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Method development and survey of Sudan I–IV in palm oil and chilli spices in the Washington, DC, area

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Abstract

Sudan I, II, III and IV dyes are banned for use as food colorants in the United States and European Union because they are toxic and carcinogenic. These dyes have been illegally used as food additives in products such as chilli spices and palm oil to enhance their red colour. From 2003 to 2005, the European Union made a series of decisions requiring chilli spices and palm oil imported to the European Union to contain analytical reports declaring them free of Sudan I–IV. In order for the USFDA to investigate the adulteration of palm oil and chilli spices with unapproved colour additives in the United States, a method was developed for the extraction and analysis of Sudan dyes in palm oil, and previous methods were validated for Sudan dyes in chilli spices. Both LC-DAD and LC-MS/MS methods were examined for their limitations and effectiveness in identifying adulterated samples. Method validation was performed for both chilli spices and palm oil by spiking samples known to be free of Sudan dyes at concentrations close to the limit of detection. Reproducibility, matrix effects, and selectivity of the method were also investigated. Additionally, for the first time a survey of palm oil and chilli spices was performed in the United States, specifically in the Washington, DC, area. Illegal dyes, primarily Sudan IV, were detected in palm oil at concentrations from 150 to 24 000 ng ml⁻¹. Low concentrations (< 21 µg kg⁻¹) of Sudan dyes were found in 11 out of 57 spices and are most likely a result of cross-contamination during preparation and storage and not intentional adulteration.

Keywords

Sudan dyes; palm oil; colour additive; LC-MS/MS; LC-DAD

Introduction

Sudans I–IV are oil-soluble azo dyes used for colouring textiles, plastics, wax, floor polish, and as a biological stain for lipids, triglycerides and lipoproteins (Hunger et al. 2000). They

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are labelled category 3 carcinogens by the IARC (IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans 1987) and are not permitted to be used in food in most countries including the United States and the European Union (Title 21 –Food and Drugs 2014; European Commission 1994, 2005). Because these dyes are bright in colour and low in cost, they have been used intentionally to adulterate palm oil and spices to enhance the perceived quality of the product (Arora & Bharti 2005). High-quality chillies naturally have a bright red colour and receive higher prices at market than those that are dull or orange and yellow (Arora & Bharti 2005). Adulteration with dyes has been used to cover up products in which the natural colour has been lost due to improper drying, storage or fungal infestations where pigment is degraded (Arora & Bharti 2005; Mishra et al. 2007). In 2003 the first detection of Sudan I in chilli powder in the European Union was reported, and the source of contamination was traced back to an adulterated chilli powder imported from India with a concentration of 4000 mg kg⁻¹ (RASFF 2003; ASTA 2005). At this time, a decision (2003/460/EC) was made that required all imports from fruit produced from plants under the genus name *Capsicum* be accompanied by an analytical report indicating the sample was free of Sudan I (European Commission 2003). In 2004, this requirement was expanded to Sudans II–IV, and in 2005, it was expanded to include palm oil and the genus *Curcuma* (turmeric) (European Commission 2005). In the UK in 2005, Sudan I was detected in a Worcester sauce (3 µg ml⁻¹) that was found to contain adulterated chilli powder (80 mg kg⁻¹) (RASFF 2005). This sauce had been used to make a wide variety of foods including soups, mince and sausage ready-meals, seafood sauces, pate, salad dressings and sauces (Sudan I Consolidated Product List from February 2005). As a result, close to 500 food products were recalled, making it the largest recall in the UK history to date. Unauthorised colours continue to be reported in the RASFF portal, with a total of 16 notifications in 2014 and 2015 (RASFF 2015).

