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Application News

MALDI-TOF Mass Spectrometry Analysis MALDI-7090

Confirmation of Pigments in Cosmetics by High-energy CID MS/MS using the MALDI-7090™ MALDI-TOF-TOF Mass Spectrometer

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User Benefits

- Minimal sample preparation which does not require labor-intensive procedures and excessive solvent use
- High-energy CID MS/MS is used to confirm the presence of pigments in cosmetic products
- Isotopic resolution and good mass accuracy provide confirmation of the pigment identity with high confidence

■ Introduction

Cosmetics is a huge industry that is still growing, with an estimated value of around USD 863 billion in 2024 [1]. The three major sectors include skin care, hair care and make-up. Colour is a fundamental property for the success of a cosmetic product, as it determines the attractiveness for consumers and boosts confidence in the body image.

Pigments are typically used as colourants in decorative make-up. They can be present in the free form, which is partially hydro-soluble and miscible; or as 'lakes', which are water-insoluble salt derivatives, imparting long-lasting properties to the make-up product.

In Europe, pigments are regulated by the European Regulation of Cosmetic Products (EC 1223/2009), which imposes that they must be reported in product labelling with their unique Colour Index (CI) number (Table 1). Besides safety, the choice of product by customers is also influenced by the origin of the ingredients based on the lifestyle or dietary practices, so this is analytically important to ascertain.

For example, the vegan/halal market forbids the use of animal-derived pigments. Among these is Carmine, a strong red-coloured pigment which is extracted from the body of Cochineal insects (Fig 1).

Besides cultural barriers to the use of this pigment, Carmine is also an allergen. In contrast with the vegan/halal market, there is also the organic market, which doesn't restrict the use of natural (and animal)-based pigments, such as Carmine, but requires the organic source of pigments is certified. For this reason, it is important to confirm the presence/absence of specific pigments in e.g. cosmetic products.

The choice of the analytical technique is based on how in-depth a sample needs to be characterised. MALDI-TOF mass spectrometry provides different benefits in terms of simplicity, robustness and cost-effectiveness. Depending on the sample complexity, or the degree of confidence in the species identification, MS/MS capability may be necessary.

Here, we demonstrate the capability of the MALDI-7090™ MALDI-TOF-TOF mass spectrometer to confirm the identity of pigments in lipsticks by MS/MS. We propose a simple and fast method consisting of the extraction of the pigments from the lipstick material and high-energy CID MS/MS analyses in negative ion mode, based on MS profiles previously obtained on the MALDI-8030 (Fig 2).

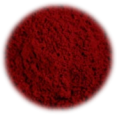

Fig 1. Carmine pigment.

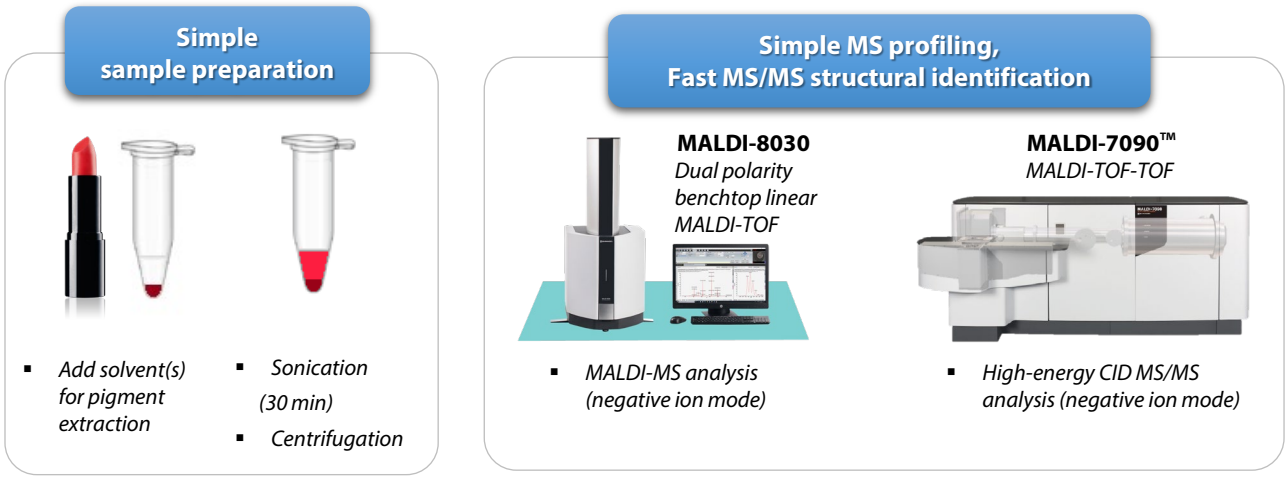

Fig 2. Sample preparation and analysis workflow for the detection and identification of pigments in cosmetics.

