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SWATH-MS screening strategy for the determination of food dyes in spices by UHPLC-HRMS

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ABSTRACT

A multi-class wide-scope screening method for the detection and identification of artificial colours and illegal dyes in spices was developed for regulatory purposes. The screening was carried out by ultra-high performance liquid chromatography hyphenated with a quadrupole/time-of-flight mass spectrometry (UHPLC-QTOF-MS) with sequential window acquisition of all theoretical fragment-ion spectra (SWATH) and was validated with forty-one compounds by spiking experiments in curry and paprika extracts. In order to detect and identify the compounds with a high level of confidence, a home-made tandem mass spectrometry (QTOF-MS/MS) database of approximately one hundred illegal dyes and artificial colours was created. The procedure was then used to screen field samples of spices and spice blends purchased from Swiss markets. Sudan IV, Sudan I, bixin (E160b) and Ponceau 4R (E124) were all detected among the eight non-compliant samples.

1. Introduction

Natural or artificial (i.e. synthetic) colours are added to many foods to enhance their attractiveness and compensate for variations or losses that can occur mainly during processing or storage (Scotter, 2015; Thomas Bechtold, 2009). Although numerous naturally-derived colours exist such as turmeric, paprika, safflower, beet extract, carotenoids, or cochineal (Oplatowska-Stachowiak & Elliott, 2017; Thomas Bechtold, 2009), the food industry often prefers artificial colours due to their effectiveness, excellent stability and relative lower cost (Scotter, 2015). Over the years, some of these artificial additives have been delisted or banned in many countries owing to their detrimental impact on consumers' health (Downham & Collins, 2000). In addition, researchers at the University of Southampton have linked the so-called "Southampton six" (i.e. Tartrazine, Allura Red AC, Ponceau 4R, Quinoline Yellow, Sunset Yellow FCF and Azorubine) to increased hyperactivity in children (McCann et al., 2007). As a result of these growing concerns about adverse health effects (Amchova, Kotolova, & Ruda-Kucerova, 2015), a major trend in the food industry is now to replace synthetic colours in food with natural ones despite the challenges regarding feasibility, stability and cost (Oplatowska-Stachowiak & Elliott, 2017; Scotter, 2015). In order to encourage the transition, the Food Standards Agency (FSA) in the UK has published guidelines for their replacement (Chapman, 2011).

Even though legislation on food additives is specific to each country, only additives explicitly mentioned in the law can be used (Lehto et al., 2017). Europe and Switzerland approve in specific conditions (i.e. in terms of concentration and type of food) the use of Tartrazine (E102), Quinoline Yellow (E104), Sunset Yellow FCF (E110), Erythrosine (E127), Allura Red AC (E129), Azorubine (E122), Amaranth (E123), Ponceau 4R (E124), Patent Blue (E131), Indigo Carmine (E132), Brilliant Blue (E133), Green S (E142) and Brilliant Black (E151) (CH/817.022.31, 2017).

Lipophilic azo dyes such as the Sudan-type ones are widely used for diverse industrial and scientific applications whether for example the colouring of fuels, textiles, oils or staining for microscopy (Oplatowska-Stachowiak & Elliott, 2017; Rebane, Leito, Yurchenko, & Herodes, 2010). However, because of their carcinogenicity, their use as food additives is banned in most countries, including the European Union and Switzerland (EFSA, 2005). In spite of this, on account of their colourfastness, low price and wide availability, they have been found to fraudulently enhance the appearance of various foods such as spices (e.g. chilli or paprika), tomato sauces, palm oil, salami, olive oil, and many others food products (EFSA, 2005; Rebane et al., 2010; Sciuto et al., 2017; Zhu et al., 2016).

One of the foodstuffs frequently adulterated by these dyes and also by artificial colours are ground spices (Galvin-King, Haughey, & Elliott, 2018; Oplatowska-Stachowiak & Elliott, 2017) as it is often difficult for consumers and importers to evaluate their quality based on sensory input alone. As for the Swiss legislation, it forbids the addition of food

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colours to spices except Quinoline Yellow (E104) that can be added to curry and tandoori preparations (CH/817.022.31, 2017).

In this context, in order to ensure consumers' trust and health, a suitable analytical method was required for the fast detection and identification in spices of both unauthorized dyes and authorised or delisted artificial colours. Furthermore, their simultaneous extraction and determination in foodstuffs has seldom been reported in literature and has usually focused on a relatively limited number of compounds analysed either by HPLC-DAD-MS (Ma, Luo, Chen, Su, & Yao, 2006) or LC-MS (Ates, Mittendorf, & Senyuva, 2011; Tsai, Kuo, & Shih, 2015). Besides these methods, a great number of other LC-MS(/MS) or LC-UV/Vis approaches exist, but are focused either on artificial colours (whether authorised or delisted) (Feng et al., 2011; Harp, Miranda-Bermudez, Baron, & Richard, 2012; Ji, Feng, Chen, & Chu, 2011; Martin, Oberson, Meschiari, & Munari, 2016; Tang et al., 2014; Yoshioka & Ichihashi, 2008) or illegal Sudan-type dyes (Botek, Poustka, & Hajšlová, 2007; Enríquez-Gabeiras, Gallego, Garcinuño, Fernández-Hernando, & Durand, 2012; Genualdi et al., 2016; Li, Wu, & Shen, 2010; Zacharis, Kika, Tzanavaras, Rigas, & Kyranas, 2011; Zhu et al., 2014).

In the last few years, full-scan screening methods using high-resolution mass spectrometry (HRMS) are proving to be a promising alternative to triple quadrupole methods as they maximise the number of analytes that can be screened and are generally designed to minimise sample preparation, analysis time and cost (López, Callao, & Ruisánchez, 2015).

Such an approach would thus be suited for both colours and dyes. Additionally, the accurate mass measurement as well as the isotopic peak pattern and, in the case of hybrid instruments, the MS/MS spectra, ensures an improved selectivity. In comparison, strategies based on the sole search of the precursor ion are liable to false negatives and false positives that need to be subsequently clarified by a confirmatory step. It is consequently highly beneficial to compare the MS/MS spectra with those of a library to ensure the correct identification. Currently, this is best achieved by building empirical in-house libraries that contain as many compounds as possible to overcome the lack of universal fragment mass spectral libraries. MS/MS spectra libraries are thus a critical tool for small molecular identification in food analysis.

Therefore, a SWATH-MS (sequential window acquisition of all theoretical fragment-ion spectra mode) method was developed as it provides simultaneous acquisition of TOF-MS and TOF-MS/MS traces. Moreover, this acquisition mode can facilitate the retrospective analysis of suspected samples which is of special interest as new “emerging” adulterants appear periodically on the markets such as Basic Red 46 (Ruf, Walter, Kandler, & Kaufmann, 2012), Reactive Red 195 (Müller-Maatsch, Schweiggert, & Carle, 2016), Rhodamine 123 and 6G (Chang et al., 2018) or Basic Fuchsin (Tatebe et al., 2014).

In conclusion, the aim of this paper is to describe the sensitive, robust and fast screening method that was developed and validated for the extraction and analysis in spices of both food colours and dyes. To the best of our knowledge, no published report has analysed a complete set of illegal, banned and authorised food colours in combination with a simple and generic extraction procedure for spices and spice blends.