Once the European Union began to require analytical reports with imported products, there came a need for analytical methods for quantifying Sudan dyes in a wide variety of foods. Many methods were developed using LC combined with UV-vis absorbance, photodiode array (PDA) detectors, or mass spectrometric detectors (MSD) (Rebane et al. 2010). For MS both electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) were used along with several mass analysers including Q-TOF, TOF, ion trap, single quadrupole and triple quadrupole (Rebane et al. 2010). For chilli powders, multiple methods were developed (Rebane et al. 2010) including a method validated for the Belgium monitoring programme that involved extraction of the spices into acetonitrile followed by LC-diode array detection (DAD) analysis (Cornet et al. 2006). This method used matrix-matched standards for calibration and the LOQs for the Sudan dyes ranged from 1.5 to 2 mg kg⁻¹ (Cornet et al. 2006). Without matrix-matched standards, the lowest LODs for spices were reported at 0.5–10 µg kg⁻¹ for Sudans I and III, and 5–100 µg kg⁻¹ for Sudans II–IV using LC-MS/MS (Schummer et al. 2013). Both methods involved a simple extraction with acetonitrile followed by filtration and dilution prior to analysis. For palm oil, only a few methods are published (Guffogg et al. 2004; Uematsu et al. 2007) including a qualitative TLC method used in monitoring by the Food Standards Agency (FSA) (Guffogg et al. 2004) and a method using DAD and clean-up by conversion of the oil to fatty acid methyl esters

(FAMES) and further clean-up by silica gel chromatography (Uematsu et al. 2007). In order for the USFDA to monitor and assess potentially contaminated palm oil samples, there was a need to develop a simple quantitative method using LC-MS/MS that could provide structural confirmation of Sudan dyes in palm oil.

Since many methods have been published on the analysis of Sudan dyes in spices using LC-MS/MS, there was no need to develop a new method and a previously developed method was validated (Tran et al. 2005). Additionally, the detection of Sudan dyes in both chilli powders and palm oil using LC-DAD was examined for strengths and weaknesses in identifying adulterated samples, since in a regulatory laboratory setting LC-DAD could be a useful screening tool for large numbers of samples. Using these methods, a survey of Sudans I–IV in palm oil and chilli spices sold in the Washington, DC, area and from online retailers was performed. The objectives of this research were: to develop a simple extraction method for Sudan dyes in palm oil; to validate an LC-MS/MS method and an LC-DAD method for palm oil and chilli spices; to investigate the suitability of LC-DAD and LC-MS/MS for Sudan dye analysis; and, for the first time, to investigate the concentrations of Sudan dyes present in these foods in the United States.

Materials and methods

Standards

Sudan I–IV analytical standards with purity > 96% were purchased from Sigma Aldrich (St. Louis, MO, USA) and deuterium-labelled d_6 -Sudan III for use as an internal standard was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Stock solutions were prepared in methanol with 10% tetrahydrofuran (THF) to ensure dissolution of the Sudan dyes. It is known that E-Z photo-isomerisation can occur when Sudans III and IV are exposed to light. The isomers formed produce additional ‘fast peaks’ in the chromatogram that elute much earlier than Sudans III and IV (Mölder et al. 2007). Unless the entire analytical process is performed in the dark, it is difficult to avoid the formation of these isomers. Mölder et al. (2007) found that once the vials were wrapped in aluminium foil and placed in the dark for up to 4.5 h, the isomers of the ‘fast peaks’ reverted back to their more stable form and were no longer present in the chromatogram. Therefore, in this study, all calibration standards and samples were stored in amber vials and kept in the autosampler tray in complete darkness for at least 1 h prior to analysis. This prevented the formation of ‘fast peaks’ that could potentially underestimate the concentrations of Sudans III and IV in samples.

Samples

Red palm oil samples were collected at supermarkets in the Washington, DC, area with a focus on those specialising in African foods. It was assumed that these oils were unrefined and the red colour was due to naturally occurring carotenes. In this case, adulteration with Sudan dyes would enhance the red colour to make the palm oil appear nutritionally superior. Based on the smell and texture of these oils, they appeared to be palm oil and not a cheaper oil substitute, but this was not investigated in this study. Additional samples were obtained

through online retailers with a focus on red palm oil samples used as dietary supplements. In total 30 samples were collected including 23 red palm oils and seven red palm oil dietary supplements sold as capsules. A majority of these samples were imported from West Africa (17), with a few samples coming from Malaysia (2) and South America (2). The country of origin of the remaining nine samples could not be identified from the labels.

