LC-MS/MS Analysis of Emerging Food Contaminants

Identification of Artificial Colors and Dyes in Food Samples using LC-HR-MS/MS

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Overview

Here we present a novel LC-HR-MS/MS method that was used to identify artificial colors and dyes in food samples.

High resolution MS and MS/MS data were collected using a SCIEX X500R QTOF system in negative Electrospray Ionization (ESI). Non-target peak finding, sample-control-comparison followed by identification based on empirical formula finding and ChemSpider database searching was performed in SCIEX OS software. In addition, statistical data processing was done in MarkerView™ software.

Introduction

Artificial colors and dyes are used in food to make it visually more appealing and “flavorful” since people associate certain colors with certain flavors.

However, some dyes are banned because they are toxic and carcinogenic. Other dyes are approved for use in foods and regulated by Codex Alimentarius, the US-FDA, EFSA etc. Nature derived color additives (pigments derived from vegetables, minerals or animals) are exempt from certification.[1-3]

Recent research shows a link between the presence of artificial colors in food and behavioral problems of children.[4-5] These findings have resulted in public concern about the use of artificial dyes.

Analytical methods used to test for the presence of banned colors and dyes in food include TLC-UV/VIS, LC-UV/VIS, and LC-MS. Such methods have limited selectivity and sensitivity and are therefore only used for target analysis. Recent advancements in LC-HR-MS technology provide the ability to perform targeted and non-targeted screening in food samples on a routine basis. The exact mass and MS/MS data provided by these instruments contain enough information to confidently identify known food ingredients and contaminants and also to identify unknown chemicals that may also be present in the sample.

Artificial colors and dyes in food samples were identified using the SCIEX X500R QTOF system. MS detection was performed using information dependent acquisition (IDA) to simultaneously collect accurate mass MS and MS/MS information.

Compounds were automatically identified. SCIEX OS was used to automatically process the data using a non-target peak finding algorithm and sample-control-comparison to locate unique peaks in the sample. MarkerView™ software and statistical data processing was used to separate matrix and sample specific signals from true contaminants. TOF-MS and MS/MS data of ions of interest were automatically processed using empirical formula finding and searched against online databases, such as ChemSpider, for identification. The SCIEX OS software offers an easy to use and intuitive workflow to tentatively identify unknown chemicals in food.

Experimental

Samples

Store-bought “Icing Colors” were diluted 10,000x using a sugar solution prepared by dissolving 10 g of sugar in 10 mL water (LC grade) to mimic the icing sugar matrix typically used for baking.

LC Separation

LC separation was performed using a SCIEX ExionLC™ AD system with a Phenomenex Luna Omega 1.6 µm Polar C18 (50 x 2.1 mm) column and a fast gradient of water and methanol with

5 mM ammonium formate buffer at a flow rate of 0.5 mL/min (see Table 1 for the gradient profile). The injection volume was 5 µL.

Table 1. Gradient conditions used for the identification of food dyes

Step	Time (min)	A (%)	B (%)
0	0.0	90	10
1	1.0	90	10
2	6.0	10	90
3	7.0	10	90
4	7.1	90	10
8	10.0	90	10

MS/MS Detection

The SCIEX X500R QTOF system with Turbo V™ source and Electrospray Ionization (ESI) was used in negative polarity.

Mass calibration was achieved using the integrated calibrant delivery system (CDS) with the TwinSprayer probe (dual ESI needle).

High resolution data were acquired using an IDA method consisting of a TOF-MS survey scan (100-1000 Da for 200 msec) and up to 10 dependent MS/MS scans (50-1000 Da for 50 msec). Declustering Potential (DP) was set to -80 V and MS/MS fragmentation was achieved using a Collision Energy (CE) of CE of -35 V with a collision energy spread of ±15 V.

Dynamic background subtraction (DBS) was activated for best MS/MS coverage. No inclusion list was used, which allowed non-target identification without the need for a second injection to acquire MS/MS data.

