Using TOF for Screening and Quantitation of Sudan Red Colorants in Food

Application

Food

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Abstract

The Sudan Reds are four commercial dyes, which could increase the risk of cancer if consumed in foods over long periods, or in large quantities. Now, they are banned in most countries. This application note presents the capability of the Agilent Time of Flight Mass Spectrometer (LC/MSD TOF) to be used as a screening, confirmation and quantitation tool-within-one analytical run of 5 min.

Introduction

Sudan Red is a red dye, traditionally used for coloring solvents, oils, waxes, petrol, and shoe and floor polishes. However, it is prohibited for use by the Regulations Relating to Food Colorants (R.1008) of the Foodstuffs, Cosmetics and Disinfectant Act 54 of 1972. Furthermore, the European Commission extended the scope of the ban on Sudan Red after it was revealed that related chemicals were also being found in chili products adulterated with Sudan Red. These chemical varieties are classified as Sudan Red I, II, III, and IV. All are considered to be carcinogenic.

There is now an emergency measure in force dictating that chili and chili products, including curry powder, can only enter an EU country provided it has proof that these illegal chemical dyes are not present. These rulings extend tight measures that have been in place since June 2003, when France alerted the European Commission that traces of Sudan Red I were found in chili and chili powder imports from India. The inclusion of curry powder, which is found extensively in European food products, could lead to a surge in product recalls for the food industry.

In the UK alone, the food industry has recalled for destruction more than 160 products from the supermarket shelves since July 2003 as part of the enforcement of the new measures. Many countries including Canada, South Africa, France, Germany, and China have also discovered traces of Sudan I in their cuisines [1]. Figure 1 shows the structures of the banned dyes.

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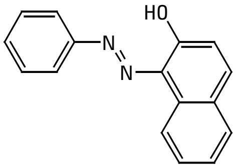
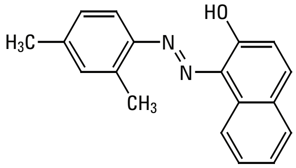
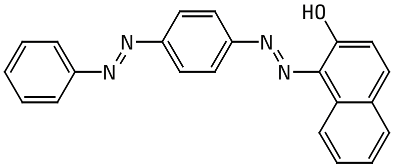
Sudan Red I



Sudan Red II



Sudan Red III



Sudan Red IV

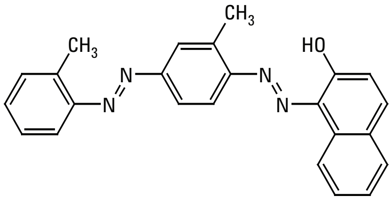


Figure 1. Structures of Sudan Reds.

Some methods exist for Sudan Red analysis. In method GB 19681-2005, the separation is complimented with a solid phase extraction, which simplifies the analysis and concentrates the sample [2].

Accurate mass measurement greatly increases the confidence of identification because it inherently limits the possible number of candidate compounds. That is, the better the precision and accuracy of the mass measurement, the fewer the number of compounds theoretically possible for a given accurate mass. Even without complicated sample preparation, good results can be obtained.

This application note provides an overview of the power of the Agilent TOF mass spectrometer for the screening, confirmation, and quantitation analysis of Sudan Reds. The TOF mass spectrometer provides accurate mass determination (<3 ppm) with good linearity, proving its use as an excellent tool for selectivity, specificity, and sensitivity for detection of analytes in different source matrices. The method used here is not intended to represent one that will determine the lowest possible level of any one particular analyte, or class of analytes, but rather is a procedure that could be expanded to cover a wider range of components used in screening analyses.

Experimental

Instrument

Agilent 1100 Series LC/MSD TOF with Agilent 1100 binary pump and well plate autosampler

LC Conditions

Column	ZORBAX XDB C18, 2.1 mm × 50 mm, 1.8 µm Agilent p/n: 922700-902
Mobile phases	A: H_2O with 5 mM NH_4OAc B: Acetonitrile
Gradient	0–3 min 95% B to 98% B 3–5 min 98% B
Post time	3 min
Flow rate	0.5 mL/min

MS Conditions

Ionization	ESI, Positive
Gas temp	350 °C
Drying gas	8 L/min
Nebulizer pressure	45 psi
Capillary V (+ve)	4000 V

Reference Mass Introduction with LC-TOF

The Agilent TOF MS uses a reference mass for the generation of reliable and high-level accurate mass spectra. The electro-spray ion source for the TOF is a unique dual-spray assembly that allows the simultaneous and constant introduction of reference mass components consisting of ~2-µM purine (*m/z* 121.050873 and HP-0921 (*m/z* 922.009798) calibrant compounds.