Spices were collected at local and international markets in the Washington, DC, area that included crushed or minced chilli, ground chilli powder, ground cayenne pepper, paprika powder, and turmeric or other chilli products that are from the genera *Capsicum* and *Curcuma*. In total, 59 spices were collected and represent imports from Africa, Korea, China and India.

Sample preparation

Palm oil—Palm oil bottles were heated in a 66°C oven for approximately 5–10 min until the oil liquefied. Then, 100 µl of the palm oil were added to 900 µl of hexane to make a 1 ml solution.

Chilli spices—For the analysis of spices, a previously developed extraction method was used (Tran et al. 2005). Briefly, 1 g of the spice and 10 ml of acetone were added to a 50 ml polypropylene Falcon tube. The vial was shaken using a Glas-Col Digital Pulse Mixer (Terra Haute, IN, USA) for 5 min at a speed of 1200 rpm. The vial was then centrifuged for 5 min at 1800 rcf. An aliquot of the supernatant (1 ml) was removed and reduced to dryness using nitrogen. The sample was then brought up to 1 ml in hexane in preparation for SPE. For turmeric, low recoveries (approximately 5%) were found when the sample was brought up in hexane, likely due to the low solubility of the turmeric residue in hexane. Therefore, the sample was brought up in 1 ml of ethyl ether instead followed by a modified SPE clean-up. This improved the average recoveries to an acceptable range of 61–119%.

Solid-phase extraction (SPE) cleanup and preparation of extracts

SPE was performed using LC-Alumina-B SPE tubes (1 g/3 ml) from Supelco (St. Louis, MO, USA) and the conditioning and elution steps were modified from a method for spices and chilli oil (Tran et al. 2005). The cartridge was conditioned with 6 ml of methanol, 6 ml of ethyl acetate and 6 ml of hexane. Then, 1 ml of the sample solution was added to the SPE column. The sample was washed with 6 ml of hexane and 6 ml wash of ethyl ether (palm oil only), followed by 2 ml of ethyl acetate. The Sudan dyes were then eluted from the column using 8 ml of 90:10 ethyl acetate:methanol solution. For turmeric, the conditioning steps were the same and the wash step consisted of 2 ml of ethyl acetate followed by elution with 90:10 ethyl acetate:methanol. The elution solvent was blown to dryness using nitrogen and brought up to 1 ml in methanol. The extract was filtered through a 0.2 µm PTFE filter and internal standard was added (10 µl of 1 ng/µl *d*₆-Sudan III solution) prior to LC-MS/MS analysis. The addition of internal standard was necessary to correct for matrix effects based on the slopes of the calibration curves with and without internal standard correction. Both *d*₅-Sudan I and *d*₆-Sudan III were investigated as internal standards and it was determined that *d*₆-Sudan III was just as effective as *d*₅-Sudan I for correcting for matrix effects for

Sudans I and II. Therefore, just d_6 -Sudan III was used as an internal standard. For LC-DAD analysis, no internal standard was added since in DAD the internal standard and native peaks are not separated. However, the addition of a small amount of internal standard (10 ng ml^{-1}) to the final extract will have little effect on the measured concentration of Sudan III using LC-DAD since this concentration is well below the lower limit of quantification (LLOQ) for LC-DAD analysis (25 ng ml^{-1}) and since adulterated samples have significantly higher concentrations (ppm range).

