-lowering and glucose-lowering products most commonly contained additives, especially those products advertised to use Chinese medicine extracts to lower blood sugar. Representative samples of blood pressure-lowering capsules showed a high rate of positive results. The main additives were atenolol, nitrendipine, nifedipine, glibenclamide, glipizide, rosiglitazone, gliclazide and other inexpensive and readily available glucose- and blood pressure-lowering drugs, impotence, anti-fatigue/immune system-enhancing additives were generally sildenafil or tadalafil. Additives take the form of one or many drugs; some additives are present in amounts several times therapeutic doses. Thus, they can be quite hazardous to consumer health.

The SCIEX X500R QTOF high resolution mass spectrometry system was used for rapid monitoring of 50 different additives in 6 types of nutritional supplements. Its high sensitivity detected small concentrations of additives, its rapid scanning and effective overcoming of complex matrix interference ensure that after sample injection, a first order mass accuracy number (TOF-MS) and second order fragmentation spectrum (TOF-MS/MS) are simultaneously obtained. Combined with the high-quality additive library, accurate qualitative screening for additives in complex matrices can be performed.

Health product additive screening methods using the X500R QTOF system are reliable, simple, and rapid. The system provides an efficient approach to additive screening of nutritional supplements, and it ensures health and safety product quality; it is critical in the fight against the use of potentially harmful additives.

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