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Identification of unlawful food dyes by thin-layer chromatography–fast atom bombardment mass spectrometry

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Abstract

A thin-layer chromatographic–fast atom bombardment mass spectrometric (TLC–FAB-MS) method incorporating an analyte condensation technique was established for the identification of the 27 food dyes consisting the twelve dyes permitted for use in foods and the fifteen unlawful dyes in Japan. The use of magic bullet [1,4-dithiothreitol–1,4-dithioerythritol (3:1)] as a matrix allowed the measurement of the FAB mass spectra of the food dyes except for Food Blue No. 2 (Indigo Carmine). The separation was performed on a C_{18}-modified silica gel TLC plate using the following two solvent systems: methanol–acetonitrile–5% aqueous sodium sulphate solution (3:3:10) and methyl ethyl ketone–methanol–5% aqueous sodium sulphate solution (1:1:1). The condensation technique for concentration of a diffuse sample spot on the TLC plate improved the detection limit 4–20-fold with good reproducibility. The method was successfully applied to the identification of unlawful dyes in imported foods.

1. Introduction

Over 50 synthetic dyes are used in foods all over the world [1]. In Japan, the following twelve dyes are permitted for use in foods; Amaranth [Color Index (C.I.) No. 16185, R-2), Erythrosin (C.I. No. 45430, R-3), Allura Red AC (C.I. No. 16035, R-40), New Coccine (C.I. No. 16255, R-102), Phloxine (C.I. No. 45410, R-104), Rose Bengal (C.I. No. 45440, R-105), Acid Red (C.I. No. 45100, R-106), Tartrazine (C.I. No. 19140, Y-4), Sunset Yellow FCF (C.I. No. 14982, Y-5), Fast Green FCF (C.I. No. 42053, G-3), Brilliant Blue FCF (C.I. No. 42090, B-1) and Indigo Carmine (C.I. No. 73015, B-2). However, unlawful dyes are also frequently detected in foods [2], so the inspection of dyes in foods has been continued by public health agencies.

The analyses of foods for dyes have been mainly achieved by thin-layer chromatography (TLC), because it is a simple and effective technique for the separation of components in a mixture. However, the only useful information obtained from a TLC plate to identify components is the R_F values, and the identification of the separated components is difficult unless an appropriate spectrometric method such as mass spectrometry (MS) is used. A stepwise operation including separation by TLC and measurement of the individual mass spectra is laborious and time consuming, because it needs extra steps such as extraction of the desired compounds from the TLC plate and elimination of adsorbents. Recently, the direct analysis of TLC spots in a mass spectrometer has been reported, and

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TLC–fast atom bombardment (FAB) and TLC–liquid secondary ion (LSI) MS have been successfully applied to the identification of drugs, their metabolites and dyes [3–9].

In the previous study [10], we successfully applied TLC–FAB-MS with a condensation technique to the identification of the twelve permitted food dyes in Japan. Although this method is highly effective, an evaluation of its applicability to unlawful dyes has not been achieved. As unlawful dyes are frequently detected in foods as mentioned above, we wished to establish an identification method for unlawful dyes using TLC–FAB-MS. In this paper, we report an identification technique for the 27 dyes constituting the above twelve permitted and the following fifteen unlawful dyes using TLC–FAB-MS: Ponceau 3R (C.I. No. 16155, R-1), Ponceau SX (C.I. No. 14700, R-4), Oil Red (C.I. No. 12140, R-5), Ponceau R (C.I. No. 16150, R-101), Eosine (C.I. No. 45380, R-103), Azo Rubine (C.I. No. 14720, R-AZ), Orange I (C.I. No. 14600, O-1), Orange RN (C.I. No. 15970, O-RN), Oil Orange SS (C.I. No. 12100, O-SS), Naphthol Yellow S (C.I. No. 10316, Y-1), Yellow AB (C.I. No. 11380, Y-2), Yellow OB (C.I. No. 11390, Y-3), Guinea Green B (C.I. No. 42085, G-1), Wool Green S (C.I. No. 44090, G-S) and Acid Violet 6B (C.I. No. 42640, V-1). Finally, the established technique was applied to the identification of unlawful dyes in important foods.

