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Illegal Color Dye in Food Matrix for Multi Compounds Analysis with Agilent 6460 QQQ and 6545 Q-TOF

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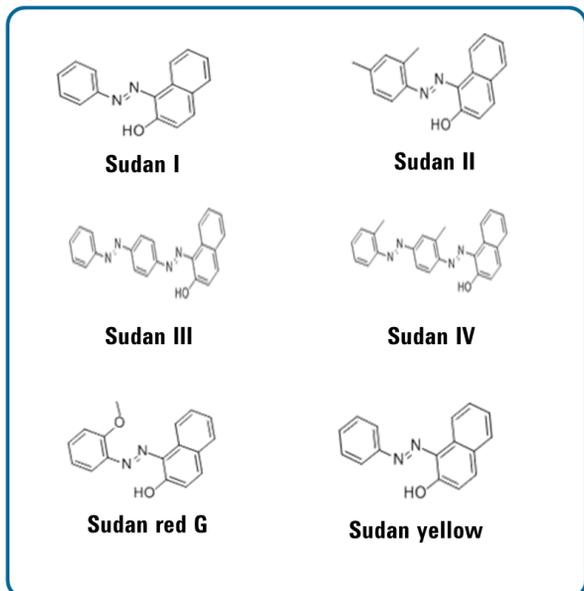
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Introduction

The color dyes, especially synthetic dyes are often used to enhance the appearance of food products. Although more and more evidence in recent years indicates that the abuse of dyes may cause cancer, many kinds of dyes are still widely used because of their low price, high effectiveness and excellent stability. For protecting the public health, many countries have their own regulations about the varieties of color dyes and their maximum levels permitted.

The triple-quadrupole (QQQ) and quadrupole-time of flight (Q-TOF) mass spectrometry systems are very powerful tools to identify and quantify the broadest range of compounds in complex food matrices and the positive result of food samples could be confirmed further using Personal Compound Database & Library (PCDL). The combination of UPLC/QQQ with Dynamic MRM acquisition enables thousands of MRMs per run for complex samples. In this study, a fast method for forty-five illegal color dyes qualitative analysis and quantitative determination in food matrix was developed using UHPLC/QQQ and Q-TOF/MS systems.

The parts of illegal Color Dyes Structures



Experimental

QuEChERS Sample Preparation

Sample information: Standards are obtained from different sources. The 1mg/mL per vial standards are dissolved in methanol and then diluted to a series concentrations as working solution.

Matrix extract: 2-5g sample were weighted into 50 mL tubes and extracted with organic solvent by vortexing 1min, and then different color dyes used different extraction procedure for cleaned up. For example, raw extracts were cleaned up by dispersive SPE kit(contained 150mg C18 powder)for base color dyes. For example of base color dyes:

Sample weight 2-5g
 ↓
 10ml Acetonitrile(1% Acetic acid)
 ↓
 Vortex 30s and Sonication 5min
 ↓
 Take upper layer 5ml and add to dispersive SPE kit
 (contained 150mg C18 powder)
 ↓
 Vortex 1min
 ↓
 Centrifuge 10000rpm,5min and Take 1ml,N2 dryness
 ↓
 Dissolved by H2O/MEOH=1/1

Agilent 1290 Infinity UHPLC Method

Column: Agilent Poroshell 120 EC-C18, 3.0*100mm 2.7um
 Agilent Poroshell 120 EC-C18, 3.0*150mm 2.7um
 Column temperature:30 °C
 Injection volumn:5ul
 Autosampler temp:4°C
 Needle wash:flushport(acetonitrile),10sec
 Mobile phase: A=5mM ammonium acetate+0.1% Formic acid in water
 B=Acetonitrile

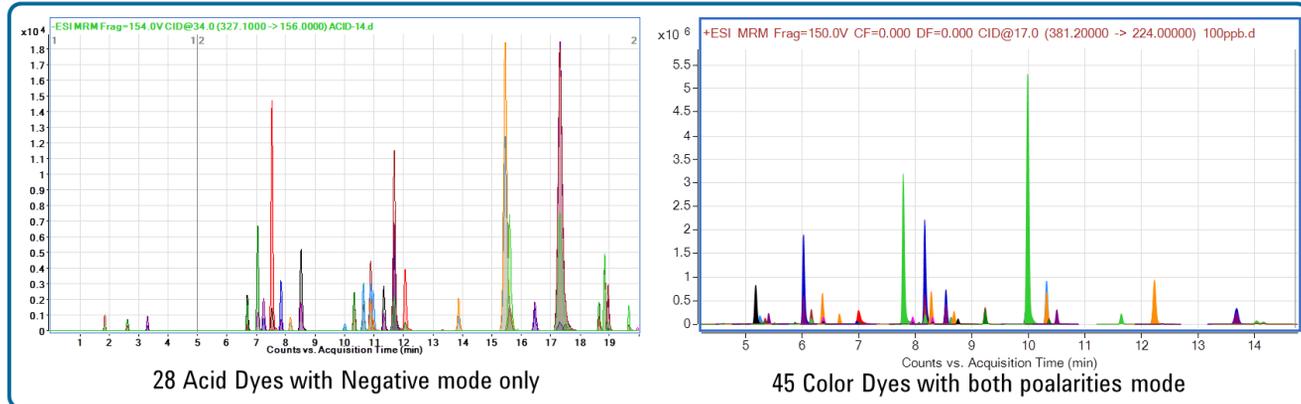
Flow rate:0.3ml/min
 Gradient: hold 2% B for 0.5min,2% to 100% in 10min, hold at 100% B for 5min,post run is 3min.The total time for each run is 18min.