Data Acquisition and Processing

All data were acquired and processed using SCIEX OS software version 1.0, which showcases a thoughtfully designed user interface that is fast to learn and delivers improved lab productivity. In addition, MarkerView™ software version 1.3 was used for statistical processing using Principal Components Analysis (PCA).

Results and Discussion

X500R Performance Characteristics and Data Acquisition Workflows

The X500R QTOF system utilizes an N-optics TOF design to maximize resolution while maintaining benchtop design and a minimized footprint. Its resolving power increases with mass range providing ~30000 to 40000 resolution for the typical molecular weight range of ingredients and contaminants in food (Figure 1).

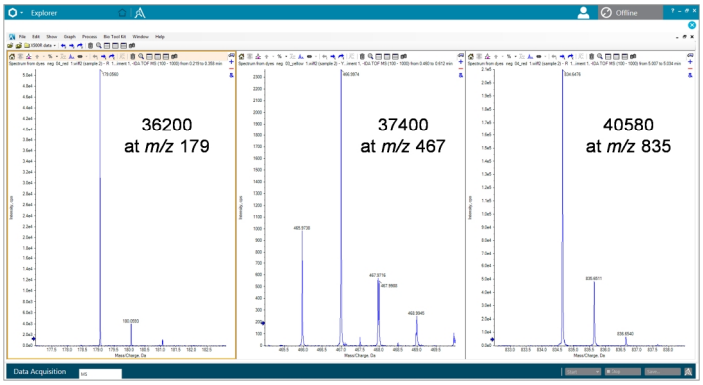

Figure 1. Resolution of different chemicals detected in negative polarity ESI in diluted dye samples

The X500R QTOF system achieves stable mass accuracy of less than 2 ppm by using a heated TOF configuration, with 6 heater drones throughout the TOF path and by using the dynamic background calibration software algorithm. The X500R QTOF's mass accuracy is supplemented by legendary dynamic transmission control and dynamic background calibration, introduced in 2010 with the TripleTOF® system and optimized over time.

In addition, the integrated CDS with the TwinSprayer probe provides an independent calibrant delivery path for reliable auto-calibration (Figure 2), maintaining mass accuracy over long periods of time by automatically calibrating in batch mode.

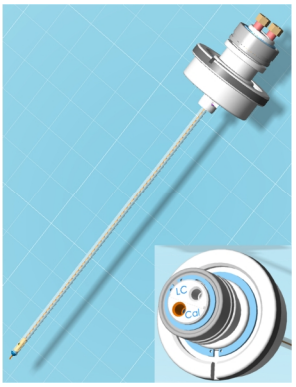

Figure 2. TwinSprayer ESI probe showing the independent inlet for LC and calibrant

The accurate mass measurement of a molecular ion is insufficient for compound identification. Single stage MS information can only be used for empirical formula finding. Because many different chemicals have identical molecular formulas accurate mass MS/MS data are absolutely necessary to identify chemical structures based on the molecular fingerprint observed in the MS/MS spectrum.

Using IDA, simultaneous acquisition of TOF-MS and MS/MS into a single data file for each sample was possible. Up to 10 MS/MS spectra were automatically collected for each chromatographic data point (Figure 3).

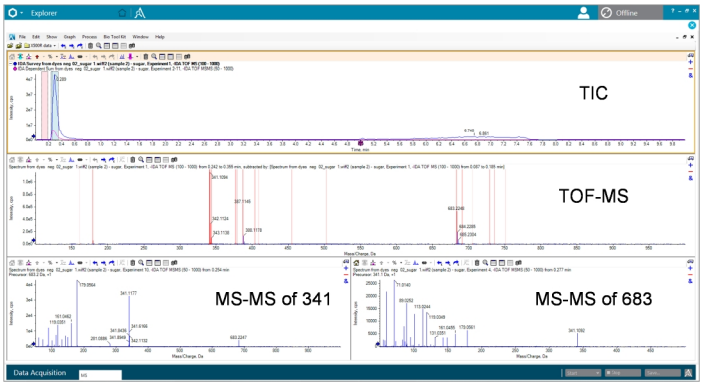

Figure 3. Simultaneous acquisition of TOF-MS and MS/MS using IDA, the example shows spectra of sucrose and sucrose dimer of the matrix

Processing Workflow for Non-Target Identification in SCIEX OS Software

Full scan chromatograms are very rich in information and easily contain thousands of ions from chemicals present in the sample, including the food matrix itself. Powerful software is needed to explore the high resolution MS and MS/MS spectra generated to get answers and results from these complex data.