2. Material and methods

2.1. Chemicals

All dyes and internal standards were purchased from Sigma-Aldrich (Buchs, Switzerland), except for 4-aminocarminic acid (EGT Chemie, Tägerig, Switzerland), Acid red 88 (Rocceline) (Chemie Brunschwig, Basel, Switzerland) and New Red (LGC Standards, Teddington, Middlesex, UK).

Tetrahydrofuran (THF) (HPLC grade) was purchased from Acros Organics (Sigma-Aldrich, Buchs, Switzerland) whereas methanol (MeOH) and acetonitrile (ACN) (both LC-MS grade) were purchased from J.T. Baker (Avantor Performance Materials, Deventer, The Netherlands), and ammonium acetate (LC-MS grade) was purchased from Sigma-Aldrich (Buchs, Switzerland). Water was generated by a Purelab ultra water purification system (Elga, High Wycombe, Buckinghamshire, UK).

2.2. Field samples

Spices were purchased from local markets and supermarkets around Switzerland. In total, 87 field samples were collected, including spices (e.g. paprika, curcuma, chilli, nutmeg, cumin, sumac) and spice blends (such as curry, tandoori, tikka masala, Ras-el-hanout or couscous).

2.3. Sample preparation

Approximately 1 g of spice or spice blend was weighed and extracted with 10 mL of the extraction solvent (H_2O/MeOH/ACN/THF, 9:1:5:5, v/v/v/v) for 30 min under magnetic stirring. The solution was centrifuged at 970*g* for 5 min, and an aliquot of the resultant supernatant was filtered through a 0.2 µm PTFE filter into an amber LC vial. If the extract was still cloudy, it was diluted with the extraction solvent (up to four fold in some cases) and filtered a second time.

2.4. Analytical method

Two microliters of the spice extracts were injected onto an ExionLC AD system coupled to an X500R QTOF system (Sciex, Framingham, Massachusetts, USA) equipped with a heated nebuliser interface. Separation was performed using a gradient on an ACQUITY UPLC BEH C18 column from Waters (1.7 µm, 2.1 × 100 mm) at 50 °C, and a mobile phase consisting of 10 mM ammonium acetate buffer (phase A) with MeOH (phase B), at a flow rate of 0.5 mL/min. The gradient started at 2% MeOH for 1 min, was increased to 95% MeOH in 10 min, then to 99% MeOH in 2 min, and remained at 99% MeOH for 0.5 min.

Analyses were performed using the TurboSpray source. For both negative and positive modes, the source temperature was set at 500 °C, and the ion source gases 1 and 2, curtain gas and collisionally activated dissociation gas were set at 45, 35 and 7 [AU], respectively. For the positive mode, the spray voltage was set at 5.5 kV and for the negative mode at −4.5 kV.

The TOF-MS survey scan was performed from 120 to 1200 Da, using the following parameters: the declustering potential, the accumulation time and the collision energy were set at 50 V, 0.1 s and 10 V, respectively, in the positive mode, whereas they were set at −80 V, 0.1 s and −10 V, respectively, in negative mode. Analytes were also detected by SWATH-MS using eight windows, according to Table 1. The declustering potential, collision energy and spread parameters were set at 50, 35 and 15 V for the positive mode, and −80, −35 and 15 V for the negative mode, respectively. The accumulation time for both modes was 0.05 s. An auto-calibration check was performed automatically by

Table 1
SWATH windows for species analysis in positive and negative modes.

	Positive mode		Negative mode	
SWATH window	Precursor ion start mass [Da]	Precursor ion stop mass [Da]	Precursor ion start mass [Da]	Precursor ion stop mass [Da]
1	120.0000	286.7077	120.0000	269.0000
2	285.7077	362.6385	268.000	310.4000
3	361.6385	446.2538	309.4000	394.6333
4	445.2538	532.6000	393.6333	506.9833
5	531.6000	604.5462	505.9833	614.6000
6	603.5462	709.4000	613.6000	735.9167
7	708.4000	787.5615	734.9167	857.7833
8	787.5615	1200.0000	856.7833	1200.0000

the instrument every five samples during the sequence, to check the resolution and maintain the correct calibration.

2.5. Library building

Since a commercial MS/MS library was not available, it was created using standards. As most dyes had purity levels below 95%, they were injected using the LC method described in Section 2.4. Six different energy collisions: (–)20, (–)30, (–)40, (–)50 V and (–)35 ± 15 V and (–)40 ± 20 V were used for this purpose. For compounds that could be ionised in positive and negative modes, both were added to the library.

The library comprised illegal dyes such as the Sudan-type (e.g., Sudan I-IV, Sudan Red B, Sudan Orange G), authorised artificial colours and the delisted ones (e.g. Ponceau SX (E125), Citrus Red 2 (E121) or Yellow 2G (E107)) and other dyes that have been discovered in various foodstuffs in the last few years such as Basic Red 46 (Ruf et al., 2012), Reactive Red 195 (Müller-Maatsch et al., 2016) or 4 amino-carminic acid (Sabatino et al., 2012). The complete list of the 96 compounds is given in Table S1.

2.6. Detection and identification criteria

A compound was deemed *detected* if the following criteria were met: the retention time (RT) was ± 0.1 min of the reference RT, the mass error was below or equal to 5 ppm, the library hit corresponded to the actual compound, and the chromatographic peak width was superior to 0.09 min. The compound was considered *identified* if either two ions (generally the precursor and one product ion) met the detection criteria or if, in addition to the detection criteria, the library spectrum hit score for the precursor was superior to 70. In this case a positive result was regarded as unambiguously identified or confirmed. Indeed, according to the EU guidelines (Commission Decision 2002/657/EC, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 2002), a minimum of three identification points is required for authorised substances and four for banned ones. In the present HRMS analysis, a minimum of 4.5 identification points was secured: 2 for the precursor and 2.5 for each product ion.

The deviation tolerance threshold for RT was set at 0.1 min, as recommended by the SANCO/12571/2013 protocol for validation of screening method (SANCO/12571/2013, 2015). An exception was made for two pairs of isobaric compounds (Azorubine/Ponceau 4R and Sudan IV/Sudan Red B) that elute closely and whose RT threshold had to be set at 0.05 min to allow differentiation and avoid false positive results.

The ion intensity ratio criteria for identification, commonly used in targeted quantitative LC–MS/MS methods, was not applied. The reason being that variations in the fragmentation performances caused by the sample matrix could occur (especially at low concentration levels (Dasenaki, Bletsou, Koulis, & Thomaidis, 2015)) so it would be extremely tedious to consider this parameter.

2.7. Qualitative validation design

Two different spices, a curry blend and ground paprika, were used for validation and a subset of forty-one compounds was selected. The full list is presented in Table 2.

The false negative and false positives rates were calculated using spiked samples that were prepared by extracting 3 g of spice with 30 mL of the extraction solution (see Section 2.3) for 30 min under magnetic stirring followed by centrifugation and filtration (0.2 µm PTFE filter). A 905 µL aliquot of the filtered extract was then spiked with 50 µL of the doping solution (mix of several dyes), 25 µL of the mix of internal standards (5 µg/mL of Sudan I-d5 and Sudan III-d6, and 40 µg/mL of Congo Red-d8) and 20 µL of either Reactive Red 195 at 750 µg/mL or E102 at 500 µg/mL. Both extracts were spiked in a manner that they contained between one and thirty-three compounds. In total, forty vials were thus prepared with each analyte added randomly in twenty of them (ten in each extract). The vial solutions were then injected onto the UHPLC-HRMS system using the conditions described in Section 2.4. The injected concentrations of the compounds (Table 2) corresponded to the limit of identification (LOI) determined during the pre-validation (Section 3.2).