2. Experimental

2.1. Chemicals

Magic bullet [a mixture of 1,4-dithiothreitol and 1,4-dithioerythritol (3:1)], glycerol, thioglycerol, diethanolamine, triethanolamine, *o*-nitrophenyl octyl ether, *m*-nitrobenzyl alcohol, R-1, R-4, R-5, R-101, R-103, R-AZ, O-1, O-RN, O-SS, Y-1, Y-2, Y-3, and G-1 were purchased from Tokyo Kasei (Tokyo, Japan), R-2, R-3, R-40, R-102, R-104, R-105, R-106, Y-4, Y-5, G-3, B-1, B-2 and V-1 from San-Ei Gen FFI (Osaka, Japan) and G-S from Aldrich (Milwaukee, WI, USA).

2.2. Thin-layer chromatography

TLC was performed on precoated glass-backed C_{18}-modified silica gel TLC plates (Merck, 15423). The following solvent systems were used: methanol–acetonitrile–5% aqueous sodium sulphate solution (3:3:10) (solvent system A) and methanol–methyl ethyl ketone–5% aqueous sodium sulphate solution (1:1:1) (solvent system B).

2.3. Extraction of unlawful dyes from foods

An amount of 20 g of the sample was dissolved in 50 ml of water and the solution was acidified by the addition of 5 ml of concentrated acetic acid. A piece of pure wool that had been throughly washed in boiling 10% ammonia solution and boiling water was added to the sample solution. The coloured wool was removed, rinsed thoroughly with water and then heated slightly in 3% ammonia solution to remove colour. The solution was evaporated to dryness and the residue was dissolved in methanol.

2.4. Sample condensation technique on TLC plate

The following sample condensation technique was used to concentrate an analyte on the TLC plate before measurement of its FAB mass spectrum (Fig. 1): (1) an area including the desired spot on the developed TLC plate was cut rectangularly; (2) a small volume of methanol was applied around the sample spot on the developed TLC plate; (3) after several tens of seconds, the sample was condensed towards the centre of the spot in a line with penetration of methanol.

2.5. Mass spectrometry

After application of the condensation technique, the TLC plate was placed on the TLC holder, a matrix was applied on the sample spot

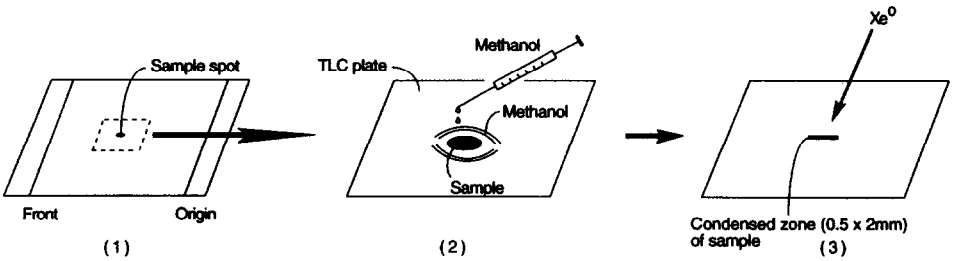

Fig. 1. Procedure for the sample condensation technique.

and the TLC–FAB mass spectrum was measured using a JMS-AX505W mass spectrometer (JEOL, Tokyo, Japan) with a TLC–FAB ion source. The FAB gun was operated with xenon gas at 5 kV using an acceleration voltage of 3 kV for measurement of all spectra.

3. Results and discussion

To apply TLC–FAB-MS successfully to the identification of food dyes, aspects that must be carefully investigated include the selection of a suitable matrix, TLC conditions and sample condensation on the TLC plate. These are discussed below.

3.1. Selection of suitable matrix

Organic dyes possess conjugated chromophores including azo, immonium and quinone groups that are generally fragile and easily reducible. It is well known that these dyes are readily reduced under FAB-MS conditions [11–13]. In our basic studies on FAB and LSIMS of the twelve permitted dyes [7], we observed these phenomena and found that only the use of magic bullet as a matrix depresses the reduction of the dyes and gives correctly molecular ion species. In this study, magic bullet was examined for the TLC–FAB-MS of the fifteen unlawful dyes using a conventional procedure. It depressed the reduction to give the molecular ion species of the dyes with the theoretical isotopic abundances. Therefore, we selected magic bullet as a matrix.