Liquid chromatography (LC)

LC was performed using a Prominence UFLC XR (Shimadzu, Kyoto, Japan). Isocratic elution was used to separate the Sudan dyes (I–IV) with an Agilent Eclipse $5 \mu\text{m}$ XDB-C18 ($4.6 \text{ mm} \times 150 \text{ mm}$) column at 40°C and a flow rate of 0.8 ml min^{-1} for 30 min. For LC separation with DAD detection, a 150 mm column along with a flow rate of at least 0.5 ml min^{-1} is needed for good separation between the natural colours and Sudan dyes. For analysis using MS/MS without the DAD detector in series, a shorter column with a smaller particle size and lower flow rate would reduce solvent and the run time and maximise sample throughput. The mobile phase was 95/5 v/v methanol: water buffered with 5 mM ammonium formate and 0.1% formic acid with an injection volume of $5 \mu\text{l}$. A 5-min column wash was added at the end of each run using 98/2 v/v isopropanol:water solution buffered with 2 mM ammonium formate and 0.03% formic acid followed by a 5-min equilibration with mobile phase solvents. Additionally, during DAD analysis, a wash of 100% isopropanol was added at the end of each sample set to remove the buffered mobile phase from the DAD detector. The LC conditions (flow rate, column selection) were optimised for use with a DAD and MS/MS detector in series on the same instrument in order to develop a method that could be easily transferred among instruments with different detectors for testing adulterated samples.

MS/MS

An AB Sciex 4500 QTRAP hybrid triple-quadrupole/linear ion-trap MS with an electrospray ESI ion source (AB Sciex, Toronto, ON, Canada) was used for MS/MS detection for the palm oil samples. Initially, the chilli powder samples were analysed using the AB Sciex 4500, but the concentrations in the samples were quite low, so the analysis was moved to the AB Sciex 5500 QTRAP hybrid triple-quadrupole/linear ion-trap MS. Individual standards for Sudans I–IV were infused into the MS to optimise MS parameters (entrance potential, CE and CXP) for individual compounds. The two most abundant MRM transitions were monitored for each analyte, except in the case of Sudans I and II where two transitions were not chosen ($249 \leftarrow 93$ and $277 \leftarrow 121$) due to interferences. The monitored transitions were: $249 \leftarrow 156$, $249 \leftarrow 128$ for Sudan I, $277 \leftarrow 156$, $277 \leftarrow 128$ for Sudan II, $353 \leftarrow 197$, $353 \leftarrow 120$ for Sudan III, $381 \leftarrow 225$, $381 \leftarrow 106$ for Sudan IV, and $359 \leftarrow 197$, $359 \leftarrow 162$ for d_6 -Sudan III. The MS source/gas parameters were: curtain gas 20 arbitrary units (au), collision gas medium, ion spray voltage 4000 V, source temperature 400, ion source gas 160 au, ion source gas 270 au, and declustering potential 75 V. A seven-point linear calibration curve ($r^2 > 0.999$) for MS/MS analysis using the AB Sciex 4500 ranged from 0.5 to 100 ng ml^{-1} on column. The seven-point calibration curve for MS/MS analysis on the AB Sciex

5500 ranged from 0.1 to 10 ng ml⁻¹ on column. Since the samples were diluted 10 times prior to analysis, the concentration range measured in the palm oil samples was 5–1000 ng ml⁻¹ and in chilli spices was 1–100 µg kg⁻¹. The range for the calibration curve for chilli spices was adjusted to measure accurately the low concentrations in the survey samples.

Diode array detector (DAD)

A UV/vis photodiode array detector (DAD) Prominence by Shimadzu (SPD-M20A) was used for the detection of the Sudan dyes. The acquisition parameters included using both a D₂ and W lamp wavelength scan from 400 to 600 nm, a wave step of 8, slit width of 8, cell temperature of 40°C, and a period of 2 s (frequency of 4.167 Hz). The λ_{max} (nm) was 480 nm for Sudan I, 496 nm for Sudan II, 504 nm for Sudan III, and 520 nm for Sudan IV. For the DAD analysis, a six-point linear calibration curve ($r^2 > 0.99$) ranged from 25 to 1000 ng ml⁻¹ on column. Because of the 10:1 sample dilution, this corresponded to a concentration range of 250–10 000 ng ml⁻¹ in palm oil and 250–10 000 µg kg⁻¹ in chilli spices.