The three labelled internal standards were used to monitor the instruments' performance during the sequence. Solvent blanks were also injected throughout the sequence to ensure that there was no carry-over. Two different operators without prior knowledge of which and how many compounds the vials contained, reprocessed the samples, and the results were then computed.

2.8. Repeatability test

Intra-day and inter-day repeatability were assessed using both curry and paprika extracts and a representative subset of 24 compounds: Acid Red 1, Acid Red 88, Acid Yellow 9, Allura Red AC, Aniline Yellow, Basic Red 46, Citrus Red 2, Erythrosine, Fast Garnet GBC, New Red, Orange II, Para Red, Ponceau 3R, Ponceau 6R, Ponceau SX, Red 10B, Rhodamine B, Sudan I, Sudan IV, Sudan Red 7B, Sudan Red G, Sunset Yellow FCF, Tartrazine and Toluidine Red. Both extracts were spiked with these analytes at the LOI that had been estimated during the pre-validation, and with the mix of internal standards. These spiked spice extracts were prepared and analysed as described in Section 2.7.

For the determination of intra-day repeatability, the samples were injected ten times in both positive and negative modes (n = 10), while for the inter-day repeatability the same samples were injected four times in each mode, over two more days each time using a new mobile phase (n = 3 × 4). The parameters monitored were the RT, the raw area with an extracted ion chromatogram window of 25 ppm, the mass error and the false negative rate (i.e. according to the detection criteria, see Section 2.6). For compounds that could be detected in both modes, the data from the most sensitive mode was used.

3. Results and discussion

3.1. Method development

3.1.1. Sample preparation

As for all multiclass or multi-residue analytical methods, a generic and simple sample preparation was needed not only because a quantitative extraction was not required but also considering the different physico-chemical properties of the compounds. Indeed they cover a wide range of polarities, from the relatively apolar lipophilic illegal dyes, such as Sudan IV (logP 8.5) to very polar dyes, like Tartrazine (logP −2.33), Amaranth (logP −2.2) and Acid Yellow 9 (logP −0.6) (data from chemicalize.com, 29.06.2018), necessitating a solvent system suitable for both types. Consequently, the quaternary solvent mixture (H_2O/MeOH/ACN/THF) was tested with water at 22, 45 and 72%. The highest ratio tested (72% water) showed the lowest recoveries for the Sudan-type dyes, while the two remaining ratios obtained similar recoveries to each other. For the artificial dyes, which are relatively more hydrophilic, no significant difference was observed between the 72 and 45% water proportions. Hence, the final selection was the mixture with 45% water, corresponding to H_2O/MeOH/ACN/THF at 9:1:5:5 (v/v/v/v) respectively.

3.1.2. Chromatographic conditions

As mentioned above, since the compounds analysed covered a wide range of polarities, different chromatographic conditions were evaluated starting with the selection of the column chemistry. The Synergi-Polar RP column (2.5 µm, 2.1 × 100 mm, Phenomenex), which is an ether-linked phenyl phase and is polar endcapped, offered a good separation of the illegal dyes but not the artificial colours, so a fused-core

Table 2
The list of compounds used for validation (in bold the most sensitive *m/z* for the compound).