Figure 4 shows Total Ion Chromatograms (TIC) of samples analyzed. It can be seen that the TIC are dominated by matrix components (sugars) eluting at ~0.3 min. The main dyes in the red and blue sample can be found, but minor components and ingredients in the yellow and brown sample are not visible.

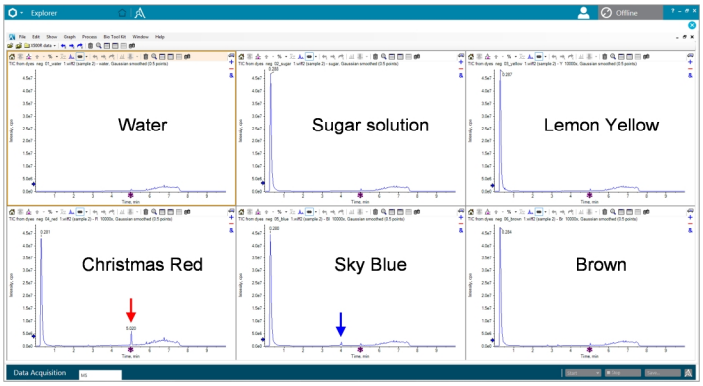

Figure 4. TIC of a blank (water), matrix (sugar solution) and for dyes samples, peak finding without software tools is very complicated or even impossible

SCIEX OS software a single platform for MS control, data processing and reporting, and provides:

- Simple software workflows that deliver reliable results
- Automated feature detection based on non-target peak finding followed by sample-control-comparison
- Automated compound identification using empirical formula finding followed by library and online database searching
- Quick data review and reporting utilizing customizable flagging and filtering of results

The workflow to setup non-target data processing is illustrated in Figures 5a and b.

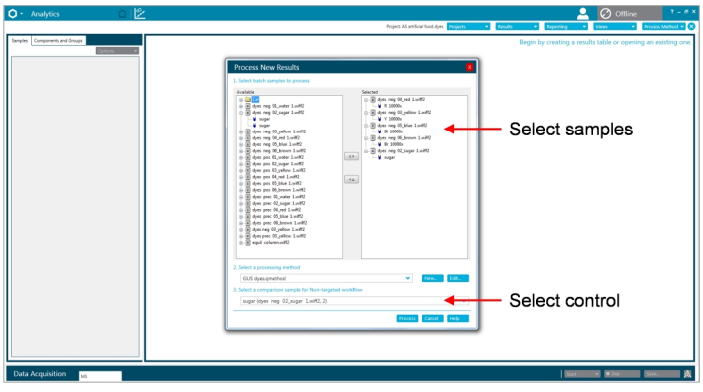

Figure 5a. Selecting unknown sample(s) and control sample for non-target data processing and sample-control comparison in SCIEX OS software

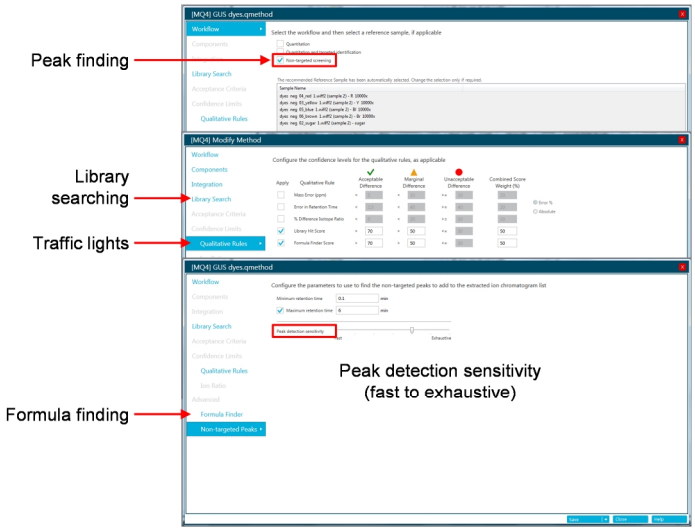

Figure 5b. Setup of non-target peak finding criteria and identification tools, including MS/MS library searching and empirical formula finding, criteria for traffic lights are set for later data review and filtering