Table 1. List of common pigments which are approved for use in cosmetics in Europe.

Common name	Colour Index (CI)	Colour
YELLOW 5 LAKE	19140	
YELLOW 6 LAKE	15985	
RED 6	15850	
RED 7	15850:1	
RED 22 LAKE	45380	
RED 27	45410:1	
RED 28 LAKE	45410:2	
RED 36	12085	
CARMINE	75470	
BLUE 1 LAKE	42090	

■ Measurement Conditions and Samples

Samples of commercial branded lipsticks were purchased in the UK: two non-vegan/non-organic, one organic (non-vegan), one vegan/halal (non-organic). The following pigment standards were purchased from Merck Life Science: Yellow 6, Yellow 5, Carmine. Red 7 and Red 36 were purchased from Tokyo Chemical Industry (TCI). Individual stock solutions of the pigment standards were prepared at 1 mg/mL in water/methanol (1:1), except for CI 12085/Red 36 which was prepared in dichloromethane.

The sample preparation workflow is illustrated in Fig 1. An amount of lipstick material was placed in a microcentrifuge tube, to which 50 µL of water/methanol (1:1), or dichloromethane for the lipstick containing CI 12085/Red 36, were added. The pigment extraction was carried out by sonication for at least 30 min, until the solvent solution became coloured and cloudy. This process facilitates the transfer of the pigments from the wax/oil medium into suspension. After centrifugation, the solution containing the extracted pigments was recovered for analysis.

For the MALDI analyses, samples were spotted with 9-Aminoacridine (9-AA, 10 mg/mL in methanol). MALDI-MS analyses were conducted in negative ion mode on the MALDI-8030 to measure the isotopic masses (data not shown). To confirm the identity of the pigment species in the samples, high-energy CID MS/MS analyses were conducted in negative mode on the MALDI-7090 for the standards and samples, and the obtained MS/MS fingerprints compared for similarities.

■ Results – High-energy CID MS/MS analysis of non-vegan/non-organic lipsticks

Table 2 provides a summary of the pigments listed in the label of the four commercial lipsticks, indicating those which were/were not detected and how their identity was confirmed. Cosmetic manufacturers often produce their product lines in different colours or shades. The same ingredients/pigments may be listed for the whole product line whereas, in fact, depending on the colour, some of the pigments may not actually be present in the formulation.

For Red 22 and Red 28 pigments, whose bromine and chlorine elements provide very distinctive isotopic distribution, a comparison between the isotopic signatures of the computed and detected species in MS mode was used to confirm the pigment identity (data not shown). The other pigments were confirmed through comparison between the MS/MS fingerprints of the standard and the sample.

Table 2. Pigments reported in the ingredient list of the four commercial lipsticks.

non-vegan/non-organic lipstick 1		
+/- May contain *	**Detected**	**ID confirmed**
CI 45380 / RED 22 LAKE	YES	*a*
CI 15850 / RED 7	YES	*b*
CI 15985 / YELLOW 6 LAKE	YES	*b*
CI 45410 / RED 28 LAKE	NO	–
CI 19140 / YELLOW 5 LAKE	NO	–
CI 42090 / BLUE 1 LAKE	NO	–
CI 75470 / CARMINE	NO	–

non-vegan/non-organic lipstick 2		
+/- May contain *	**Detected**	**ID confirmed**
CI 45410 / RED 28 LAKE	YES	*a*
CI 15850 / RED 7	YES	*b*
CI 15985 / YELLOW 6 LAKE	YES	*b*
CI 19140 / YELLOW 5 LAKE	YES	*b*
CI 45380 / RED 22 LAKE	NO	–
CI 42090 / BLUE 1 LAKE	NO	–
CI 75470 / CARMINE	NO	–

Organic (non-vegan) lipstick 3		
Contains	**Detected**	**ID confirmed**
CI 75470 / CARMINE	YES	*b*

Vegan/Halal (non-organic) lipstick 4		
Contains	**Detected**	**ID confirmed**
CI 15850:1	YES	*b*
CI 12085	YES	*b*

* Depending on the shade, some pigments may/may not be present.
a confirmed by isotope distribution (MALDI-8030).
b confirmed by MS/MS (MALDI-7090).

