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Application News

MALDI-TOF Mass Spectrometry Analysis MALDI-8030

Negative Mode Detection of Sulfonated Azo Colourants in Sweets/Candies using the MALDI-8030 Dual Polarity Benchtop MALDI-TOF Mass Spectrometer

S. Salivo

User Benefits

- Simple easy analysis of sulfonated azo dyes on an affordable benchtop MALDI-TOF
- Quality spectra with good resolution and accuracy in negative ion mode
- Workflow useful for detecting regulated colourants in food

■ Introduction

Synthetic colourants are a type of additive that are widely used in food, pharmaceutical and cosmetics manufacturing. In foods, colourants are used to: i) make food more attractive and appetizing; ii) provide or enhance colours already present; iii) correct natural variations in colour. Among them, sulfonated azo dyes are popular because of their stability, solubility in water and low cost.

Colourants are subject to stringent regulations by different bodies around the world over their safety and adverse effects on human health. For example, in the United States (US), the FDA is responsible for the approval of colourants for use in foods, drugs and cosmetics, while in Europe they are regulated by the European Food Safety Authority.

Among the various approved food colourants, there are some which have been flagged following scientific research, over a possible link to attention deficit hyperactivity disorders (ADHD) in children: Sunset Yellow FCF (E110), Tartrazine (E102) and Allura Red AC (E129). While these dyes have not been completely banned in the EU, food manufacturers are required to apply warning labels on products containing the dyes of concern.

Here, we demonstrate the capability of the dual polarity MALDI-8030 benchtop linear MALDI-TOF mass spectrometer to detect the presence of sulfonated azo colourants in commercial sweets/candies, known to contain these colourants (Table 1). The dyes are selectively extracted via ion-pair extraction [1], and analysed in negative ion mode (Fig 1).

Table 1 List of colourants reported in the ingredients of the commercial candy used for this study

Common Name(s)	E number[b]	Status	Colour
Sunset Yellow FCF (FD&C[a] Yellow 6)	E110	Warning labels in EU. Approved in US.	
Tartrazine (FD&C Yellow 5)	E102	Warning labels in EU. Approved in US.	
Allura Red AC (FD&C Red 40)	E129	Warning labels in EU. Approved in US.	
Brilliant Blue FCF (FD&C Blue 1)	E133	Approved in EU and US.	
Indigo carmine (FD&C Blue 2)	E132	Approved in EU and US.	

a: approved for use in foods, drugs and cosmetics by FDA (US).
b: approved for use in foods by the European Food Safety Authority.

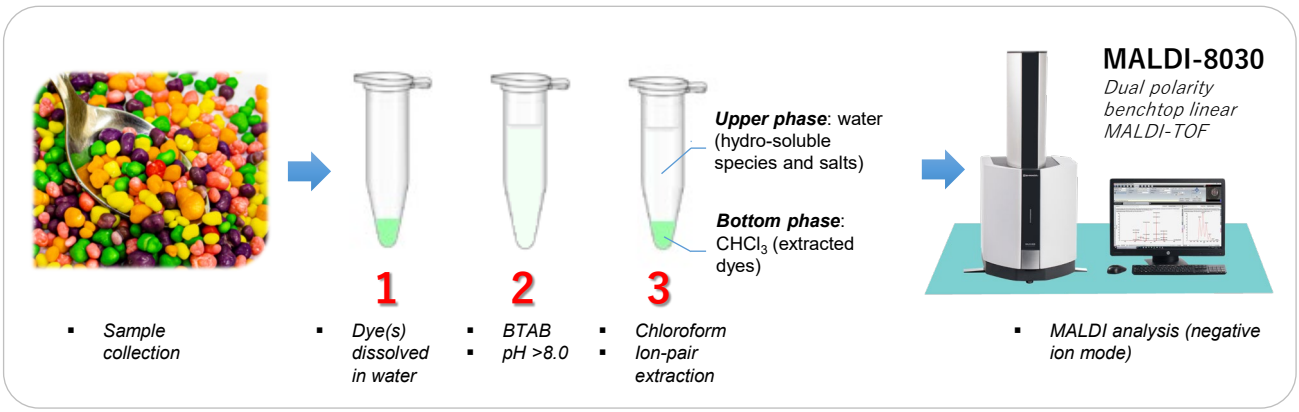

Fig. 1 Sample preparation and analysis workflow for the detection of sulfonated azo colourants in commercial sweets/candies