Data Review during Non-Target Identification in SCIEX OS Software

After non-target peak finding and sample-control-comparison the results are displayed (Figure 6). The results table can be sorted and filter using the traffic lights. The Peak review will automatically provide XIC, TOF-MS and MS/MS data for both the sample and control sample.

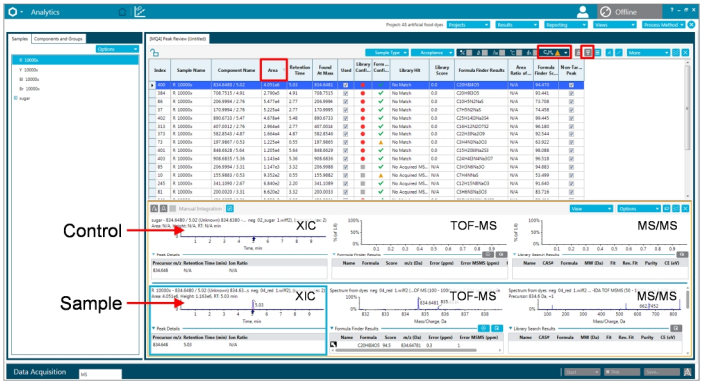

Figure 6. Results display after non-target peak finding and sample-control comparison, results were filtered by formula finding score (>70%) and sorted by intensity

Zooming into the TOF-MS spectrum provides details of formula finding, including mass error in TOF-MS and MS/MS and the number of structures found in ChemSpider for each possible formula (Figure 7).

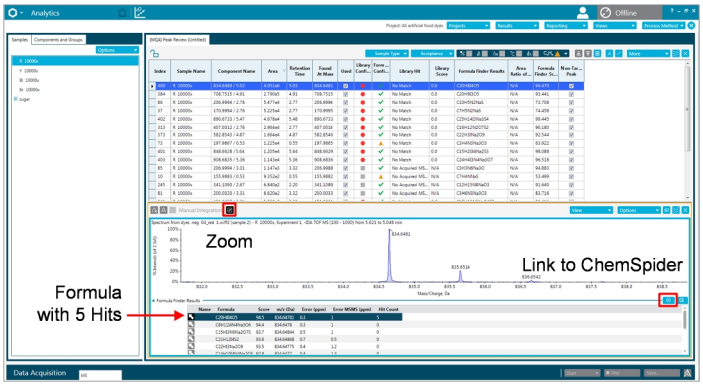

Figure 7. Zoom into TOF-MS to display detailed results of formula finding, the most likely formula has a mass error of 0.3 ppm in TOF-MS an average mass error of 1 ppm of all fragments in MS/MS and 5 matching structures in ChemSpider

From the TOF-MS display the formula can be linked to ChemSpider. The ChemSpider display will list all matching structures, automatically sorted by number of references. The selected structure is automatically fragmented in-silico and compared against the accurate mass MS/MS spectrum.

Using this workflow the main ingredient in the red food coloring was quickly identified as Erythrosine (Figure 8).