Class	Compound	Color index	E number	Chemical formula	Adduct/Charge	*m/z* precursor	Fragment *m/z*	LOI ng/mL	RT ± SD [min]
Artificial	Acid Red 1 (Red 2G)	18050	E128	C18H13N3H2O8S2	**[M+H] +**	**466.0373**	424.0268; 93.0573	400	5.09 ± 0.01
Artificial	Acid Yellow 17 (Yellow 2G)	18965	E107	C16H10Cl2N4H2O7S2	[M+H]+	506.9597	266.0241; 173.0141	500	5.04 ± 0.01
Artificial	Acid Yellow 17 (Yellow 2G)	18965	E107	C16H10Cl2N4H2O7S2	**[M−2H]2−**	**251.9689**	170.9996	500	5.04 ± 0.01
Artificial	Acid Yellow 9	13015	E105	C12H9N3H2O6S2	**[M+H] +**	**358.0162**	185.0026; 109.076	2000	1.69 ± 0.05
Artificial	Acid Yellow 9	13015	E105	C12H9N3H2O6S2	[M−H]−	356.0017	170.9996; 276.0448	2000	1.70 ± 0.06
Artificial	Allura Red AC	16035	E129	C18H14N2H2O8S2	**[M+H] +**	**453.0421**	217.0404; 202.018	1000	4.86 ± 0.01
Artificial	Allura Red AC	16035	E129	C18H14N2H2O8S2	**[M−2H]2−**	**225.0101**	205.9917; 206.9996	1000	4.86 ± 0.01
Artificial	Amaranth	16185	E123	C20H11N2H3O10S3	**[M−2H]2−**	**267.9833**	206.0043; 221.0152	3000	1.84 ± 0.04
Artificial	Azorubine	14720	E122	C20H12N2H2O7S2	[M+H]+	459.0315	223.0298; 442.0299	100	6.27 ± 0.01
Artificial	Azorubine	14720	E122	C20H12N2H2O7S2	**[M−2H]2−**	**228.0048**	221.015	100	6.27 ± 0.01
Natural	Bixin	75120	E160b	C25H30O4	**[M+H] +**	**395.2217**	145.1012; 157.1012	1000	10.78 ± 0.01
Natural	Carminic Acid	75470	E120	C22H20O13	**[M−H] −**	**491.0831**	447.0922; 357.061	1000	2.03 ± 0.03
Artificial	Chrysoine S	14270	E103	C12H9N2HO5S	**[M−H] −**	**293.0238**	155.9887; 170.9996	50	3.80 ± 0.01
Artificial	Citrus red 2	12156	E121	C18H16N2O3	**[M+H] +**	**309.1234**	153.0784; 138.055	100	10.81 ± 0.01
Artificial	Erythrosine	45430	E127	C20H6I4H2O5	[M+H]+	836.6623	–	100	7.95 ± 0.01
Artificial	Erythrosine	45430	E127	C20H6I4H2O5	**[M−H] −**	**834.6478**	662.745; 536.848	100	7.94 ± 0.01
Artificial	Ponceau 4R (Acid Red 18)	16255	E124	C20H11N2H3O10S3	**[M−2H]2−**	**267.9833**	206.00430	2000	3.69 ± 0.01
Artificial	Ponceau 6R	16250	E126	C20H12N2H2O7S2	**[M+H] +**	**459.0315**	143.07300	1000	6.20 ± 0.01
Artificial	Ponceau 6R	16250	E126	C20H12N2H2O7S2	**[M−H] −**	**457.017**	301.95500	1000	6.19 ± 0.01
Artificial	Quinoline Yellow-1	47005	E104	C18H9NH2O8S2	[M+H]+	433.9999	353.0352	1000	3.64 ± 0.01
Artificial	Quinoline Yellow-1	47005	E104	C18H9NH2O8S2	**[M−2H]2−**	**215.489**	79.9574	1000	3.64 ± 0.01
Artificial	Quinoline Yellow-2	47005	E104	C18H9NH2O8S2	[M+H]+	433.9999	352.0274	1000	4.30 ± 0.01
Artificial	Quinoline Yellow-2	47005	E104	C18H9NH2O8S2	[M−H]−	431.9853	352.0285	1000	4.30 ± 0.01
Artificial	Sunset Yellow FCF	15985	E110	C16H10N2H2O7S2	[M+H]+	409.0159	392.013; 236.0012	500	4.11 ± 0.01
Artificial	Sunset Yellow FCF	15985	E110	C16H10N2H2O7S2	**[M−2H]2−**	**202.997**	206.0043; 170.9996	500	4.11 ± 0.01
Artificial	Tartrazine	19140	E102	C16H9N4H3O9S2	**[M+H] +**	**469.01118**	200.0008; 450.9996	10,000	1.03 ± 0.03
Artificial	Tartrazine	19140	E102	C16H9N4H3O9S2	[M−H]−	466.9973	172.0074; 198.0071	10,000	1.03 ± 0.04
Artificial	Tartrazine	19140	E102	C16H9N4H3O9S2	**–**	**197.9868**	134.0201; 79.9574	10,000	1.03 ± 0.04
Delisted artificial	Ponceau SX (Food Red 1)	14700	E125	C18H14N2H2O7S2	[M−2H]2−	217.0127	170.0281; 199.0309	100	6.64 ± 0.01
Delisted artificial	Ponceau SX (Food Red 1)	14700	E125	C18H14N2H2O7S2	**[M−H] −**	**435.0326**	355.0758	100	6.64 ± 0.01
Illegal	4-amino carminic acid			C22H21O12N	**[M−H] −**	**490.0991**	356.076; 326.066	1000	1.30 ± 0.05
Illegal	Acid Red 88 (Rocceline)	15620		C20H13N2HO4S	**[M−H] −**	**377.0602**	221.0152	10	8.90 ± 0.02
Illegal	Aniline Yellow	11000		C12H11N3	**[M+H] +**	**198.1026**	77.0386	1000	8.39 ± 0.01
Illegal	Basic Red 46	110825		C18H21N6	**[M] +**	**321.1822**	196.1121; 91.0542	500	7.17 ± 0.01
Illegal	Congo Red	22120		C32H22N6H2O6S2	[M+H]+	653.1272	353.1397; 418.1094	400	7.30 ± 0.01
Illegal	Congo Red	22120		C32H22N6H2O6S2	**[M−2H]2−**	**325.0527**	152.0369; 416.0949	400	7.30 ± 0.01
Illegal	Congo Red	22120		C32H22N6H2O6S2	[M−H]−	651.1126	571.1558	400	7.30 ± 0.01
Illegal	Dimethyl yellow	11020		C14H15N3	**[M+H] +**	**226.1339**	77.0386; 120.0808	80	10.16 ± 0.02
Illegal	Fast garnet GBC	11160		C14H15N3	**[M+H] +**	**226.1339**	121.076; 91.0542	250	9.59 ± 0.02
Illegal	New Red			C18H12N3H3O11S3	**[M+H] +**	**545.9941**	503.987; 172.0074	5000	1.78 ± 0.06
Illegal	New Red			C18H12N3H3O11S3	[M−2H]2−	271.4862	172.0074; 291.0081	5000	1.78 ± 0.07
Illegal	Oil Orange SS	12100		C17H14N2O	**[M+H] +**	**263.1179**	107.073; 128.0621	100	11.26 ± 0.01
Illegal	Orange II	15510		C16H11N2HO4S	**[M−H] −**	**327.0445**	170.9996; 155.9887	50	7.85 ± 0.02
Illegal	Para Red	12070		C16H11N3O3	[M+H]+	294.0873	156.0444; 277.0846	50	10.40 ± 0.01
Illegal	Para Red	12070		C16H11N3O4	**[M−H] −**	**292.0728**	122.024; 138.019	50	10.39 ± 0.01
Illegal	Ponceau 3R	16155		C19H16N2H2O7S2	**[M+H] +**	**451.0628**	135.1043; 369.0915	500	6.90 ± 0.01
Illegal	Reactive Red 195			C31H19ClN7H5O19S6	**[M−2H]2−**	**511.4497**	237.0101; 561.0059	15,000	3.79 ± 0.02
Illegal	Red 10B	17200		C16H11N3H2O7S2	**[M+H] +**	**424.0268**	342.0543; 251.0121	750	4.25 ± 0.01
Illegal	Red 10B	17200		C16H11N3H2O7S2	[M−H]−	422.0122	248.9976	750	4.24 ± 0.01
Illegal	Rhodamine B	45170		C28H31N2O3	**[M] +**	**443.2329**	399.1703	10	9.38 ± 0.03
Illegal	Sudan I	12055		C16H12N2O	**[M+H] +**	**249.1022**	93.0573; 156.0444	50	10.84 ± 0.01
Illegal	Sudan II	12140		C18H16N2O	**[M+H] +**	**277.1335**	121.0886; 260.1308	50	11.64 ± 0.01
Illegal	Sudan III	26100		C22H16N4O	**[M+H] +**	**353.1397**	77.0386; 197.0947	50	12.01 ± 0.01
Illegal	Sudan IV	26105		C24H20N4O	**[M+H] +**	**381.171**	224.1182; 91.0542	20	12.78 ± 0.01

(*continued on next page*)

Table 2 (*continued*)

Class	Compound	Color index	E number	Chemical formula	Adduct/Charge	*m/z* precursor	Fragment *m/z*	LOI ng/mL	RT ± SD [min]
Illegal	Sudan Orange G	11920		C12H10N2O2	[M + H] +	215.0815	93.0573; 122.0237	10	8.78 ± 0.01
Illegal	Sudan Orange G	11920		C12H10N2O2	**[M − H] −**	**213.067**	121.0159; 120.0081	10	8.77 ± 0.01
Illegal	Sudan Red 7B	26050		C24H21N5	**[M + H] +**	**380.187**	183.0917	10	12.52 ± 0.01
Illegal	Sudan Red B	26110		C24H20N4O	**[M + H] +**	**381.171**	91.0542; 224.1182	50	12.69 ± 0.01
Illegal	Sudan Red G	12150		C17H14N2O2	**[M + H] +**	**279.1128**	123.0679; 108.0444	100	10.78 ± 0.01
Illegal	Toluidine red	12120		C17H13N3O3	**[M + H] +**	**308.103**	156.0444; 244.0995	50	10.69 ± 0.01
ISTD	*Congo red d8*			*C32H16D8N6O6S2*	*[M − H] −*	*659.1623*	579.2100	NA	7.27 ± 0.01
ISTD	*Congo red d8*			*C32H16D8N6O6S2*	***[M − 2H]2 −***	***329.0772***	424.1500	NA	7.27 ± 0.01
ISTD	*Congo red d8*			*C32H16D8N6O6S2*	*[M + H] +*	*661.1818*	426.1600	NA	7.27 ± 0.01
ISTD	*Soudan I-d5 pos*			*C16H8D5N2O*	***[M + H] +***	***254.1336***	98.0900	NA	10.82 ± 0.01
ISTD	*Soudan III-d6 pos*			*C22H10D6N4O*	***[M + H] +***	***359.1774***	197.1000	NA	11.98 ± 0.01

Kinetex biphenyl column (2.6 µm, 2.1 × 100 mm, Phenomenex) and an ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 × 100 mm, Waters) were evaluated for improving the peak shape and separation. The ACQUITY UPLC BEH C18 column proved to be the best choice even if some peaks still showed signs of tailing, for example Basic Red 46 or Azure B. Considering the vast range of compounds this could not be avoided.