Results

Shown in Figure 2 is the total ion chromatogram (TIC) and the corresponding MS spectra for Sudan Red I, Sudan Red II, Sudan Red III, and Sudan Red IV. Note that all the compounds can be separated well within 5 min for a high-throughput analysis, which corresponds to at least half the time seen in most other methods.

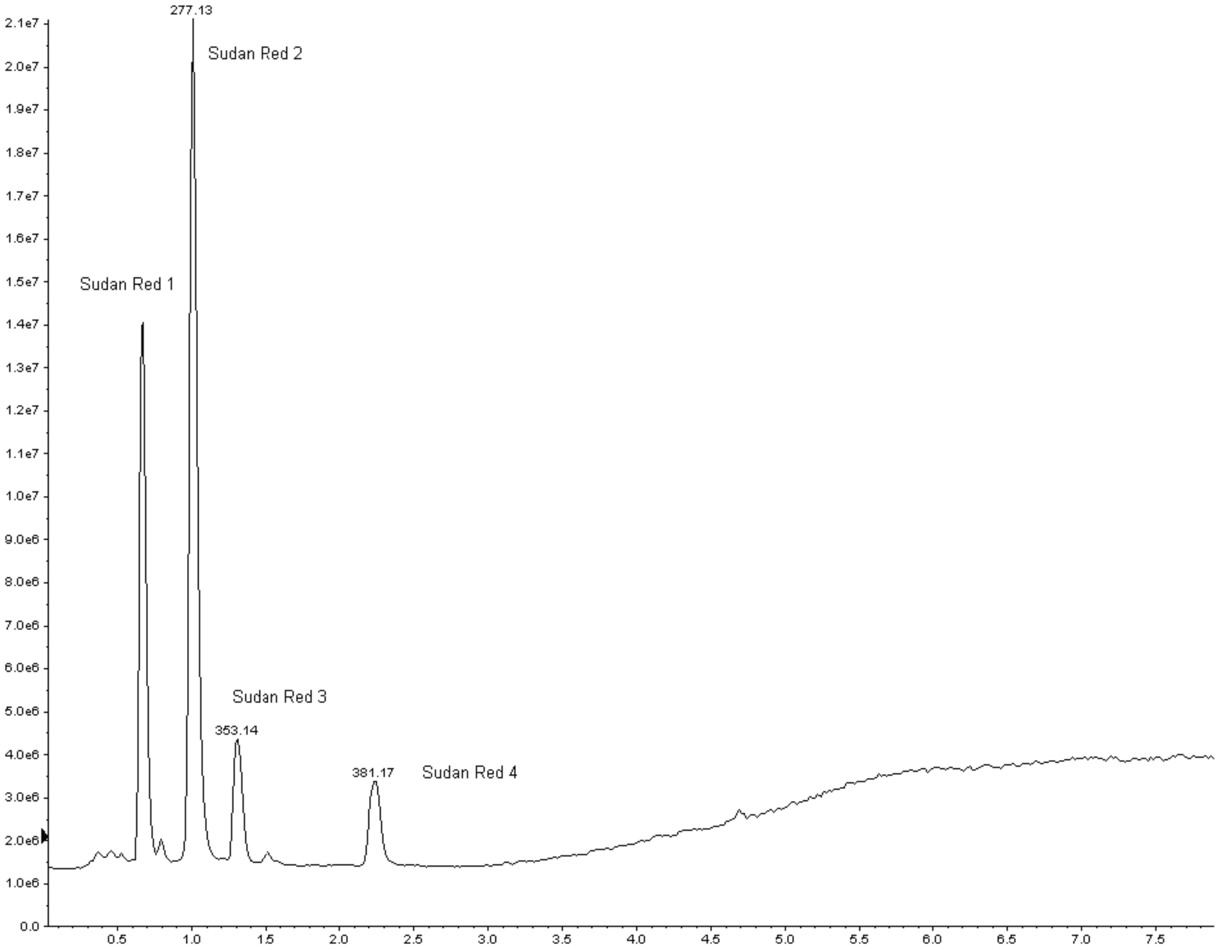


Figure 2. TIC showing the four peaks of the Sudan Red dyes along with the corresponding mass spectum of each peak.