For lipstick 1 (non-vegan/non-organic), the following pigments were confirmed by high-energy CID MS/MS analyses: CI 15850/Red 7 (*m/z* 385.049; Fig 3A); CI 15985/Yellow 6 Lake (*m/z* 407.001; Fig 3B). The insets in Figure 3 show the (-)MS/MS spectra of the corresponding pigment standards. As can be seen, there is a high degree of similarity between the standard and the sample, allowing the identity of the pigment species to be positively confirmed. All fragment ions are isotopically resolved thanks to the ASDF™ (Axial Spatial Distribution Focussing) technology [2].

For CI 15850/Red 7 pigment (Fig 3A), the additional fragments at *m/z* 192 and 326 detected in the lipstick may suggest a potential isobaric species which is co-fragmenting with the pigment, since they were not detected in the standard. However, this did not constitute an impediment to the confirmation of the pigment identity, as the fragmentation patterns and ion ratios overall match. Similar considerations can be applied to CI 15985/Yellow 6 Lake (Fig 3B), due to the fragment at *m/z* 363 which is not detected in the (-)MS/MS spectrum of the corresponding pigment standard.

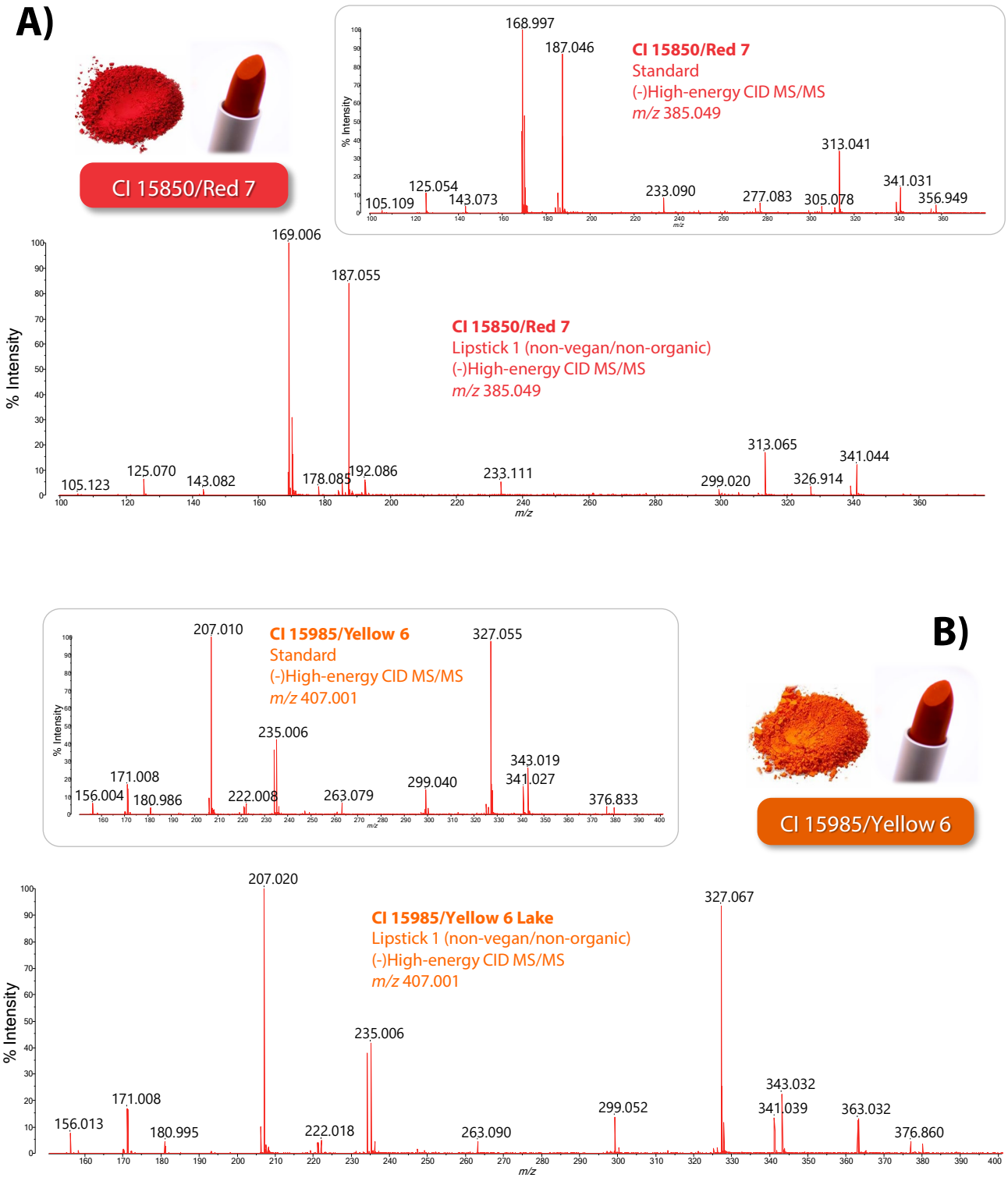

Fig 3. A) negative mode high-energy CID MS/MS spectrum of CI 15850/Red 7 (*m/z* 385.049) in lipstick 1 (non-vegan/non-organic). B) negative mode high-energy CID MS/MS spectrum of CI 15985/Yellow 6 Lake (*m/z* 407.001) in lipstick 1 (non-vegan/non-organic). The insets in A) and B) show the negative mode high-energy CID MS/MS spectra of the corresponding pigment standards.

For lipstick 2 (non-vegan/non-organic), the following pigments were confirmed by high-energy CID MS/MS analyses: CI 15850/Red 7 (*m/z* 385.049; not shown); CI 15985/Yellow 6 Lake (*m/z* 407.001; not shown); CI 19140/Yellow 5 Lake (*m/z* 466.997; Fig 4). The poorer signal intensity of the (-)MS/MS spectrum of CI 19140/Yellow 5 Lake in the lipstick, compared to the standard, suggests the pigment is present in small amount. However, the instrument was capable of producing a good quality spectrum in which the key pigment fragments were detected, thanks to the high MS/MS sensitivity.

■ Results – High-energy CID MS/MS analysis of organic/non-vegan lipstick

Fig 5 shows the negative mode high-energy CID MS/MS spectrum of CI 75470/Carmine pigment (*m/z* 491.083), expected in lipstick 3 (organic (non-vegan)). The fragmentation pattern of Carmine in the sample matches that of the corresponding standard. Note that Carmine is considered to be 'haram' or not permissible in Islamic religion [3], so confirmation of its absence has particular significance in certain markets.

