

Determination of Industrial Dyes in Food by LCMS-IT-TOF

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1. Introduction

Industrial dyes is a class of dyes which has been widely used in the production of textile, fur and leather, wood and china, which using as food additive is forbidden because of potential toxic effects. Industrial dyes illegally added in food were reported frequently, therefore departments like China FDA focused on the determination of industrial dyes in food, and they needed to develop a quick, high sensitivity and high accuracy qualitative and quantitative method. The

food samples were extracted by a mixed organic solution, cleaning up on an SPE column, then the extracts were analyzed by LCMS-IT-TOF. In this study, 10 basic industrial dyes and 9 acidic industrial dyes in food were analyzed. External standard method was used for quantitative analysis, and MSⁿ (n>2) results were used for qualitative analysis.

2. Experimental

Sample pretreatment method:

Basic industrial dyes—The food sample was extracted with acetonitrile, and then skimmed with acetonitrile and hexane. In the end, the sample was purified by Oasis WCX column.

Acidic industrial dyes—The food sample was extracted with a mixed solution (methanol/water = 80/20, V/V), and then skimmed with acetonitrile. In the end, the sample was purified by Oasis WAX column.

After above three steps, the final extract was injected and detected with LCMS-IT-TOF.

UHPLC/MS parameters:

The analysis was performed on a Prominence UFLC (Shimadzu, Kyoto, Japan) equipped with LC-20AD pumps, a CTO-20A column oven, an SIL-20A autosampler, a DGU-20As degasser, a CBM-20A communication bus module and an LCMS-IT-TOF mass spectrometer.

Analytical conditions for UHPLC

Column : Shim-pack XR-ODSII
(100 mmL. × 2.0 mmi.d., 2.2 μm)
Flow rate : 0.2 mL/min
Column oven : 30°C
Elution mode : Gradient elution
Mobile phase : For basic dyes;
A: 5 mmol/L ammonium acetate,
B: 0.1% formic acid in acetonitrile
For acidic dyes;
A: 10 mmol/L ammonium acetate,
B: methanol

Analytical conditions for MS

Ionization : ESI
Polarity : basic dyes; positive
acidic dyes; negative
Mass range : basic dyes *m/z* 250-510
acidic dyes *m/z* 210-700
Nebulizer gas : N₂ 1.50 L/min;
Drying gas : N₂ 10 L/min;
CDL temperature : 200°C
Heat block temperature : 200°C

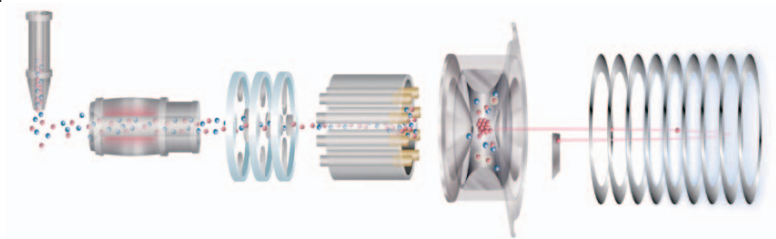


Fig. 1 Schematic diagram of the LCMS-IT-TOF

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3. Results and Discussion

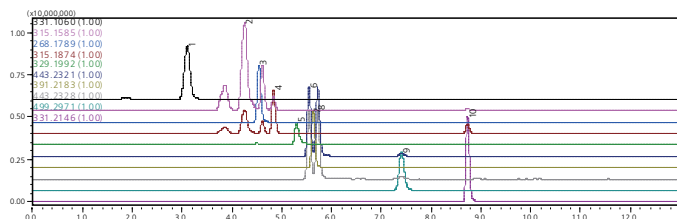


Fig. 2 Mass chromatograms of 10 basic dyes
(1. Rhodamine 110, 2. Basic red 2, 3. Auramine O,
4. Astrazon Orange G, 5. Malachite Green, 6. Rhodamine B,
7. Astrazon Orange R, 8. Rhodamine 6G,
9. Butyl Rhodamine B, 10. Leucomalachite Green)