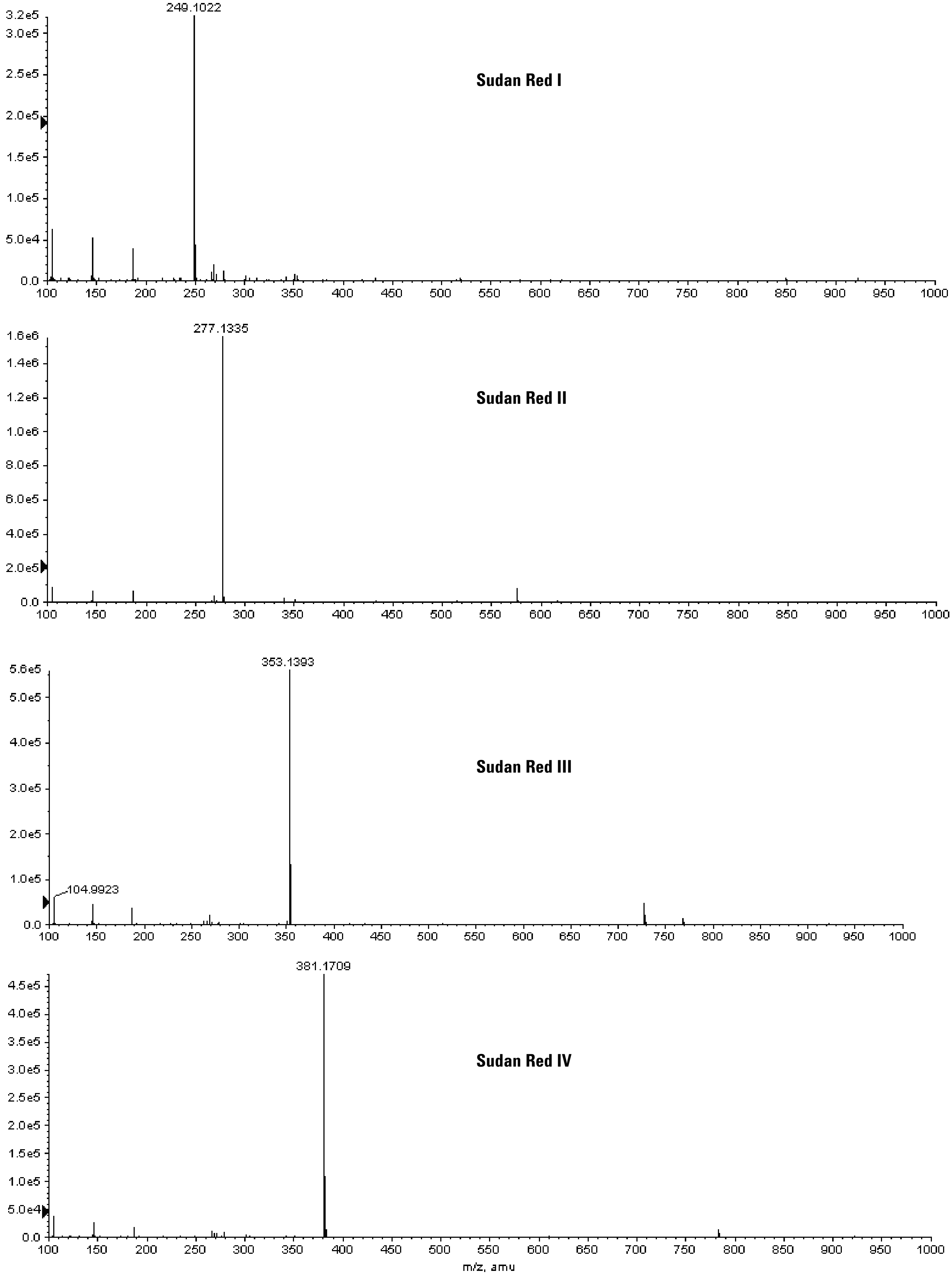


Figure 2. TIC showing the four peaks of the Sudan Red dyes along with the corresponding mass spectum of each peak.