■ Measurement Conditions and Samples

Samples of US commercial candies were purchased in the UK at a local supermarket. The following sulfonated azo dye standards were purchased from Merck Life Science: Sunset Yellow FCF (dye content 90 %); Allura Red AC (dye content 80 %); Tartrazine (dye content ≥85 %); Brilliant Blue FCF. The reagents used for the ion-pair extraction were: benzyltributylammonium bromide (BTAB) 10 mM [1], Sodium hydroxide (NaOH) 1M for pH adjustment. Individual stock solutions of the dye standards were prepared at 1 mg/mL in 1:1 water/methanol.

The sample preparation workflow is illustrated in Fig 1. The ion-pair extraction method was first optimised and validated with the dye standards. The dyes in the candies were dissolved in water through vortexing until the inner core of the candy was exposed (Fig 1, step 1). Each coloured candy was extracted separately. The aqueous solution containing the dissolved dyes was aspirated and transferred to a microcentrifuge tube, where BTAB (10 mM) was added and the pH adjusted to >8.0 with NaOH (1M) (Fig 1, step 2). Chloroform was then added, and the bi-phasic solution agitated to facilitate the formation of ion-pairs of the sulfonated azo dyes and the subsequent extraction in chloroform (Fig 1, step 3). After centrifugation, the upper (aqueous) phase was discarded, and the bottom (organic) phase containing the extracted dyes recovered for analysis.

For the MALDI analyses, samples were spotted with 9-Aminoacridine (9AA, 10 mg/mL in methanol). All analyses were conducted in negative ion mode on the MALDI-8030.

■ Results of Sulfonated azo dyes (standards)

Fig 2 shows the negative mode MALDI spectra obtained for the sulfonated azo dye standards following ion-pair extraction: Sunset Yellow FCF (FD&C Yellow 6, E110; Fig 2a); Allura Red AC (FD&C Red 40, E129; Fig 2b); Tartrazine (FD&C Yellow 5, E102; Fig 2c); Brilliant Blue FCF (FD&C Blue 1, E133; Fig 2d). The m/z detected correspond to the intact species after removal of the sodium ions as result of the ion-pair extraction. All standard dyes were successfully detected as monoisotopic species along with good mass accuracy: i) Sunset Yellow FCF (m/z 407.001 exact; m/z 407.071 detected; -0.07 Da error); ii) Allura Red AC (m/z 451.027 exact; m/z 451.008 detected; 0.019 Da error); iii) Tartrazine (m/z 466.997 exact; m/z 467.017 detected; -0.02 Da error); iv) Brilliant Blue FCF (m/z 747.151 exact; m/z 747.152 detected; -0.001 Da error).