Agilent QQQ & Q-TOF Method

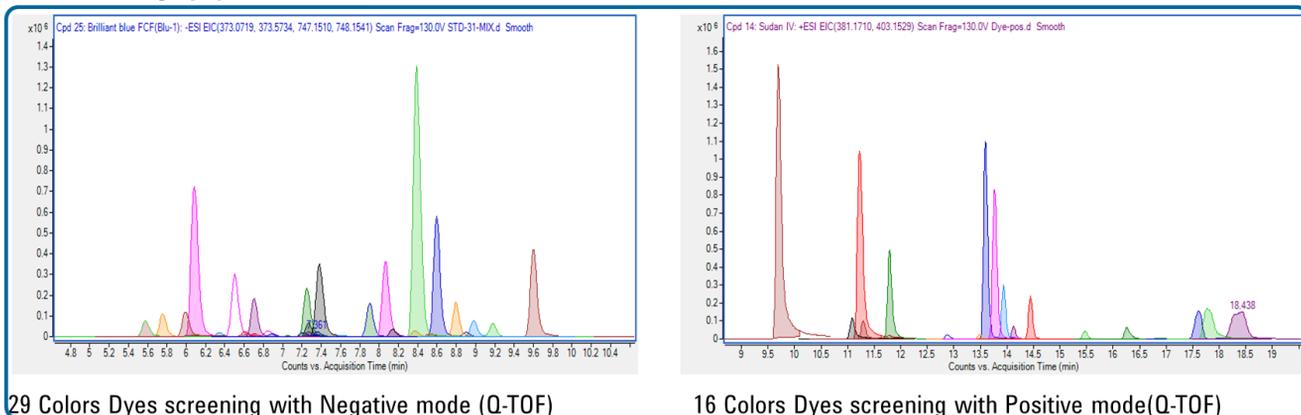
6460QQQ		6545Q-TOF	
Gas Temp	325°C	Gas Temp	325°C
Drying Gas	6L/min	Drying Gas	8L/min
Nebulizer Gas	45psi	Nebulizer Gas	45psi
Sheath Gas Temp	350°C	Sheath Gas Temp	350°C
Sheath Flow	11L/min	Sheath Flow	11L/min
Capillary Voltage	3500(pos)/3000(neg)	Capillary Voltage	4000(pos)/3500(neg)
Nozzle Voltage	500(pos)/1500(neg)	Nozzle Voltage	250(pos)/1500(neg)
Cycle Time	500ms	Fragment	130V

Results and Discussion

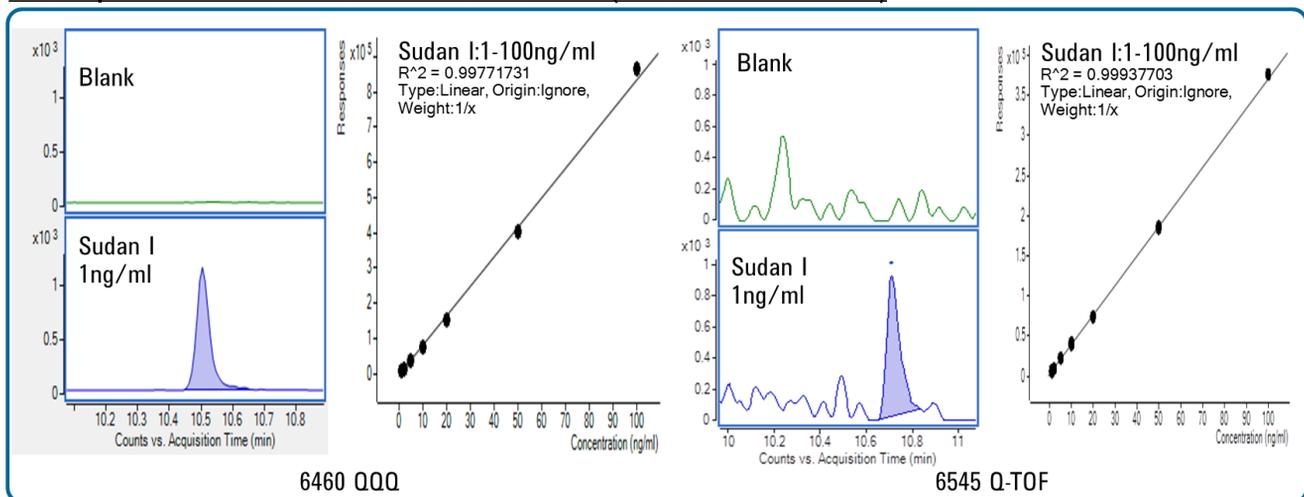
The Chromatography of 6460 QQQ



The Chromatography of 6545 QTOF



The comparison of matrix-match calibration with Sudan I (6460 QQQ vs. 6545 Q-TOF)