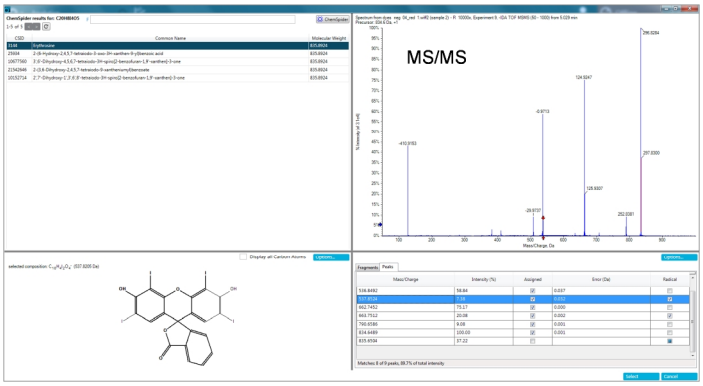

Figure 8. ChemSpider search results and in-silico fragmentation assisting to quickly identify Erythrosine in red food coloring

Results of Analyzing Food Coloring

Identified artificial dyes and by-products are summarized in Table 2. Figures 9, 10 and 11 show further examples of identification based in ChemSpider searching and MS/MS elucidation.

Table 2. Artificial dyes and by-products identified in samples

Sample	*m/z* - RT	Area %*	Formula	Formula Finder Score (%)	Mass error (ppm)	MS/MS error (ppm)	Identification
Red	834.6480 / 5.02	88.5	C20H8I4O5	94.5	0.3	1.0	**Erythrosine**
	708.7515 / 4.91	6.1	C20H9I3O5	93.4	0.5	0.9	Erythrosine-I
	890.6733 / 5.47	1.0	C23H12I4O6	92.0	0.8	0.8	Erythrosine+C3H4O
	407.0012 / 2.76	0.6	C16H12N2O7S2	96.2	0.1	1.0	**Sunset Yellow**
	582.8543 / 4.87	0.4	C20H10I2O5	92.1	0.1	2.1	Erythrosine-I2
Yellow	197.9867 / 0.53		$C7H4NO4S^-$	88.9	0.7	2.0	in-source fragment
	466.9974 / 0.53	90.3	C16H12N4O9S2	92.0	0.2	2.0	**Tartrazine**
	224.0134 / 0.53		$C8H6N3O3S^-$	93.6	0.7	0.5	in-source fragment
	501.9503 / 0.56	9.7	contains 2 Cl^-				not identified
Blue	747.1508 / 4.01	90.1	C37H36N2O9S3	86.9	0.3	3.3	**Brilliant Blue**
	577.1473 / 4.49	5.4	C30H30N2O6S2	92.5	0.2	1.7	Brilliant Blue - C7H6O3S (by-product)
	184.9909 / 0.52	4.5	C7H6O4S	78.1	2.1	2.3	3-Formylbenzene-sulfonic acid (by-product)
Brown	407.0012 / 2.76	33.9	C16H12N2O7S2	93.3	0.2	1.6	**Sunset Yellow**
	451.0277 / 3.31	24.8	C18H16N2O8S2	86.6	0.5	3.0	**Allura Red**
	834.6480 / 5.02	19.1	C20H8I4O5	92.8	0.1	1.9	**Erythrosine**
	501.9503 / 0.56	9.3	contains 2 Cl^-				not identified

* Area % includes monoisotopic peak, isotopes, adducts (i.e. Na+), multiply charged ions and in-source-fragments

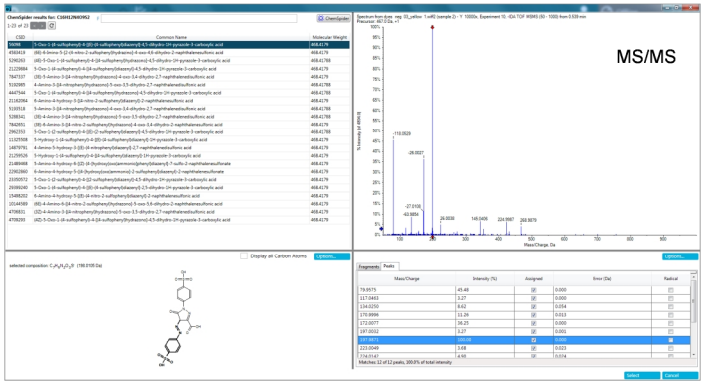

Figure 9. ChemSpider search results and in-silico fragmentation assisting to quickly identify Tartrazine in yellow food coloring