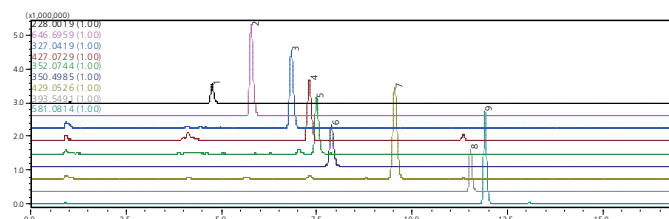


Fig. 3 Mass chromatograms of 9 acidic dyes
(1. Azorubin, 2. Acid Red 87, 3. Orange 2, 4. Acid Yellow A4-R,
5. Acid Yellow 36, 6. Tracid Brilliant Red B, 7. Orange 3,
8. Tracid Brilliant Red 10B, 9. Acid Orange 67)

Table 1 Quantitative result of basic dyes

No.	Basic Dyes	R.T. (min)	m/z	Linear Equation	Linear Range (µg/L)	Correlation coefficient (r)	RSD/% (n=7)
1	Rhodamine 110	3.034	331.1060	Y = 726481.7X + 3355202	1-100	0.9944	5.0
2	Basic red 2	4.147	315.1585	Y = 939612.0X + 6832046	1-100	0.9924	2.9
3	Auramine O	4.55	268.1789	Y = 478763.8X + 2212455	1-100	0.9954	4.4
4	Astrazon Orange G	4.867	315.1874	Y = 416215.4X + 1143541	1-100	0.9988	3.8
5	Malachite Green	5.386	329.1992	Y = 351826.8X + 408018.1	1-100	0.9989	4.1
6	Rhodamine B	5.669	443.2321	Y = 519311.1X + 3287386	1-100	0.9935	3.1
7	Astrazon Orange R	5.76	391.2183	Y = 432170.3X + 2682160	1-100	0.9931	2.5
8	Rhodamine 6G	5.801	443.2328	Y = 551295.2X + 3627538	1-100	0.9932	2.9
9	Butyl Rhodamine B	8.069	499.2971	Y = 608686.3X + 3178368	1-100	0.9944	8.5
10	Leucomalachite Green	8.827	331.2146	Y = 570237.4X + 4472225	1-100	0.9915	4.6

Table 2 Quantitative results of acidic dyes

No.	Acidic Dyes	Matrix	R.T. (min)	m/z	Linear Equation	Linear Range (mg/L)	correlation coefficient (r)	RSD/% (n=7)
1	Acid Red 87	Condiment	5.755	646.6959	Y = 18712.66X - 2188798	0.1-4	0.9960	4.4
2	Acid Yellow 36	Condiment	7.465	352.0744	Y = 120547.8X - 205766.1	0.01-0.4	0.9996	1.9
3	Orange 2	Condiment	6.810	327.0419	Y = 151089.4X + 147236.4	0.01-0.4	0.9997	1.6
4	Azorubin	Condiment	4.740	228.0019	Y = 1680.244X - 94578.77	0.2-8	0.9996	3.8
5	Orange 3	Condiment	9.500	429.0526	Y = 2059937X + 1033213	0.001-0.04	0.9989	2.2
6	Acid Orange 67	Condiment	11.900	581.0814	Y = 69577.60X + 759077.10	0.02-0.8	0.9972	4.7
7	Tracid Brilliant Red 10B	Condiment	11.515	393.5491	Y = 18895.15X - 1572,776	0.05-2	0.9919	3.3
8	Acid Yellow A4-R	Condiment	7.275	427.0729	Y = 117428.3X - 167170.2	0.01-0.4	0.9996	2.1
9	Tracid Brilliant Red B	Condiment	7.830	350.4985	Y = 12013.91X - 1654,808	0.1-4	0.9969	2.0

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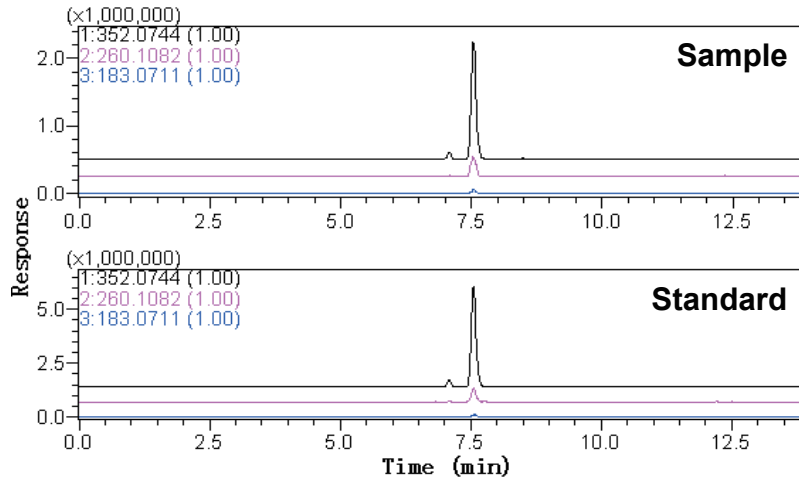