3.2. TLC conditions

For the separation of food dyes by TLC, silica gel, polyamide and C_{18}-modified silica gel have been used as stationary phases [1,14]. TLC–FAB-MS on a normal-phase plate usually provides a higher sensitivity than is observed with a reversed-phase plate [8]. On the other hand, a reversed-phase plate gives better results than normal-phase plates such as silica gel and polyamide plates for the separation of the dyes. Our previously reported C_{18}-modified silica gel TLC method with two solvent systems can separate the twelve permitted dyes completely with no tailing spots and good reproducibility [14,15]. Further, the separation is not affected by coexisting substances from foods and the spots of dyes extracted from foods always give the same R_F values as the authentic standards [15]. Therefore, we applied C_{18}-modified silica gel TLC in this study. The chromatograms gave roundish spots of the dyes with no tailing and good reproducibility. Table 1 gives the R_F values for the 27 food dyes studied. Only five and eight isolated spots of the dyes could be observed using solvent systems A and B, respectively. However, the combined use of both solvent systems enabled us to distinguish nineteen dyes. Although unsatisfactory results were obtained for distinguishing dyes between R-3 and R-103, between R-5 and O-SS and among R-1, R-101, R-AZ and G-S, it is not always necessary to differentiate between overlapping dyes, because these dyes have different molecular masses and they can be identified by the measurement of FAB mass spectra after TLC separation. Therefore, the TLC conditions for TLC–FAB-MS of

Table 1
R_F values of dyes on reversed-phase TLC plates

Dye	R_F	
	Solvent system A[a]	Solvent system B[b]
Ponceau 3R (R-1)	0.16	0.86
Amaranth (R-2)[c]	0.71	1.00
Erythrosine (R-3)[c]	0.00	0.40
Ponceau SX (R-4)	0.21	0.89
Oil Red (R-5)	0.00	0.06
Allura Red AC (R-40)[c]	0.34	1.00
Ponceau R (R-101)	0.22	0.89
New Coccine (R-102)[c]	0.58	1.00
Eosine (R-103)	0.00	0.42
Phloxine (R-104)[c]	0.00	0.15
Rose Bengal (R-105)[c]	0.00	0.19
Acid Red (R-106)[c]	0.04	0.77
Azo Rubine (R-AZ)	0.19	0.88
Orange I (O-1)	0.15	0.75
Orange RN (O-RN)	0.05	0.61
Oil Orange SS (O-SS)	0.00	0.09
Naphthol Yellow S (Y-1)	0.42	0.90
Yellow AB (Y-2)	0.00	0.16
Yellow OB (Y-3)	0.00	0.13
Tartrazine (Y-4)[c]	0.79	1.00
Sunset Yellow FCF (Y-5)[c]	0.45	1.00
Guinea Green B (G-1)	0.03	0.73
Fast Green FCF (G-3)[c]	0.17	1.00
Wool Green S (G-S)	0.22	0.90
Brilliant Blue FCF (B-1)[c]	0.14	1.00
Indigo Carmine (B-2)[c]	0.66	1.00
Acid Violet 6B (V-1)	0.01	0.67

[a] Solvent system A = methanol–acetonitrile–5% sodium sulphate solution (3:3:10).
[b] Solvent system B = methanol–methyl ethyl ketone–5% sodium sulphate solution (1:1:1).
[c] Permitted dyes for use in foods in Japan.

the food dyes were standardized to the use of a C_{18}-modified silica gel plate with two solvent systems as described under Experimental.

3.3. *Condensation of spots of dyes on TLC plates*

When a matrix is deposited on a sample spot on a TLC plate, diffusion of the sample usually occurs with spreading of the matrix used, so that no satisfactory spectrum is obtained with good sensitivity unless a large amount of sample is applied to the TLC plate. In previous work, to prevent diffusion of the analyte and to obtain higher sensitivity in the TLC–FAB mass spectrum, we developed a sample condensation technique [4,7,8]. Although the diffusion of a sample on a reversed-phase plate is not as great as is observed using normal-phase plates when a matrix is deposited on the spot, no satisfactory spectra were obtained unless more than 20 μg per spot of the dyes was applied to the TLC plate without the condensation technique. To obtain high sensitivity, the developed spot was reconcentrated on the TLC plate using the concentration technique described under Experimental.