The choice of the mobile phase (10 mM ammonium acetate buffer, pH ~ 6.7) was based on the improved peak shape and sensitivity of most of the compounds when compared to 10 mM ammonium formate (pH ~ 3.3) or 0.1% formic acid (pH ~ 2.7). In addition, Black 7984 and Brilliant Black were only detected with this specific mobile phase due to the poor peak shape obtained with the other ones, as illustrated in Fig. S1.

Of the two organic solvents (MeOH and ACN) evaluated, MeOH was selected because of the comparatively higher sensitivity obtained for most compounds and the improved separation of Sudan Red B and Sudan IV, as shown in Fig. S2. Indeed, since these illegal dyes are isobaric and have the same MS/MS spectra, chromatographic separation was required to differentiate them.

In conclusion, the best separation and peak shape for most of the dyes that were added to the library were obtained using an ACQUITY BEH C18 column at 50 °C, and 10 mM ammonium acetate buffer (phase A) with MeOH (phase B) at a flow rate of 0.5 mL/min.

3.1.3. Mass spectrometric conditions

SWATH-MS is a data-independent acquisition (DIA) mode that uses fixed or variable width Q1 (mass selective quadrupole) isolation windows. All precursors of these windows are transferred to the collision cell, fragmented and then analysed by the TOF detector. The Q1 windows cover the entire mass range needed, resulting in the comprehensive acquisition of high-resolution MS/MS spectra for every precursor ion in a sample. Since it has been shown that improved identification can be obtained when relatively narrower windows are used (to account for the mass regions of higher ion density), windows of variable width were applied (Zhang et al., 2015). However, the optimal width of the windows is sample-dependent due to the intrinsic compositional differences of the various spices and spice blends. Therefore, to determine the ion densities over the studied mass range and, consequently, the width of the windows, four spices (paprika, curcuma, sweet paprika, hot chilli) and eight spice blends (tandoori, garam masala, couscous, curry, ras el-hanout, cajun, satay and a “seven spices” mix) were selected and extracted according to the procedure described in Section 2.3. They were then injected using the LC-MS method in both negative and positive modes. The TOF-MS data was used to calculate the ion density in each sample for each mode over the mass range scanned. The resulting SWATH-MS windows were calculated using the SWATH variable window calculator (version 1.1), which is an Excel spreadsheet template provided by Sciex, that generates the windows by computing the number of precursor ions and taking into account their intensities as a weighting factor (Zhang et al., 2015). Since the resulting profiles were relatively similar for all spices, whether in the positive or negative mode, it was decided to use the same SWATH-MS windows for all types of spices (see Table 1).

3.1.4. Limit of identification (LOI)

The LOI was established as the lowest concentration tested for which a compound was satisfactorily identified in all spiked samples. The identification criteria are described in Section 2.6. In order to determine this LOI, standard solutions of the dyes were prepared in the appropriate solvent at 1 mg/mL and successively diluted till reaching 10 ng/mL. The concentration below which the identification criteria were no longer met was determined as the LOI in the solvent. This concentration was then confirmed by spiking both the paprika and curry extracts. If the results were unreliable, the concentrations were increased until the response became reliable. This concentration

threshold in the matrix was then determined as the LOI.

The full list of compounds and their corresponding LOI is given in Table 2. The least sensitive compounds were Reactive Red 195, Tartrazine, New Red, Amaranth, Ponceau 4R and Acid Yellow 9, with estimated LOIs at 15, 10, 5, 3, 2 and 2 µg/mL respectively. Low MS sensitivities for Amaranth (E123), Ponceau 4R (E124) and Tartrazine (E102) have already been documented in other studies, where lower limits of detection (LOD) were obtained using a DAD compared to an MS/MS detector (Ji et al., 2011; Ma et al., 2006). Their poor sensitivity might have been improved by optimising the MS parameters or chromatographic conditions, but these had to be generic ones because of the wide range of compounds analysed.

The illegal dyes, in particular Sudan Red 7B, Sudan Orange G, Rhodamine Band Acid Red 88, were the most sensitive, with an LOI estimated at 10 ng/mL. Similar observations in the limits of quantitation were reported in another study that also included both types of dyes (artificial and illegal) (Tsai et al., 2015). Indeed, these compounds eluted at the end of the chromatogram with high concentration of MeOH, improving the desolvation process and therefore the MS sensitivity, whereas the least sensitive compounds eluted with high concentration of water.

3.2. Qualitative validation

The aim of a qualitative analysis is to ensure the presence or absence of an analyte in a sample at a certain concentration level. Qualitative validation of screening methods is useful and necessary to assess the applicability and potential limitations of the procedure applied, as well as improve the knowledge on the analyte concentration limits and specificity of the method. This type of validation has been widely applied to veterinary drugs, doping, pesticides or antibiotics analysis but less frequently in the field of food analysis, except in the case of pesticides and veterinary drugs residues. As no quantitation is necessary, method recovery, accuracy and precision were not considered. Selectivity is regarded as the ability of the method to discriminate between the analyte and other compounds that could be present in the spices. This parameter was guaranteed by the presence of characteristics *m/z* ions, measured at the accurate mass for each compound, the RT check, the isotopic distribution of the precursor ion and the MS/MS spectra obtained at specific collision energies.

Validation was carried out by spiking two different spices: a curry and paprika. The curry was composed of red chilli, coriander, cumin, fennel, fenugreek, turmeric, black pepper, clove, cinnamon and curry leaves. A subset of forty-one compounds was selected for the validation based on their colour and relevance to the study (mostly Sudan-type dyes and yellow, orange or red artificial or natural colours). Forty vials were prepared from both curry and paprika extracts (twenty of each), as described in Section 2.7. In total, each analyte was randomly added to ten curry extract and ten paprika extract. Thus, for each analyte, twenty positive (i.e. with the compound) and twenty negative samples were prepared. Three labelled internal standards, Sudan I-d5, Sudan III-d6 and Congo Red-d8, were added and used as internal quality controls to ensure the reliability of the analysis and monitor the instruments' performance. The negative mode was monitoredusing the Congo red-d8 results, while Sudan I-d5 and Sudan III-d6 were used for the positive mode. The relative standard deviation (RSD) of the raw area was calculated at 25% for Sudan I-d5, 19% for Sudan III-d6 and 12% for Congo Red-d8. The mass error of the internal standards was always below 5 ppm, except in three samples for Sudan I-d5 and one different sample for Sudan III-d6, with mass errors between −5.6 and −5.1 ppm. The RSD of the RT for all quality controls was below 0.2%, showing the good method robustness. Therefore, the method performances were deemed reliable.