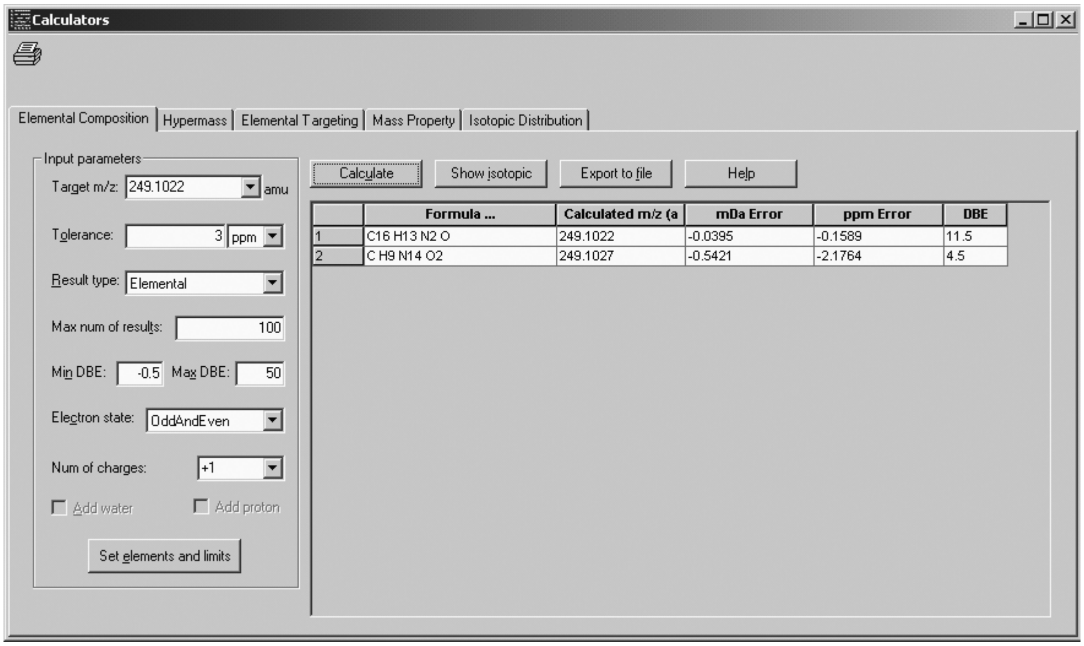
The ability to closely match the expected mass and the observed mass provides the analyst with a higher level of confidence in the assignment given to a chromatographic peak. In the screening for compounds in matrices such as food, this additional confidence is of great importance. This capability also allows the possibility of using this technique as a screening tool for a wide range of components.

To demonstrate the use of high mass accuracy in confirming the presence of compounds like the Sudan Red dyes, the mass of Sudan Red I, from Figure 3, which is 249.1022 is entered into the calculator of the TOF software to determine the empirical formula. (See Table 1.)

So, given the accurate mass of 249.1022, mass accuracy of 3 ppm, and assumption that the compound only contains carbon (C), hydrogen (H), nitrogen (N), and Oxygen (O), only two empirical formulas are determined. However, the second match with only one carbon does not make any sense for the type of compounds being examined here and can be discarded so that only $C_{16}H_{13}N_2O$ is possible, which just happens to be the expected formula for the pseudo-molecular ion, $(M+H)^+$, of Sudan Red I.