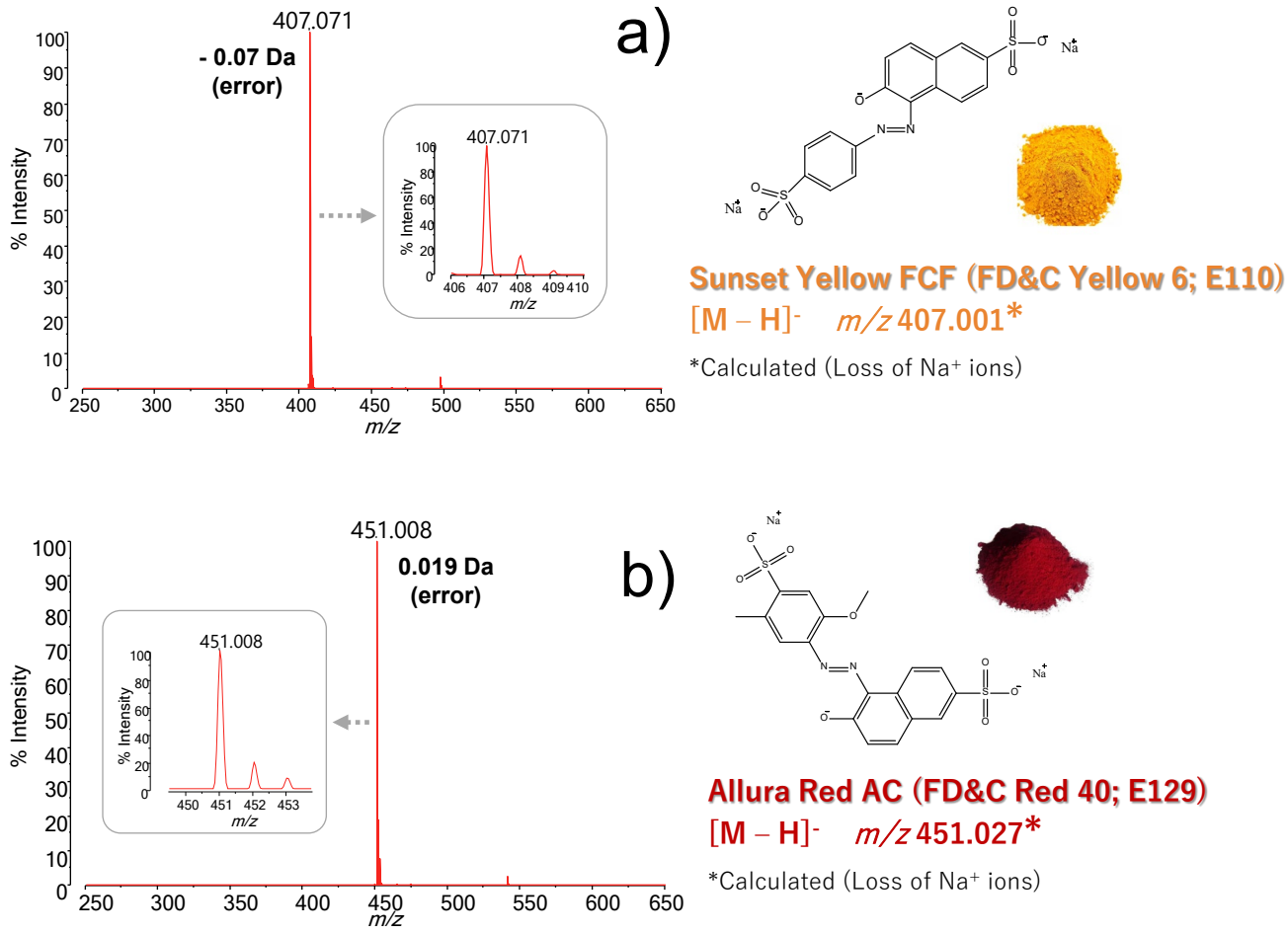

Fig. 2 (continued on next page). Negative mode MALDI spectra of standard sulfonated azo dyes after ion-pair extraction. a) Sunset Yellow FCF (FD&C Yellow 6; E110); b) Allura Red AC (FD&C Red 40; E129)