Data processing was performed in a manner to determine if the analyte was detected and if it could be identified, according to the criteria described in Section 2.6. The false negative rate corresponds to the number of false negatives divided by the sum of true positives and false negatives, whereas the false positive rate corresponds to the number of false positives divided by the sum of true negatives and false positives. A true positive is when the qualitative method gives a positive output for a sample that is indeed positive (the analyte is present in the sample), whereas, a false negative is when the method gives a negative result for sample that is actually positive. A true negative is when the qualitative analysis gives a negative output for sample that is indeed negative, while a false positive is obtained when the method gives a positive result for a sample that is negative. The screening method was considered as satisfactorily validated at the tested concentration when the compound was detected in at least 95% of the spiked samples, i.e., compounds for which the false negative rate was lower or equal to 5%, recommended by European legislation (Commission Decision 2002/657/EC, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 2002).

For the detection of the dyes in species, the false positive rate was 0%, except for Aniline Yellow and Oil Orange, with rates of 5%. The false negative rate was 0% for all compounds, except Amaranth (E123), with a rate of 5%. At the identification stage, the false positive rate was 0% for all compounds and the false negative rate was 0%, except for Reactive Red 195 and Amaranth (E123) that had rates of 5 and 10%, respectively. For all compounds, except Amaranth, the concentration tested corresponded to the LOI.

As expected at the identification stage, when two ions were detected (according to the criteria stated in Section 2.6) no false positives were determined due to the increased specificity compared to when only one ion was used. However, even when only one ion was used (such as at the detection stage), the false positive rate was also low because of the exact mass measurement at the specific RT. Only two substances (Aniline Yellow and Oil Orange) were false positives with rates of 5%.

The mass error of the precursor ion did not exceed ± 2 ppm for 81% of the measurements in the negative mode (n_{total} = 494) and 63% in the positive mode (n_{total} = 646). Only 2% of the negative mode measurements and 3% in the positive mode were above ± 5 ppm but lower than ± 10 ppm.

The findings validated the screening method of the forty-one compounds, given the rate of false negatives was lower or equal to 5%. No regulatory demand is required regarding the rate of false positives. Positives samples, i.e. non-compliant, would have to be confirmed anyhow for example using orthogonal LC conditions. The deviation tolerance threshold for the RT was also within the SANCO/12571/2013 requirements and the mass measurements lower than ± 5 ppm in 98% of the cases in negative mode and 97% in positive mode. The results thus demonstrated that the system is sensitive and accurate enough to detect most dyes and colours.

3.3. Repeatability test

Intra-day and inter-day repeatability were assessed using both curry and paprika extracts and a representative subset of 23 compounds: Acid Red 1, Acid Yellow 9, Allura Red AC, Aniline Yellow, Basic Red 46, Citrus Red 2, Erythrosine, Fast Garnet GBC, New Red, Orange II, Para Red, Ponceau 3R, Ponceau 6R, Ponceau SX, Red 10B, Rhodamine B, Sudan I, Sudan IV, Sudan Red 7B, Sudan Red G, Sunset Yellow FCF, Tartrazine and Toluidine Red. Both extracts were spiked with these analytes at the LOI as described in Section 2.8.

For both extracts, the intra-day and inter-day RT coefficients of variation (CV or RSD) were on average below 1% for all compounds, except Tartrazine and Acid Yellow 9, as illustrated in Tables 3 and 4. However, despite having standard deviations similar to the others compounds, the CV was comparatively higher as they eluted first. These results meant the method was robust and confirmed the suitability of using the RT as an identification parameter.

Regarding the raw areas, the highest intra-day CVs were obtained

Table 3
Intra- and inter-day repeatability results with the curry extract.

Compound	Intra-day						Inter-day					
	RT		Area		Nb of false negatives		RT		Area		Nb of false negatives	
	average	RSD [%]	average	RSD [%]	± 5 ppm	± 10 ppm	average	RSD [%]	average	RSD [%]	± 5 ppm	± 10 ppm
Acid red 1	5.12	0.08	4894	5	0	0	5.12	0.2	3533	29	0	0
Acid Yellow 9	1.82	0.39	2640	21	0	0	1.79	1.2	1926	44	0	0
Allura red AC	4.88	0.29	5265	17	0	0	4.88	0.3	3767	25	1	1
Aniline Yellow	8.46	0.04	84085	9	0	0	8.45	0.1	97428	17	1	0
Basic red 46	7.19	0.04	231050	12	0	0	7.21	0.3	99301	49	0	0
Citrus red 2	10.85	0.03	18351	4	0	0	10.85	0.1	18023	6	0	0
Erythrosine	7.94	0.15	9729	4	0	0	7.95	0.2	6593	35	2	0
Fast garnet GBC	9.65	0.03	26668	11	0	0	9.64	0.1	26673	17	1	0
New red	1.89	0.57	1231	26	0	0	1.87	0.8	941	51	1	0
Orange II	7.88	0.16	6086	22	0	0	7.88	0.2	4049	44	0	0
Para red	10.44	0.13	9248	7	0	0	10.44	0.1	6771	29	1	1
Ponceau 3R	6.92	0.05	30204	4	0	0	6.91	0.1	23053	26	0	0
Ponceau 6R	6.22	0.21	34761	2	0	0	6.22	0.2	29465	15	0	0
Ponceau SX	6.65	0.22	1477	31	1	0	6.65	0.2	1084	20	4	0
Red 10B	4.30	0.07	5677	6	0	0	4.29	0.3	4471	25	0	0
Rhodamine	9.41	0.04	84656	3	0	0	9.40	0.1	56562	37	0	0
Sudan I	10.89	0.04	4596	10	0	0	10.89	0.1	5546	17	4	0
Sudan IV	12.86	0.04	18413	18	0	0	12.85	0.1	15753	19	2	0
Sudan Red 7B	12.59	0.03	9515	5	0	0	12.58	0.1	7830	19	0	0
Sudan red G	10.83	0.03	23228	5	0	0	10.82	0.1	21842	5	2	0
Sunset Yellow FCF	4.14	0.32	1535	7	0	0	4.14	0.3	1143	26	1	0
Tartrazine	1.09	2.47	3490.70	10	0	0	1.07	3.1	2354	43	0	0
Toluidine red	10.74	0.03	46319	7	0	0	10.73	0.1	31633	35	0	0

for Ponceau SX (31%), New Red (26%), and Orange II (22%) in the curry extract, and Basic Red 46 (26%), Acid Yellow 9 (25%) and New Red (22%) for the paprika extract. Conversely, 57 and 65% of the investigated compounds in the paprika and curry extract, respectively, had CVs below 10%. No correlation was observed between the CV and the RT or the intensity of the peak. As no area correction was undertaken (e.g., using stable isotope labelled internal standards) the CV of the area increased significantly between days being above 25% for 61 and 74% of the compounds in the curry and paprika extract, respectively. A CV below 10% was observed for just two compounds in the curry extract, Citrus Red 2 (6%) and Sudan Red G (5%), and only for Sudan Red G (8%), in the paprika extract.

In terms of false negative results for the intra-day repeatability, Para Red and Ponceau SX did not meet the criteria for detection in one instance (n = 10) whether in the paprika extract or the curry extract, as the mass errors of −6.4 and −7.5 ppm, respectively, were above 5 ppm. In comparison, the detection criteria for the inter-day repeatability were not met more frequently: 21 times for the curry extract (once for Acid red 88, Allura red AC, Aniline Yellow, Fast Garnet GBC, New red, Para red, twice for Erythrosine, Sudan IV and Sudan red G,

Table 4
Intra- and inter-day repeatability results with the paprika extract.