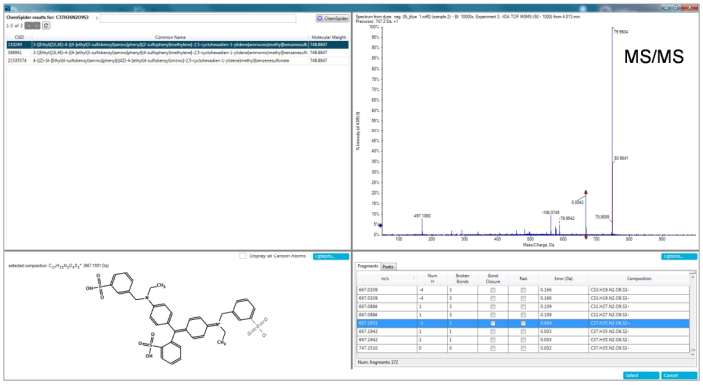

Figure 10. ChemSpider search results and in-silico fragmentation assisting to quickly identify Brilliant Blue in blue food coloring

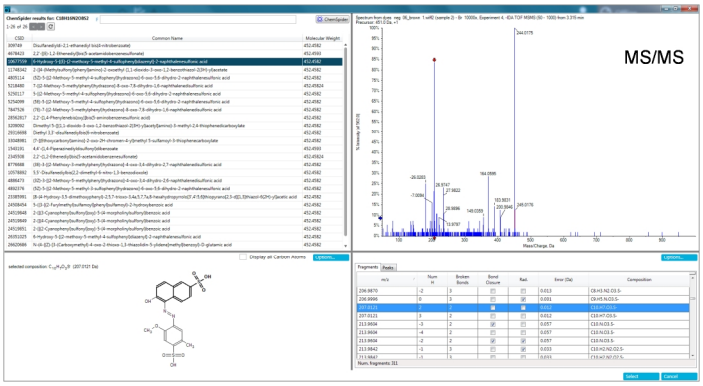

Figure 11. ChemSpider search results and in-silico fragmentation assisting in the quick identification of Allura Red in the brown food coloring

Statistical Data Analysis to Identify Unknowns

Statistical data analysis is an alternative to simple sample-control-comparison. Tools, such as Principal Components Analysis (PCA), can be used to identify characteristic markers in complex samples and at lower levels. Figure 12 shows an example of PCA performed in MarkerView™ software to find ingredients in food dyes.

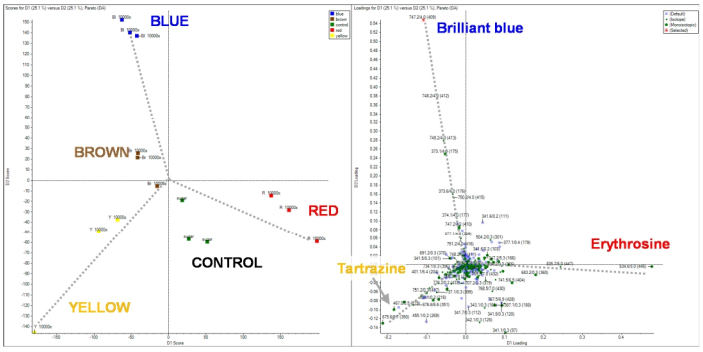

Figure 12. PCA as an alternative to sample-control comparison quickly finds differences between samples (score plot shown left) and helps to identify characteristic *m/z*-RT using the above described tools

Summary

A new non-target LC-HR-MS/MS based approach to quickly identify artificial colors in food samples was developed using the SCIEX X500R QTOF system.

Negative polarity ESI TOF-MS and MS/MS data acquired using information dependent acquisition were processed in SCIEX OS and MarkerView™ software. Characteristic *m/z*-RT were further processed using empirical formula finding and ChemSpider searching. The major compounds in food coloring were quickly identified using automated and intuitive software workflows in SCIEX OS.

References

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