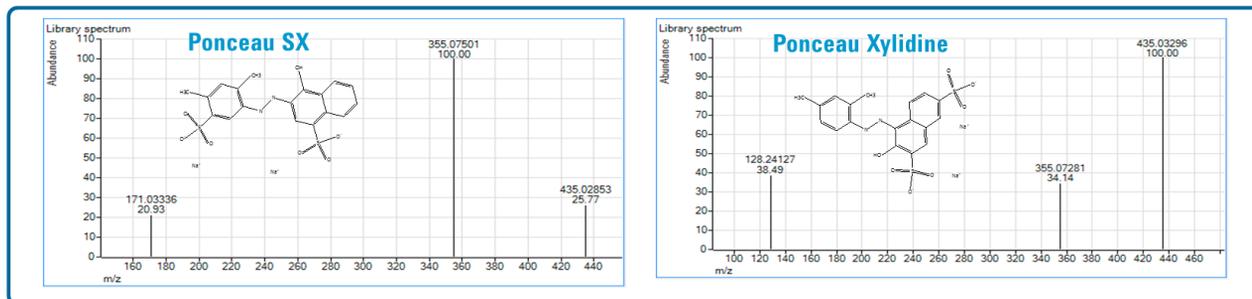


Results and Discussion

Agilent Workflow of Q-TOF Targeted Screening



Agilent PCDL for hundreds of Color Dyes



MassHunter Personal Compound Database and Library software(PCDL) allowing you to edit, search, and create libraries of MS/MS centroid spectra acquired on a Q-TOF instrument. The PCDL that contain mass spectra are regarded as spectral libraries or libraries. We acquired MS/MS spectrum using color dyes standards and added them to the PCDL database. Followed by searches of the database for the compounds in matrix sample.

Summary Table of 6545 Q-TOF (Show some results only)

Compound Name	Range (ng/mL)		LOQ (ppb)	LOD (ppb)	LOI (ppb)	Recovery, %-Matrix I	Spike-10ppb.RSD(%)
AURAMINE O	1-500	Y=55480.594201X+114819.155459 (0.999)	1.0	0.5	5.0	81.9	8.83
Leucomalachite Green	0.2-20	Y=69713.750086X+8805.081415 (0.997)	0.2	0.1	1.0	101.7	6.03
Malachite Green	0.2-20	Y=66374.494015X+16584.401954 (0.997)	0.2	0.1	1.0	15.6	5.30
sudan IV	10-500	Y=565.808018X-327.592034 (0.995)	10.0	5.0	20.0	132.2	6.49
Butter yellow (Methyl Yellow)	1-500	Y=23147.644676X+66562.356697 (0.999)	1.0	0.5	2.0	89.8	1.55
Sudan Black B	5-200	Y=2524.823568X+687.609375 (0.999)	5.0	2.0	10.0	13.5	4.22
Chrysoidine G	0.1-20	Y=71975.837965X+14994.923341 (0.998)	0.1	0.05	1.0	132.8	5.21
Rhodamine B	0.2-10	Y=77685.231815X+7611.540839 (0.999)	0.1	0.05	0.5	51.5	6.01
sudan orange G	5-200	Y=3043.571724X+21297.215011 (0.994)	5.0	2.0	10.0	93.2	0.05
sudan red 7B	0.5-100	Y=7076.809973X+11367.598184 (0.998)	0.5	0.2	2.0	115.2	2.78
sudan red G	1-200	Y=20853.273698X-413.662803 (0.999)	1.0	0.5	2.0	84.5	1.00
sudan I	5-500	Y=3581.970568X+15567.375424 (0.999)	5.0	2.0	10.0	86.2	15.1
sudan II	1-200	Y=4281.334319X+11520.951470 (0.995)	1.0	0.5	5.0	78.1	2.39
Citrus Red 2	1-500	Y=13109.473733X+35976.753572 (0.998)	1.0	0.5	10.0	87.5	1.05

Conclusions

All color dyes were separated well by UHPLC in 20 minutes. The calibration curve of all color dyes were constructed over a concentration range of 1.0 - 500 µg/L with correlation coefficients (r) more than 0.990, respectively. The recoveries of spiked color dyes were found in the range of 50–120%. Good precision and linearity were obtained for quantitative determination in food matrix by the proposed UHPLC/QQQ MS method and UHPLC/Q-TOF MS method. Moreover, the sample analysis result obtained from QTOF MS system was almost the same as that from QQQ MS system.

The method was established for sensitive and accurate qualitative analysis and quantitative determination of multi color dyes in food matrix.

Acknowledgements

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