Results

Method validation

Palm oil—The analytical method was validated in palm oil by performing spike and recovery experiments in three different brands of palm oil at three spikes close to the LLOQ. For the MS/MS method, spikes were performed at 10, 20 and 30 ng ml⁻¹ in palm oil and for the DAD analysis the spikes were performed at 350, 450 and 550 ng ml⁻¹ in palm oil. The chromatograms palm oil spiked at 1 ng ml⁻¹ can be seen in Figure 1 for LC-MS/MS analysis and in Figure 2 for LC-DAD analysis. Results of the method recoveries can be seen in Figure 3. The average recoveries among all three matrices ranged: 77–84% for Sudan I, 57–62% for Sudan II, 73–92% for Sudan III, and 64–76% for Sudan IV for the MS/MS analysis; and: 66–83% for Sudan I, 60–74% for Sudan II, 67–81% for Sudan III, and 65–68% for Sudan IV for the DAD analysis. Since these recoveries were determined close to the LOD, they fall within the acceptable recovery range of 50–120% (Codex Alimentarius Commission 1993). Method recovery spikes at the concentration of suspected adulteration (approximately 1 µg ml⁻¹) were higher and averaged 71–99% for Sudan I–IV in palm oil. The method detection limits (MDLs) were calculated by performing seven low-level spikes at 10 ng ml⁻¹ in oil (1 ng ml⁻¹ on column, $S:N = 3:1-8:1$) for LC-MS/MS analysis and 350 ng ml⁻¹ in oil (35 ng ml⁻¹ on column, $S:N = 3:1-8:1$) for LC-DAD analysis and multiplying the standard deviation of the replicates by 3.14 (t -value for seven replicates where $1 - \alpha = 0.99$) (40 CFR Appendix B part 136) (Definition and Procedure for the Determination of the Method Detection Limit –Revision 1.11). The MDLs in palm oil were 2.4 ng ml⁻¹ for Sudan I, 4.4 ng ml⁻¹ for Sudan II, 2.0 ng ml⁻¹ for Sudan III, and 3.7 ng ml⁻¹ for Sudan IV with LC-MS/MS analysis. The MDLs for LC-DAD analysis were 184 ng ml⁻¹ for Sudan I, 232 ng ml⁻¹ for Sudan II, 193 ng ml⁻¹ for Sudan III, and 237 ng ml⁻¹ for Sudan IV in palm oil.

Chilli spices—Method validation was performed for the Sudan dyes in spice samples by performing spike and recovery experiments in three different spices at concentrations of 1, 2 and 3 µg kg⁻¹ in the spices and for the diode array analysis at 8, 9 and 10 mg kg⁻¹ in the