Table 1. Theoretical Accurate Mass, Observed Mass and Mass Error

Compound	Monoisotopic mass	Retention time	Adduct	Observed mass	Adduct accurate mass	Mass (ppm)
Sudan Red I	248.0950	0.66	[M+H]+	249.1022	249.1022	–0.1589
Sudan Red II	276.1263	1.01	[M+H]+	277.1340	277.1335	–0.1434
Sudan Red III	352.1318	1.30	[M+H]+	353.1393	353.1390	0.6993
Sudan Red IV	380.4482	2.24	[M+H]+	381.1709	381.1709	–0.2306



Target m/z: 249.1022 amu
Tolerance: 3 ppm
Result type: Elemental
Max num of results: 100
Min DBE: -0.5 Max DBE: 50
Electron state: OddAndEven
Num of charges: +1
☐ Add water ☐ Add proton

	Formula ...	Calculated m/z (a	mDa Error	ppm Error	DBE
1	C16 H13 N2 O	249.1022	-0.0395	-0.1589	11.5
2	C H9 N14 O2	249.1027	-0.5421	-2.1764	4.5

Figure 3. Empirical formula calculator of TOF software determines two possible formulas given the observed mass of 249.1022 and known mass accuracy of <3 ppm.

A greater than two-fold increase in sensitivity for many components is seen with the narrowing of the extracted ion chromatogram (EIC) window. To demonstrate this, Figure 4 shows the reduction in noise that is observed with the extraction of a smaller mass range for Sudan Red I. As a result, the ability of TOF-MS to accurately determine the presence of the components at low level may assist with investigations into reporting the illegal use of the synthetic dyes, and prove to be a critical factor in confirmation when dealing with complex matrixes.

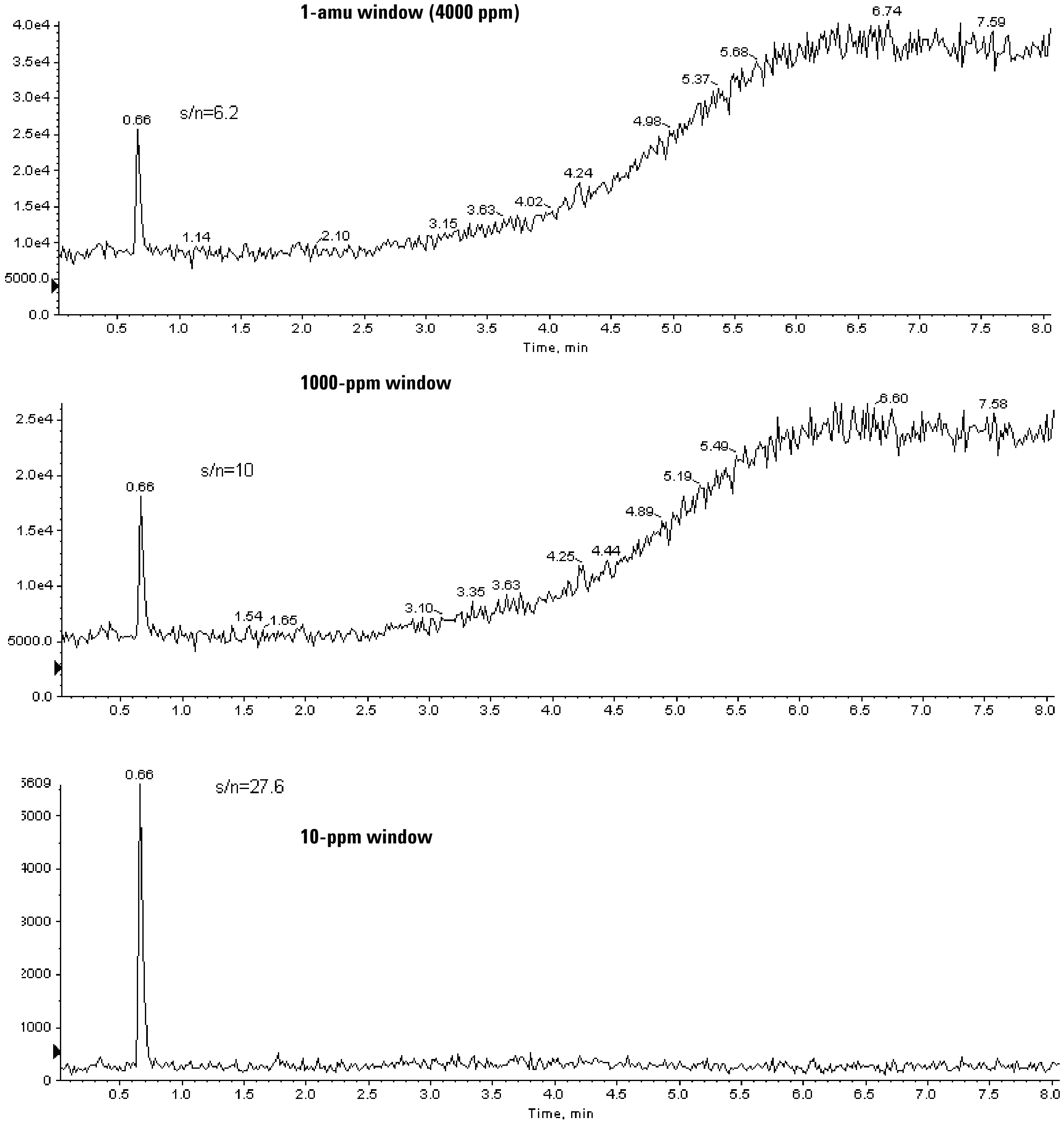
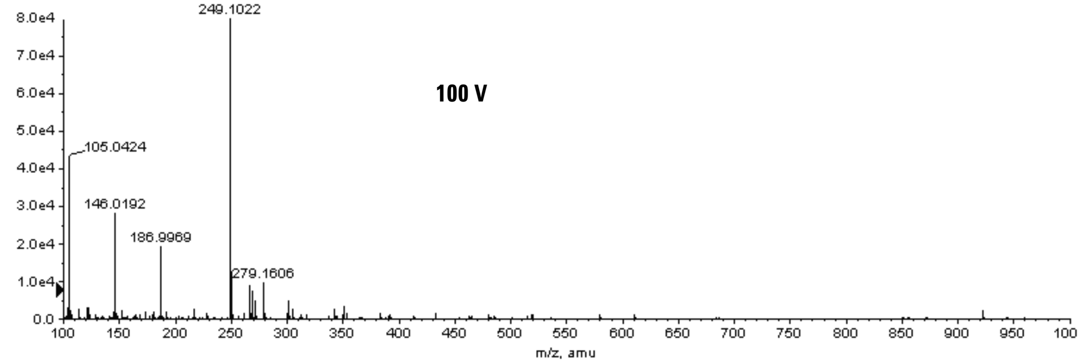