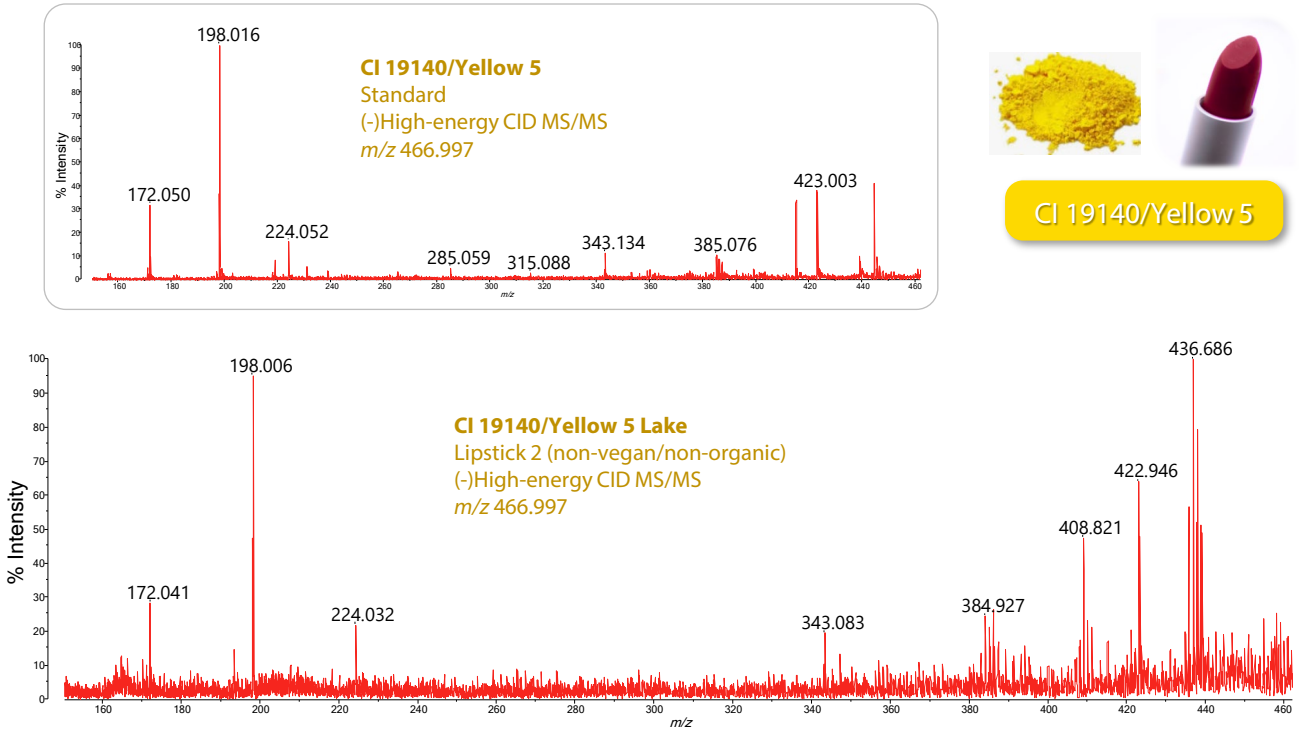

Fig 4. Negative mode high-energy CID MS/MS spectrum of CI 19140/Yellow 5 Lake (*m/z* 466.997) in lipstick 2 (non-vegan/non-organic). The inset shows the negative mode MS/MS spectrum of the corresponding pigment standard.