spices. The percentage recoveries for the LC-MS/MS method can be seen in Figure 3, and ranged: 88–100% for Sudan I, 89–104% for Sudan II, 89–93% for Sudan III, and 66–79% for Sudan IV. The recoveries for the diode array analysis are also shown in Figure 3, and ranged: 76–83% for Sudan I, 76–81% for Sudan II, 80–86% for Sudan III, and 70–76% for Sudan IV. The MDLs for Sudan dyes in chilli spices was calculated by performing seven low-level spikes at $1 \mu\text{g kg}^{-1}$ in chilli spices (0.1 ng ml^{-1} on column) and multiplying the standard deviation of the replicates by 3.14 (t -value for seven replicates where $1 - \alpha = 0.99$) (40 CFR Appendix B part 136) (Definition and Procedure for the Determination of the Method Detection Limit – Revision 1.11). The MDLs in chilli spices were $0.7 \mu\text{g kg}^{-1}$ for Sudan I, $0.5 \mu\text{g kg}^{-1}$ for Sudan II, $0.7 \mu\text{g kg}^{-1}$ for Sudan III, and $1.0 \mu\text{g kg}^{-1}$ for Sudan IV for LC-MS/MS analysis. The analysis of spices by LC-DAD is more challenging than in palm oil due to the large amounts of natural colours present with similar polarities as the Sudan dyes. For example, in Figure 2, the DAD chromatogram for palm oil extract spiked at 1 mg kg^{-1} is much cleaner than the chilli powder spiked at 1, 5 and 10 mg kg^{-1} . A spiked concentration of at least 5 mg kg^{-1} is needed in order to quantify the peaks based on retention time and spectra quality. Also, spices vary in the amounts and types of natural colours present, as seen in Figure 4. The DAD chromatograms of the chilli powder and crushed red pepper spiked at 10 mg kg^{-1} is much cleaner than the paprika chromatogram, which has a colour eluting just under 6 min that results in a maximum absorbance around 250 mAU compared with 40 mAU. Previous research has identified higher LODs ($1.5\text{--}2 \text{ mg kg}^{-1}$) of Sudan dyes in spices using LC-DAD with matrix-matched calibration curves (Cornet et al. 2006) as well as interferences with paprika and turmeric (Tateo & Bononi 2004). Additionally, carotenoids present in *Capsicum* can absorb in the same range as Sudan dyes and give false-positives (Hoenicke 2006). Because of these natural colours, the method LODs were much higher for chilli spices than that of the palm oil and calculated to be 3.6 mg kg^{-1} for Sudan I, 4.1 mg kg^{-1} for Sudan II, 3.1 mg kg^{-1} for Sudan III, and 4.6 mg kg^{-1} for Sudan IV in chilli spices based on an 8 mg kg^{-1} spike. This amount was chosen for MDL calculations based on the spice (paprika) with the DAD chromatogram with the most interferences (Figure 4). Studies have shown that at least 120 mg kg^{-1} of Sudan I would be needed to increase the extractable colour one ASTA colour unit using the ASTA method 20.1 (ASTA 2005), and researchers in Europe have found spices identified as adulterated ranging in concentration from 25 to 3500 mg kg^{-1} (Hoenicke 2006). Therefore, this method with MDLs from 3.1 to 4.6 mg kg^{-1} would be an appropriate screening method for spice samples that are potentially contaminated. Suspected contaminated samples should be verified using LC-MS/MS to confirm there are no false-positives.

Survey results

Palm oil—Twenty-two red palm oil samples and eight red palm oil dietary supplements were analysed for Sudans I–IV. Since one sample was highly contaminated (Ivory Coast 1), two additional bottles from different retailers were also purchased to determine if the concentrations were consistent (Ivory Coast (2) and (3)). In total, 20 different brands of palm oil were tested and, of those, four different brands had detections of Sudan IV. The concentrations ranged from 150 to $24\,000 \text{ ng ml}^{-1}$ by LC-MS/MS detection and from 240 to $25\,000 \text{ ng ml}^{-1}$ by LC-DAD detection (Table 1). Based on the high concentrations measured

in contaminated samples, both methods are suitable for analysis. In four out of five of the samples, low level detections of Sudans I and III were found ($0.5\text{--}30\text{ ng ml}^{-1}$), which are likely attributed to impurities in the Sudan IV dye. Although the first three samples were purchased from different retailers, they were the same brand name and all imported from Ivory Coast. It is unclear from their labels whether or not they come from the same lots. The sample from Ivory Coast (1) had the highest contamination of Sudan IV at $24\,000\text{ ng ml}^{-1}$, while the other two samples had lower concentrations at $5000\text{--}7000\text{ ng ml}^{-1}$. The other samples that were contaminated were two samples from Ghana with concentrations of 6400 and 1500 ng ml^{-1} and in another sample from West Africa at 150 ng ml^{-1} of Sudan IV, but the exact country of origin is unknown. Of these four contaminated samples, three were purchased from retail stores in the Washington, DC, area that contain grocery items primarily imported from Africa, and one of the contaminated samples was purchased from an online retailer. Although the presence of any colour additive not approved for use in food (European Commission 1994) is considered adulteration, it was proposed in 2006 at a European Union meeting of the Standard Committee on the Food Chain and Animal Health that an action limit of 500 ppb be adopted. This was due to very low concentrations of Sudan dyes found in products that were thought to be cross-contamination and not intentional adulteration. In this case, three out of these four samples would exceed this limit.