Compound	Intra-day						Inter-day					
	TR		Area		Nb of false negatives		TR		Area		Nb of false negatives	
	average	RSD [%]	average	RSD [%]	± 5 ppm	± 10 ppm	average	RSD [%]	average	RSD [%]	± 5 ppm	± 10 ppm
Acid red 1	5.11	0.26	6043	3	0	0	5.11	0.2	4267	32	0	0
Acid Yellow 9	1.82	0.81	3095	25	0	0	1.79	1.5	1911	44	0	0
Allura red AC	4.88	0.28	7367	18	0	0	4.88	0.2	5663	43	0	0
Aniline Yellow	8.44	0.13	112754	16	0	0	8.45	0.1	144948	27	3	0
Basic red 46	7.19	0.42	145978	26	0	0	7.23	0.8	88073	45	0	0
Citrus red 2	10.85	0.11	16802	8	0	0	10.85	0.1	13833	15	0	0
Erythrosine	7.95	0.11	8171	7	0	0	7.95	0.1	5190	40	2	1
Fast garnet GBC	9.64	0.11	34834	9	0	0	9.64	0.1	35750	15	0	0
New red	1.90	0.81	1371	22	0	0	1.87	1.2	929	50	4	0
Orange II	7.88	0.11	6854	19	0	0	7.88	0.1	4905	41	1	1
Para red	10.45	0.09	8114	10	1	0	10.44	0.1	5329	36	4	1
Ponceau 3R	6.91	0.16	34231	3	0	0	6.91	0.1	25099	31	0	0
Ponceau 6R	6.22	0.15	40110	3	0	0	6.22	0.2	26292	28	0	0
Ponceau SX	6.66	0.15	2063	7	0	0	6.65	0.1	1522	33	2	0
Red 10B	4.29	0.28	7658	9	0	0	4.29	0.2	5324	41	0	0
Rhodamine	9.40	0.12	79681	5	0	0	9.40	0.1	54741	32	0	0
Sudan I	10.89	0.10	4032	19	0	0	10.88	0.1	5210	27	7	0
Sudan IV	12.85	0.08	16161	17	0	0	12.84	0.1	14535	14	0	0
Sudan Red 7B	12.58	0.10	10236	8	0	0	12.58	0.1	8132	21	0	0
Sudan red G	10.82	0.10	19504	12	0	0	10.82	0.1	16815	8	1	0
Sunset Yellow FCF	4.15	0.21	1606	7	0	0	4.14	0.2	1209	29	1	0
Tartrazine	1.10	3.13	3809.50	6.6	0	0	1.07	4.1	2433	50	0	0
Toluidine red	10.73	0.11	69490	5	0	0	10.73	0.1	72708	10	0	0

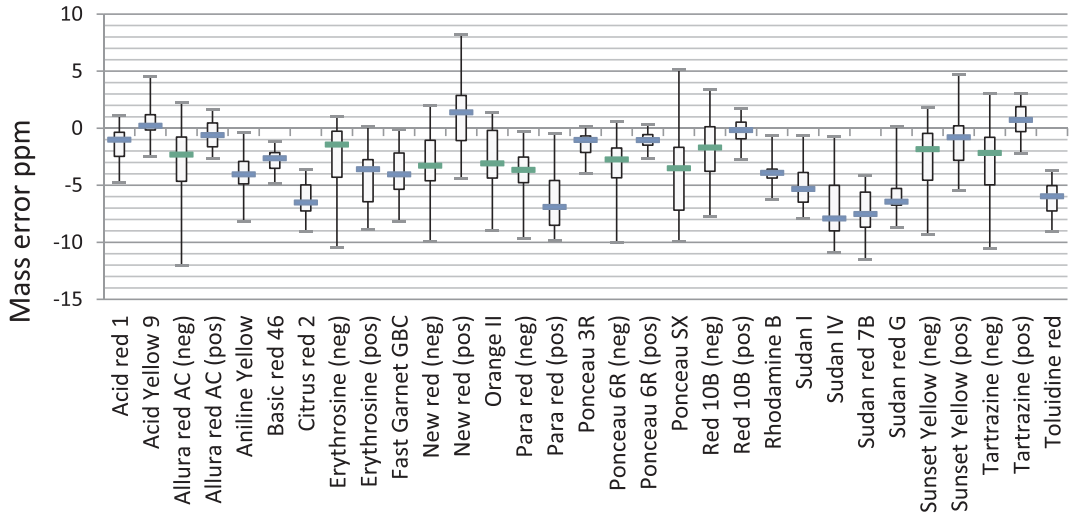

Fig. 1. Box plots of the mass error dispersion for curry and paprika extracts. The green and blue lines represent the median value whether in the negative or the positive mode, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and four time for Ponceau SX and Sudan I), and 25 times for the paprika extract (once for Orange II, Sudan red G and Sunset Yellow FCF, twice for Erythrosine and Ponceau SX, three time for Aniline Yellow, four times for New red and Para red, and seven times for Sudan I). These data corresponded to false negative rates of 7 and 9%, respectively. The cause for this was mainly the mass error which was above the 5 ppm criteria set for detection (see Section 2.6). By setting the tolerance at 10 ppm the number of false negatives decreased with only three remaining for both extracts. Given that the false positive rate obtained in the qualitative validation was not affected when 10 ppm was used instead of 5 ppm, a tolerance of 10 ppm was established for routine analyses.

Fig. 1 illustrates the dispersion of the mass error obtained from the repeatability evaluation for the compounds. The mass error dispersion was compound-dependent and not correlated to the *m/z*, the intensity of the signal (area), or the ionisation mode. As an example, the dispersion obtained for Allura Red AC, Ponceau 6R, Red 10B and Tartrazine were spread over 10 ppm or more in the negative mode but less than 5 ppm in the positive one, whereas Erythrosine, New Red, Para Red and Sunset Yellow FCF had similar dispersions in both modes.

Based on the findings and considering the main purpose of a qualitative screening method is to detect and identify positive samples at given concentrations the method developed was considered as satisfactorily validated.

3.4. Application to field samples

To evaluate the applicability of the proposed method in routine analysis, field samples were collected in Switzerland with approximately ninety spices and spice blends analysed. Three labelled internal standards, Sudan I-d5, Sudan III-d6 and Congo Red-d8, were added to the sample and used as internal quality controls to ensure the reliability of the analysis. The RSDs of the RT were highly reproducible, as they were below 0.25% for all internal standards. The mass accuracy was also evaluated to ensure the reliability of the analyses and the interpretation of the results. In the positive mode, the mass errors for Sudan I-d5 and Sudan III-d6 were always below 10 ppm, except once for both, but in different analyses, so it was considered that there was no significant shift of the mass accuracy. For the negative mode, 91% of the Congo Red-d8 mass error was below 5 ppm and only 2% were slightly above −10 ppm, at −11.1 and −10.4 ppm, respectively, which is acceptable.

The CVs of the raw area calculated for the internal standards were important probably due to the matrix effect, 68% for Sudan I-d5, 97% for Sudan III-d6 and 31% for Congo Red. These CVs were higher than those determined during the validation, most likely because of the higher diversity of matrices than the two used for the validation. However, this parameter is not a criterion to check the reliability of the analyses, only the RT and mass accuracy, so the results obtained for the real sample were deemed relevant.