Table 2 shows the molecular ion species and the detection limits of the dyes using TLC–FAB-MS. The $[M + H]^+$ ion appeared clearly for all

Table 2
Molecular ion species in the TLC–FAB mass spectra of the dyes and their detection limits

Dye	Molecular ion species[a]				Detection limit (μg per spot)
	$[M+Na]^+$	$[M+H]^+$	$[M-Na+2H]^+$	$[M-2Na+3H]^+$	
Ponceau 3 R (R-1)	517 (38)	495 (100)	473 (31)	ND[c]	5
Amaranth (R-2)[b]	627 (33)	605 (100)	583 (85)	ND	5
Erythrosine (R-3)[b]	903 (19)	881 (100)	859 (73)	837 (35)	1
Ponceau SX (R-4)	503 (24)	481 (100)	459 (30)	ND	5
Oil Red (R-5)	ND	277 (100)	ND	ND	0.5
Allura Red AC (R-40)[b]	519 (31)	497 (100)	ND	ND	5
Ponceau R (R-101)	503 (28)	481 (100)	459 (43)	ND	5
New Coccine (R-102)[b]	627 (39)	605 (100)	583 (51)	ND	5
Eosine (R-103)	715 (20)	693 (100)	671 (87)	649 (73)	0.5
Phloxine (R-104)[b]	853 (30)	831 (100)	809 (47)	787 (37)	0.5
Rose Bengal (R-105)[b]	1041 (14)	1019 (100)	997 (27)	975 (15)	0.5
Acid Red (R-106)[b]	603 (43)	581 (100)	559 (16)	ND	0.03
Azo Rubine (R-AZ)	525 (30)	503 (100)	481 (28)	ND	5
Orange I (O-1)	373 (82)	351 (100)	329 (175)	ND	1
Orange RN (O-RN)	373 (143)	351 (100)	329 (246)	ND	1
Oil Orange SS (O-SS)	ND	263 (100)	ND	ND	0.5
Naphthol Yellow S (Y-1)	381 (30)	359 (100)	337 (20)	ND	5
Yellow AB (Y-2)	ND	248 (100)	ND	ND	0.5
Yellow OB (Y-3)	ND	262 (100)	ND	ND	0.5
Taratrazine (Y-4)[b]	557 (25)	535 (100)	ND	ND	5
Sunset Yellow FCF (Y-5)[b]	475 (37)	453 (100)	ND	ND	5
Guinea Green B (G-1)	713 (89)	691 (100)	669 (48)	ND	1
Fast Green FCF (G-3)[b]	831 (68)	809 (100)	787 (61)	ND	1
Wool Green S (G-S)	599 (68)	577 (100)	555 (25)	ND	1
Brilliant Blue FCF (B-1)[b]	815 (54)	793 (100)	771 (37)	ND	1
Indigo Carmine (B-2)[b]	ND	ND	ND	ND	ND
Acid Violet 6B (V-1)	756 (23)	734 (100)	712 (70)	ND	1

[a] m/z values with relative abundances (%) in parentheses.
[b] Permitted dyes for use in foods in Japan.
[c] ND = not detected.

dyes except for B-2 and some of them also showed $[M+Na]^+$, $[M-Na+2H]^+$ and $[M-2Na+3H]^+$. These molecular ion species are considered to be useful for the identification of the food dyes. The detection limits of the molecular ion species vary with their chemical structures. Xanthene dyes (R-3, R-103, R-104, R-105 and R-106) showed high sensitivity, ranging from 0.03 to 1.0 μg per spot. Triphenylmethane dyes (G-1, G-3, G-S, B-1 and V-1) gave molecular ion species at concentrations of 1.0 μg per spot. Although azo dyes having carboxylate and/or sulphate groups (R-1, R-2, R-4, R-40, R-101, R102, R-AZ, O-1, O-RN, Y-4 and Y-5) showed a lower sensitivity of 1.0–5.0 μg per spot, oil-soluble azo dyes having no carboxylate and/or sulphate groups (R-5, O-SS, Y-2 and Y-3) showed a moderate sensitivity of 0.5 μg per spot. Naphthalene dye (Y-1) gave a low sensitivity of 5.0 μg per spot. Hence the technique can improve the detection limits of dyes 4–20-fold with good reproducibility.