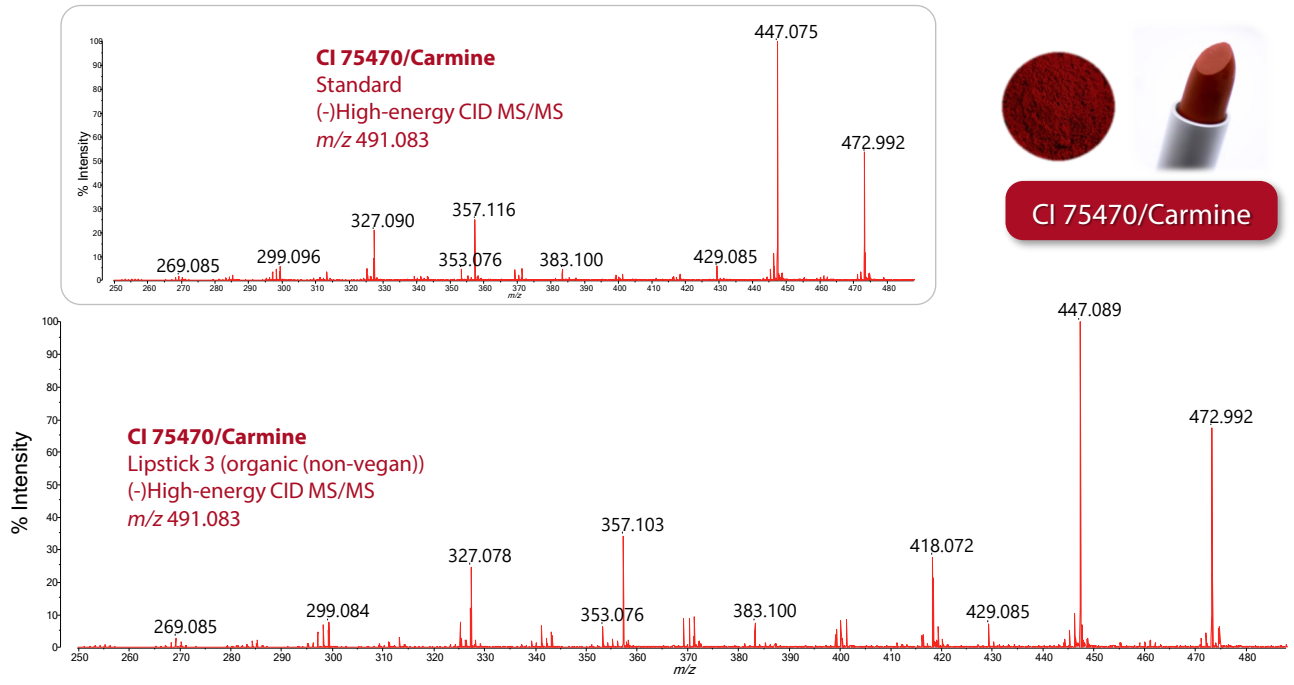

Fig 5. Negative mode high-energy CID MS/MS spectrum of CI 75470/Carmine (*m/z* 491.083) in lipstick 3 (organic (non-vegan)). The inset shows the negative mode MS/MS spectrum of the corresponding pigment standard.