Spices—Spices that fell into the categories that would require an analytical report to accompany import into the European Union were collected from local grocery stores including those specialising in international foods. Out of 59 samples, 11 were contaminated with at least one illegal dye with concentrations less than $21\text{ }\mu\text{g kg}^{-1}$ (Table 2). These concentrations were determined using LC-MS/MS with an AB Sciex 5500 QTRAP hybrid triple-quadrupole/linear ion-trap MS, since on the AB Sciex 4500 only one out of 11 of the results was above the LOQ. Additionally, the LC-DAD could not be used for this analysis since the MDL in spices is close to 1 mg kg^{-1} . In total, four spices were contaminated with Sudan I, none with Sudan II, two with Sudan III, and eight with Sudan IV. Since these concentrations are so low, it is unlikely that any of these spices were intentionally adulterated. The concentrations of illegal dyes in adulterated samples detected in the European Union ranged from 25 to 3500 mg kg^{-1} (Hoenicke 2006), and in order to enhance visually the colour of the spice, $100\text{--}1000\text{ mg kg}^{-1}$ of dye needs to be added (ASTA 2005). An analytical laboratory in Germany also detected very low levels of Sudan dyes in spices. In order to investigate the cause of the concentrations, they performed two case studies. One identified $\mu\text{g ml}^{-1}$ concentrations of Sudan I in a red lubricant used for greasing extraction plants as the source of Sudan I measured in spices ranging from 10 to $120\text{ }\mu\text{g kg}^{-1}$ (Hoenicke 2006). The second case study found mg kg^{-1} amounts of Sudan IV in red mesh bags used for storing and transporting chilli pods. In the corresponding samples, the concentration of Sudan IV was $10\text{--}20\text{ }\mu\text{g kg}^{-1}$ (Hoenicke 2006). As a result, it appears that the most likely source of the low concentrations measured in the spice samples obtained in the Washington, DC, region is related to cross-contamination during sample processing and storage.

Discussion

A method for the extraction and analysis of Sudans I–IV in palm oil using LC-MS/MS was developed and validated. The benefits of this palm oil method compared with previous methods (Guffogg et al. 2004, Uematsu et al. 2007) are the simple preparation and extraction steps, low levels of detection ($2.0\text{--}4.4\text{ ng ml}^{-1}$), and structural confirmation by MS/MS. The use of LC-MS/MS is preferred for confirmation, but the LC-DAD results are comparable (Table 1) and had clean chromatograms (Figure 2) with the disadvantage of higher LODs ($184\text{--}237\text{ ng ml}^{-1}$). For the spices, the LC-MS/MS method is clearly advantageous due to the many natural colours present that result in a LC-DAD chromatogram with a poor background, potential interferences and false-positives. But considering the high concentrations typically found in adulterated spices ($> 25\text{ mg kg}^{-1}$), the LC-DAD method performs well at these concentrations and would provide a suitable screening method for routine analysis. Previous methods that involve the extraction of spices with acetonitrile followed by filtration and dilution are excellent for use with LC-MS/MS (Schummer et al. 2013). For DAD analysis, it was determined that SPE clean-up was necessary to reduce the interferences and improve LODs. The validation of these methods for the analysis of illegal dyes in palm oil and chilli spices will allow the regulatory analysis of samples imported into the United States using either LC-MS/MS or LC-DAD detection. Based on the survey results in the Washington, DC, area, adulterated palm oil is present in the marketplace at concentrations up to $24\,000\text{ ng ml}^{-1}$. Red palm oil is popular in the diets of people from West Africa, the Caribbean and South America, and can be consumed in large amounts (several cups) daily in the form of soup or stew. As a result, these populations are at a higher risk of being exposed to unapproved and, therefore, illegal dyes in their diets. Additionally, red palm oil for use as a dietary supplement has the potential to be adulterated. In this limited survey, Sudan dyes were not detected above the MDL in any of the dietary supplements. The amount of illegal dyes found in the spices purchased in the Washington, DC, area was less than $21\text{ }\mu\text{g kg}^{-1}$, which is well below the $500\text{ }\mu\text{g kg}^{-1}$ proposed action limit in the European Union for Sudans I–IV in spices and palm oil. Considering these samples represent a regional subset of palm oil and chilli products available in the US market, the validation of the LC-DAD and LC-MS/MS methods presented ensures that future adulterated products can be tested and regulated.