Fig. 4 Mass chromatograms of Yellow 36 in red pepper

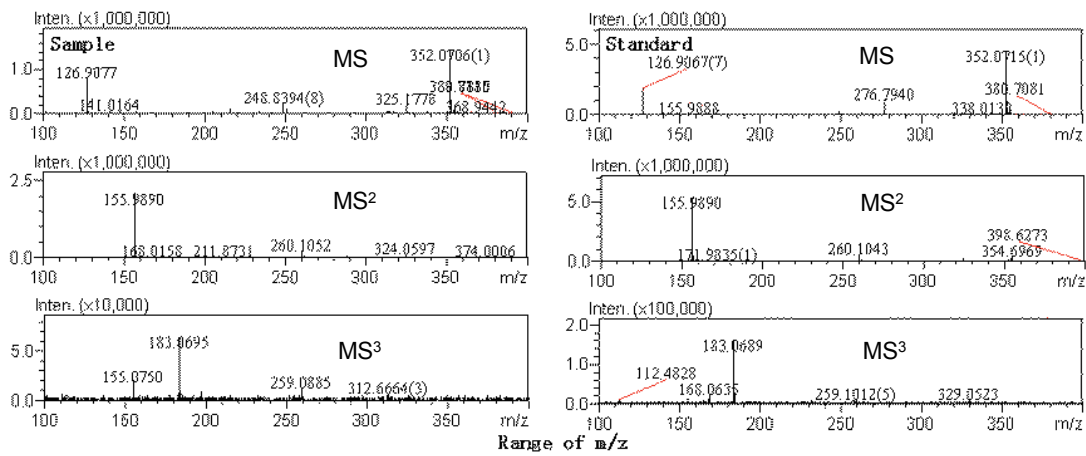


Fig. 5 MS3 spectrum of Acid Yellow 36

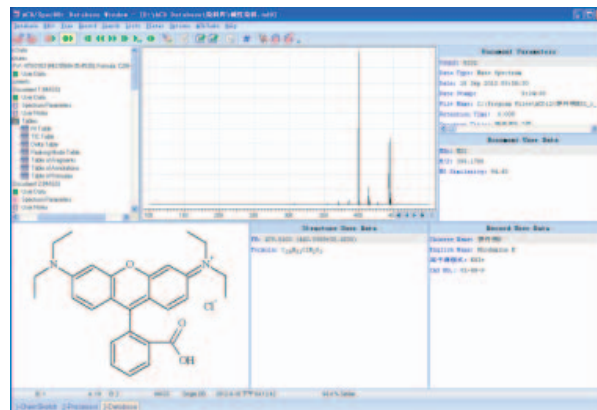


Fig. 6 ACD MS Manager database

4. Conclusions

10 basic industrial dyes and 9 acidic industrial dyes in food were determined by LCMS-IT-TOF, and quantified by external standard method. The developed methods show a good linearity range, and in the certain concentration ranges with their correlation coefficients (r) more than 0.99. For basic industrial dyes analysis, the relative standard deviations ($n=7$) were less than 9%. The average recoveries of the ten basic dyes at three levels (2, 10 and 25 $\mu\text{g}/\text{kg}$) were ranged from 65.3% to 119.2%. For acidic industrial dyes analysis, average recoveries of acid dyes in the spiked samples at three different levels

varied from 64.8% to 106.0% with the relative standard deviations ($n=5$) better than 6.7%.

In general, the methods established in our study were simple, rapid, and highly sensitive. They were suitable for the simultaneous determination of basic dyes or acidic dyes residue in food. An MS^n database (ACD MS Manager database) for these industrial dyes had been created, which provided a quick and easy way for accurate qualitative and screening of industrial dyes in food.