■ Results – High-energy CID MS/MS analysis of vegan/halal lipstick

For the vegan/halal (non-organic) lipstick 4, the following pigments were confirmed by high-energy CID MS/MS analyses: CI 15850/Red 7 (*m/z* 385.049; not shown); CI 12085 (*m/z* 326.034; Fig 6). The inset in Fig 6 shows the (-)MS/MS fingerprint of the corresponding pigment standard. Once again, it was possible to confirm the pigment identities with high confidence.

■ Conclusion

This application demonstrates the capability of the MALDI-7090 to structurally identify pigments in cosmetics. The examples shown are representative of the different markets e.g., vegan/halal, organic, non-vegan/non-organic.

The simple extraction method proposed, combined with the high-resolution MS/MS capability of the MALDI-7090, offers a quick and reliable analytical solution to confirm the identity of pigments in decorative make-up products with high confidence, in order to confirm their suitability for use according to the cultural demands.

■ References

[1] Zion Market Research. 2018. 'A Global Cosmetic Products Market Size, Share 2017: Industry Trends, Growth Analysis and Forecast, 2024." [online]. [Accessed 20 December 2020]. Available from: http://www.globenewswire.com/news-release/2018/06/22/ 1528369.

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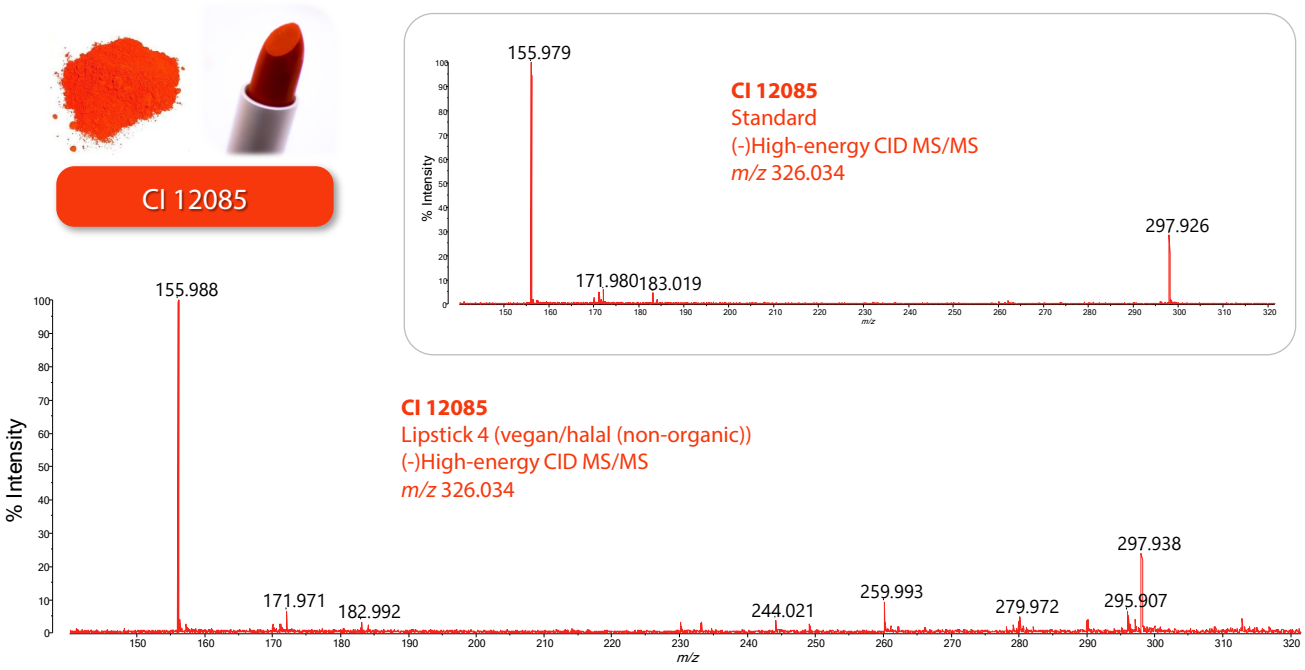

Fig 6. Negative mode high-energy CID MS/MS spectrum of CI 12085 (*m/z* 326.034) in lipstick 4 (vegan/halal (non-organic)). The inset shows the negative mode MSMS spectrum of the corresponding pigment standard.

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