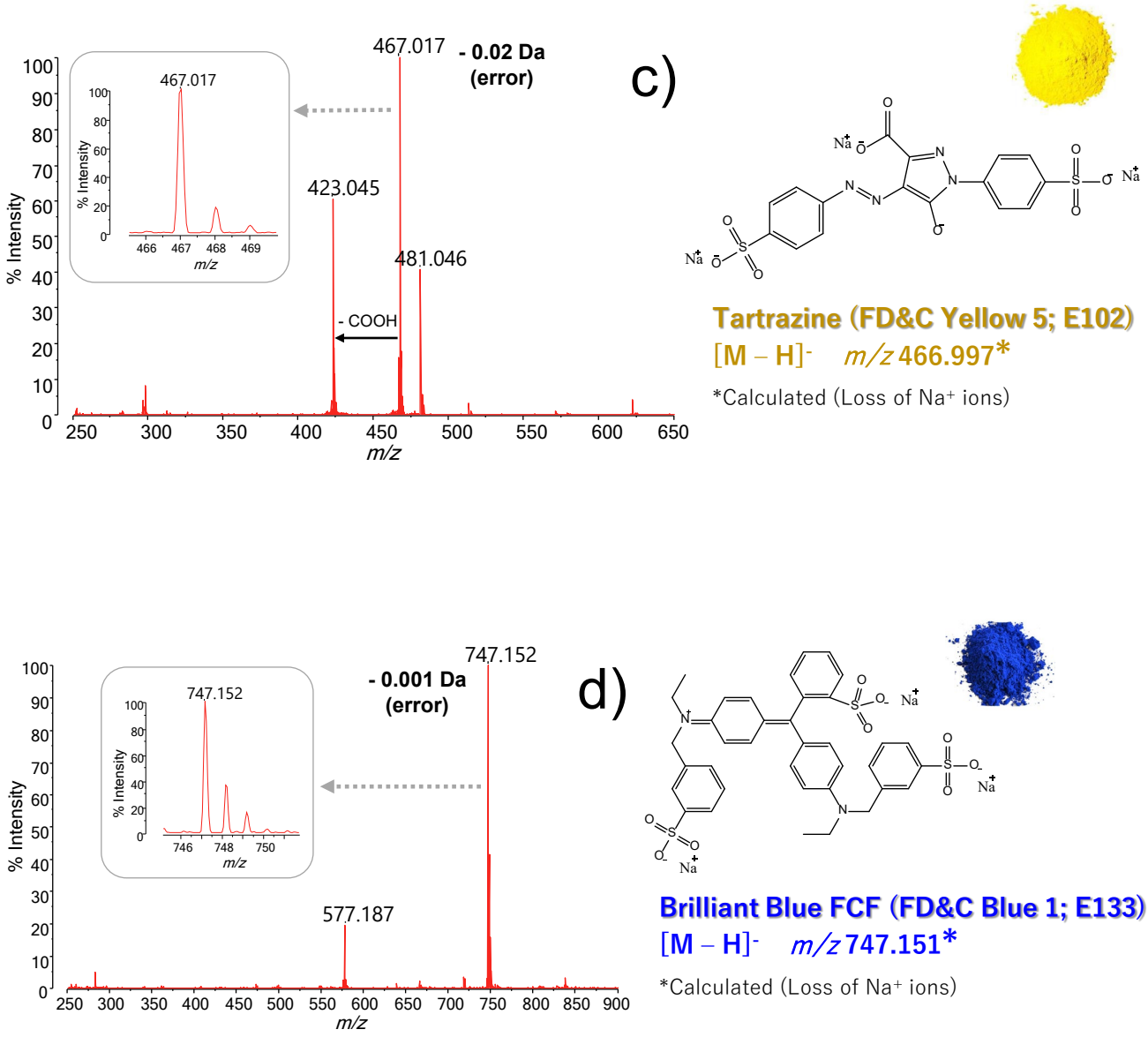

Fig. 2 (continued from previous page). Negative mode MALDI spectra of standard sulfonated azo dyes after ion-pair extraction. c) Tartrazine (FD&C Yellow 5; E102); d) Brilliant Blue FCF (FD&C Blue 1; E133). The exact *m/z* values are calculated for the monoisotopic species after removal of sodium ions. Mass errors (Da) are also provided.

■ Results of Sulfonated azo dyes (candies)

Fig 3 shows the negative mode MALDI spectra obtained for the sulfonated azo dyes extracted from the candies. All dyes found in each candy are consistent with its colour: i) the 'Orange' candy contains the orange Sunset Yellow FCF dye (FD&C Yellow 6, E110); ii) the 'Pink' candy contains the red Allura Red AC dye (FD&C Red 40, E129); iii) the 'Purple' candy contains a combination of Brilliant Blue FCF (FD&C Blue 1, E133) and Indigo carmine (FD&C Blue 2; E132) blue dyes, plus the red Allura Red AC dye (FD&C Red 40, E129); iv) the 'Yellow' candy contains the yellow Tartrazine dye (FD&C Yellow 5, E102); v) the 'Green' candy contains a combination of yellow Tartrazine (FD&C Yellow 5, E102) and blue Brilliant Blue FCF (FD&C Blue 1, E133) dyes.