3.4. *Identification of unlawful dyes in foods*

In order to evaluate the capability of the proposed method, TLC–FAB-MS was finally applied to the identification of unlawful food dyes in imported candy and powdered juice. The dyes were extracted with degreased wool from

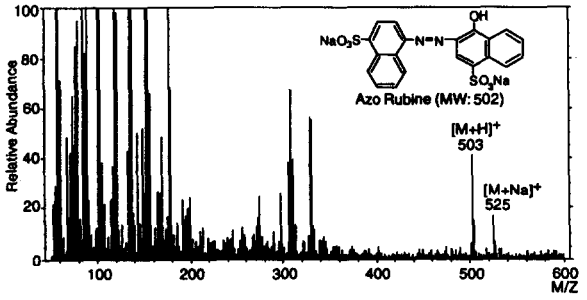

Fig. 2. TLC–FAB mass spectrum of an unknown red dye from a candy.

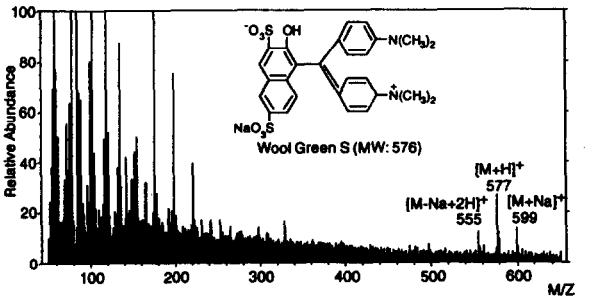

Fig. 4. TLC–FAB mass spectrum of an unknown green dye from powdered juice.

foods as described under Experimental. The residues were dissolved in methanol and the solutions were subjected to TLC–FAB-MS. The candy contained a red food dye which was suspected to be R-1, R-101 or R-AZ from the results of TLC analyses with solvent system A. As shown in Fig. 2, the two molecular ion species at m/z 525 ($[M + Na]^+$) and m/z 503 ($[M + H]^+$) are clearly observed in the TLC–FAB mass spectrum. After comparison with the authentic standard, this dye was identified as Azo Rubine (R-AZ), which is an unlawful dye in Japan. The imported powdered juice contained three dyes, as shown in Fig. 3, and we suspected them to be Y-4, Y-5 and G-S from the R_F values in Table 2. Because G-S is not permitted for use in foods in Japan, we focused on the identification of this green spot. The spectrum obtained is shown in Fig. 4. Three molecular ion species, m/z 599 ($[M + Na]^+$), m/z 577 ($[M + H]^+$) and m/z 555 ($[M - Na + 2H]^+$), clearly appear and this dye was identified as Wool Green S (G-S). Hence the effectiveness of the proposed method for the identification of unlawful dyes in foods was confirmed.

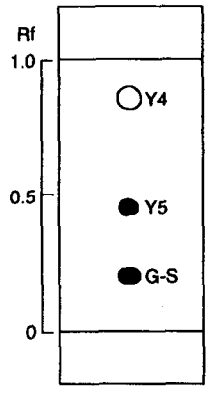

Fig. 3. TLC separation of the dyes extracted from powdered juice. Plate: C_{18}-modified silica gel. Solvent system: methanol–acetonitrile–5% aqueous sodium sulphate solution (3:3:10) (solvent system A).

4. Conclusions

A TLC–FAB-MS method with a sample condensation technique was developed for the identification of 27 food dyes constituting the twelve permitted dyes for use in foods and the fifteen unlawful dyes in Japan. The method was performed under the following conditions: TLC plate, C_{18}-modified silica gel; mobile phases, methanol–acetonitrile–5% sodium sulphate solution (3:3:10, solvent system A) and methanol–methyl ethyl ketone–5% sodium sulphate solution (1:1:1) (solvent system B); matrix, magic bullet; condensation of spot, with methanol. The method has been successfully applied to the identification of unlawful dyes in imported foods.

5. Acknowledgement

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