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Figure 1. LC-MS/MS chromatograms of (A) palm oil spiked at $1 \mu\text{g mL}^{-1}$ and (B) paprika spiked at 1 mg kg^{-1} .

Figure 2.

LC-DAD chromatograms of (A) palm oil spiked at 1 mg kg^{-1} , (B) chilli spice spiked at 1 mg kg^{-1} , (C) chilli spice spiked at 5 mg kg^{-1} and (D) chilli spice spiked at 10 mg kg^{-1} with Sudan dyes labelled as 1–4.

Figure 3.
Method recoveries for the extraction of Sudan dyes from palm oil by (A) LC-MS/MS and (B) LC-DAD; and chilli spices by (C) LC-MS/MS and (D) LC-DAD.

Figure 4.

LC-DAD chromatograms of (A) chilli powder at 10 mg kg⁻¹, (B) crushed red pepper at 10 mg kg⁻¹, and (C) paprika at 10 mg kg⁻¹ with Sudan dyes labelled as 1–4. The maximum *y*-axis for (C) is 250 mAU, while the maximum for (A) and (B) is 40 mAU.

Table 1

Concentration of Sudan dyes in palm oil by both LC-MS/MS and LC-DAD.

Palm oil country of origin	Mass spectrometry data				DAD data	
	Sudan I	Sudan II	Sudan III	Sudan IV	Sudan I	Sudan IV
	ng ml ⁻¹				ng ml ⁻¹	
Ivory Coast (1) ^a	30	< MDL	3.4	24 000	25 000	
Ivory Coast (2) ^a	20	< MDL	2.2	4800	7400	
Ivory Coast (3) ^a	20	< MDL	16	7000	6900	
Ghana	0.5	< MDL	11	6400	6600	
Ghana	13	< MDL	40	1500	2300	
West Africa	< MDL	< MDL	< MDL	150	240	

Note:

^a Sample is the same brand name.

Table 2

Concentrations of Sudan dyes in spices by LC-MS/MS.

Spice	Sudan I	Sudan II	Sudan III	Sudan IV
	(µg kg ⁻¹)			
Red pepper powder	1.1	< MDL	< MDL	21
Red pepper powder	< MDL	< MDL	0.75	3.6
Paprika powder	< MDL	< MDL	1.0	< MDL
Crushed red chillies	< MDL	< MDL	< MDL	0.25
Curry powder	1.2	< MDL	< MDL	< MDL
Turmeric	< MDL	< MDL	< MDL	0.58
Cayenne powder	< MDL	< MDL	< MDL	1.9
Cayenne powder	< MDL	< MDL	< MDL	2.3
Mixed spice	0.94	< MDL	< MDL	2.7
Mixed spice	1.8	< MDL	< MDL	< MDL
Mixed spice	< MDL	< MDL	< MDL	0.99