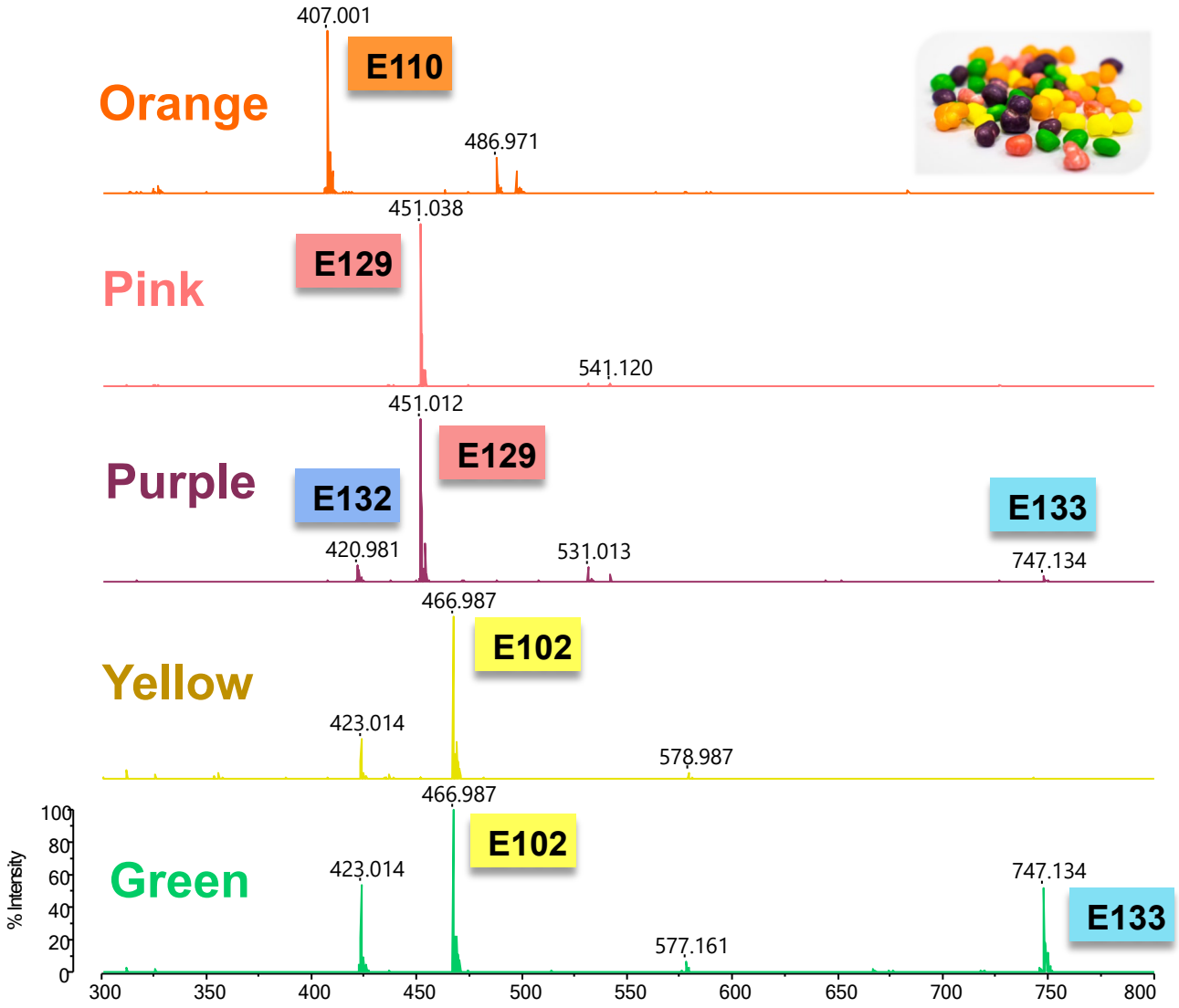

Fig. 3 Negative mode MALDI spectra of sulfonated azo dyes extracted from the commercial candies. The dye content detected with the MALDI analysis is consistent with the colour of the candy.

■ Conclusion

This application demonstrates the capability of the dual polarity MALDI-8030 to detect sulfonated azo colourants in sweets/candies.

The selective extraction method proposed, combined with the negative ion mode detection, offer a simple and fast way to obtain qualitative information on the azo colourant content of confectionery products, which are strictly regulated for their safe use in the food industry.

■ References

[1] Arroyo Negrete, M.A. et al., Anal Bioanal Chem 411, 5833–5843 (2019).

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