Eight samples were found to be either adulterated or contaminated with unauthorised dyes. Sudan IV was detected in four spices: a ground paprika, two chili peppers and a curry which was also tainted by Sudan I. For these samples, the mass error of the precursor ion and product ions were below 5 ppm, and the library score, which was above 99.5% indicating a nearly perfect match, supported the identification as illustrated in Fig. 2. These findings were confirmed by using our in-house validated quantification method carried out on a ThermoScientific triple quadrupole LC-MS/MS equipped with a Phenomenex Synergi Polar-RP column and 0.1% acid formic in water and MeOH as the mobile phases.

Two other samples were contaminated with an artificial colour, Ponceau 4R (E124). One of them was ground sumac from Iran and the second one was a mix of seven spices from Turkey. In both instances, the molecular ion was detected with a mass error below 3 ppm but either with no library match or a poor library score (below 50). Consequently, a confirmation was required and both samples were analysed by our in-house validated quantification HPLC-DAD method that uses solid-phase extraction for sample clean-up and thus allows for the injection of a more concentrated extract. The concentrations were determined at approximately 10 mg/kg for both samples, roughly four times lower than the LOI, thus explaining the poor library matches that were obtained. Moreover, for the UHPLC-HRMS analysis, the extracts had to be diluted two-fold as they were cloudy after the first extraction.

For two additional samples (a couscous spice mix and crushed chili) bixin, an apocarotenoïd extracted from annatto, was detected and identified. Although it is an authorised natural colorant with the E number E160b, its presence is not allowed in spices in the current Swiss legislation. It is not the first time this colorant has been reported (Scotter, 2011). The mass error of the precursor ion for the crushed chilli sample was −3.6 ppm and the library score was 94.8 (Fig. 2). Identity was also confirmed by the isotopic ratio, the MS/MS spectra and two SWATH fragments at 145.1030 and 157.1032 *m/z*, which were included in the reprocessing method, and whose mass errors were −0.8 and 0.1 ppm respectively.

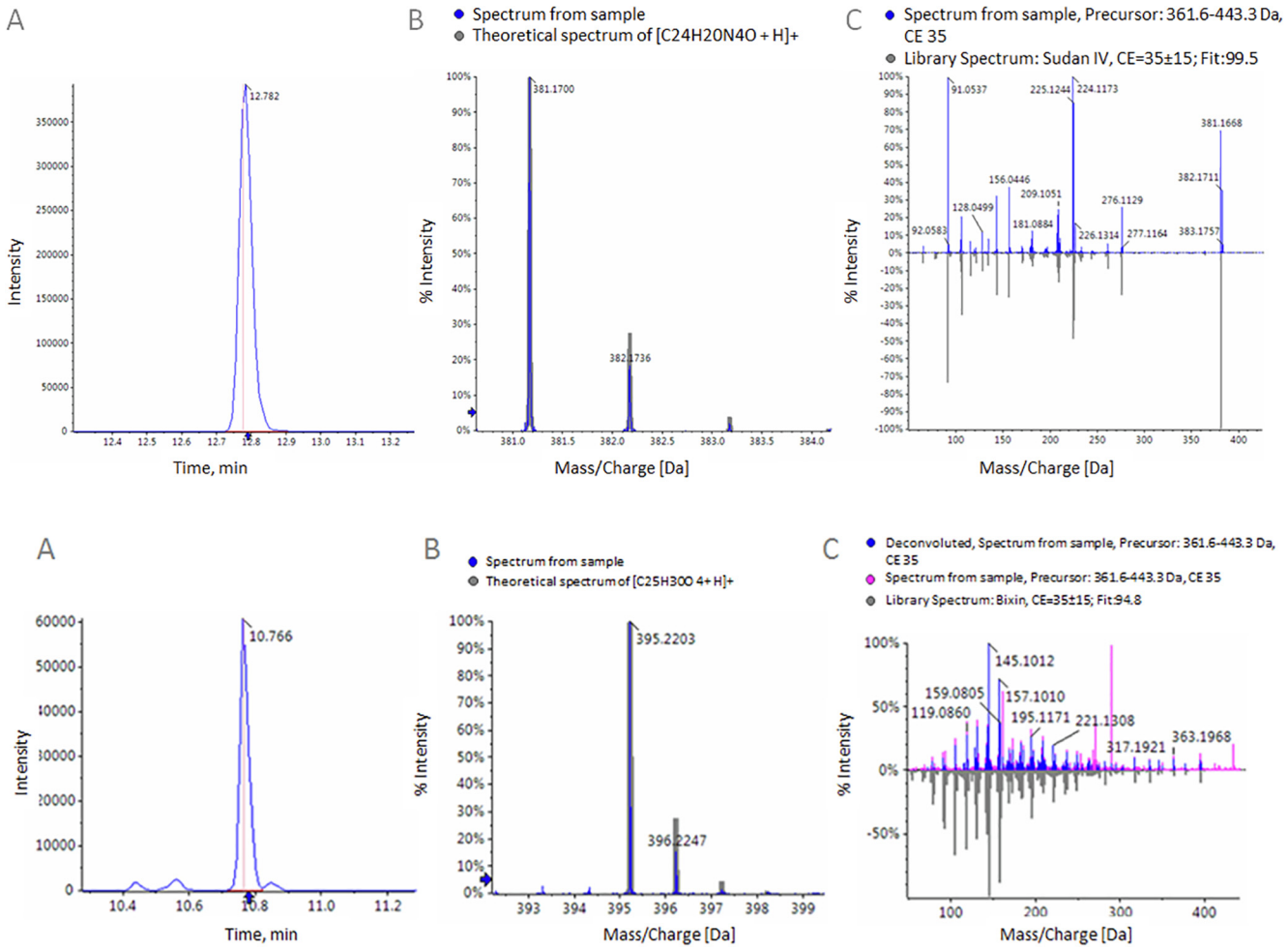

Fig. 2. Top: Reprocessing results for Sudan IV in chili peppers, with (A) the chromatogram extracted to the exact mass 381.1710 ± 25 ppm, (B) the isotopic distribution, (C) the deconvoluted MS/MS spectrum (in blue) compared to the library MS/MS spectrum (in grey) and Bottom: the results for bixin in the contaminated crushed chili with (A) the chromatogram extracted to the exact mass 395.2217 ± 25 ppm, (B) the isotopic distribution and (C) the deconvoluted MS/MS spectrum (in blue) compared to the library MS/MS spectrum (in grey). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

Hybrid instruments such as the QTOF used in this study are an attractive tool for the detection and identification of compounds in challenging matrices as they provide not only mass accuracy but also MS/MS spectra information and thus increased selectivity. These performance characteristics are paired with improved sensitivities that are close to those of MS/MS instruments. The results obtained in the present study with the analysis of a wide-range of dyes and artificial colours in spices demonstrate that the SWATH-MS screening technique coupled with a fast and generic sample preparation is an ideal tool for compliance monitoring in regulatory laboratories. A high degree of mass accuracy was obtained with the system at a sufficient mass resolution regardless of the spice or spice blend. The data processing software automatically identified the compounds against our in-house library using the TOF-MS and MS/MS data thus simplifying the routine workload especially as the need for the injection of reference compounds was only required in the case of confirmatory analyses.

In conclusion, the HRMS improvements over the last years in terms of hardware and software make it a perfectly suitable technique for the challenges encountered in the field of food analysis.

Declaration of interests

None.

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Conflicts of Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2019.100009.

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