Figure 4. Effect of extracted ion range on noise-Sudan Red I in a real sample of Chinese Source spice oil.

It can be seen from Figure 4 that the ability to narrow the mass extraction window greatly reduces the noise for a given mass, and with the retention time information, can provide a high level of confidence in the assignment of a component.

Furthermore, the TOF can use in-source collision-induced dissociation (CID) to generate fragment ions for confirmation as well. These additional ions can be seen in both the unextracted standards and the compounds in the food matrix. Such spectra obtained with the unextracted standards are shown in Figure 5.

Sudan Red I at different voltages



Sudan Red II at different voltages

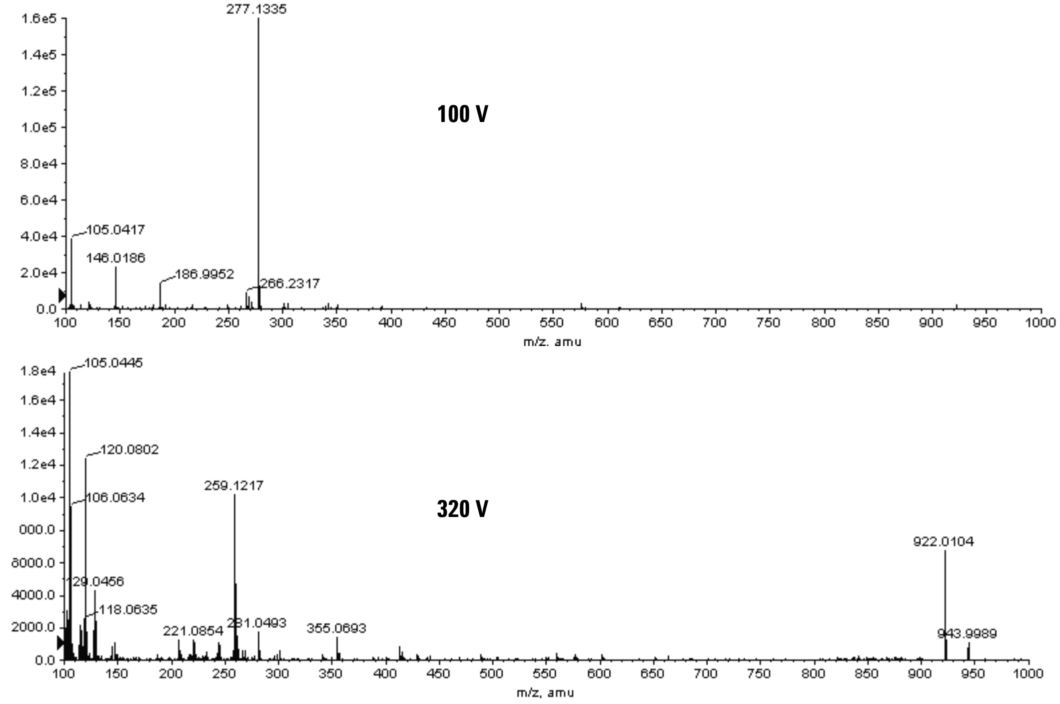
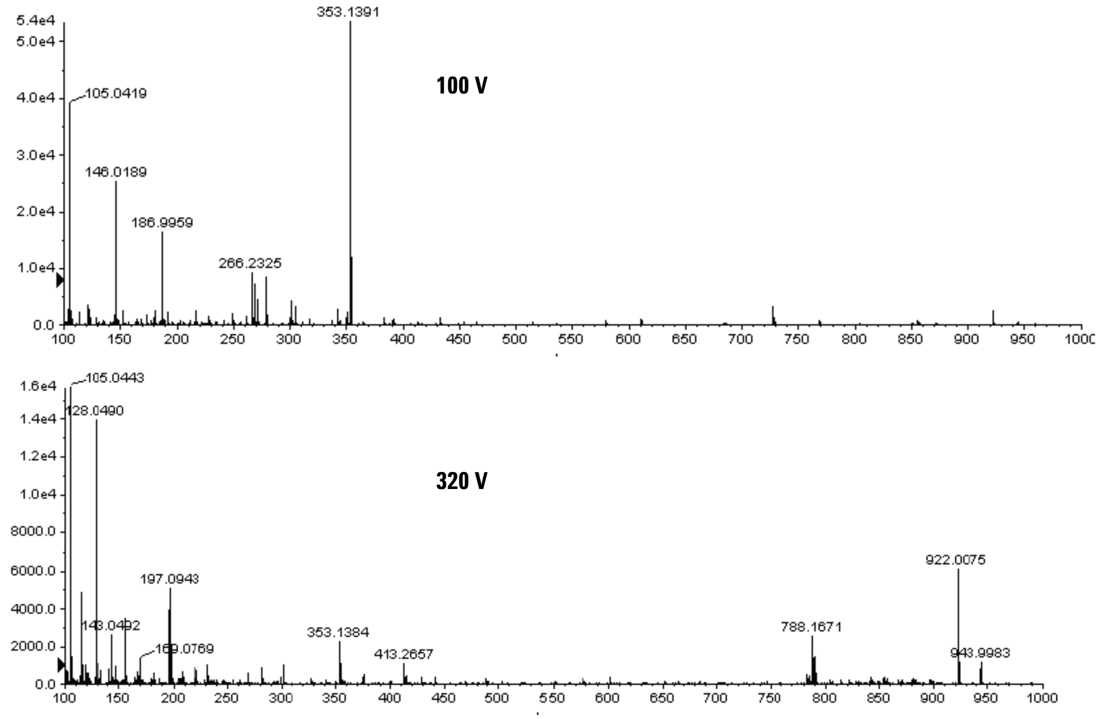


Figure 5. Sudan Red I, II, III, and IV at different in-source CID voltages.

Sudan Red III at different voltages



Sudan Red IV at different voltages

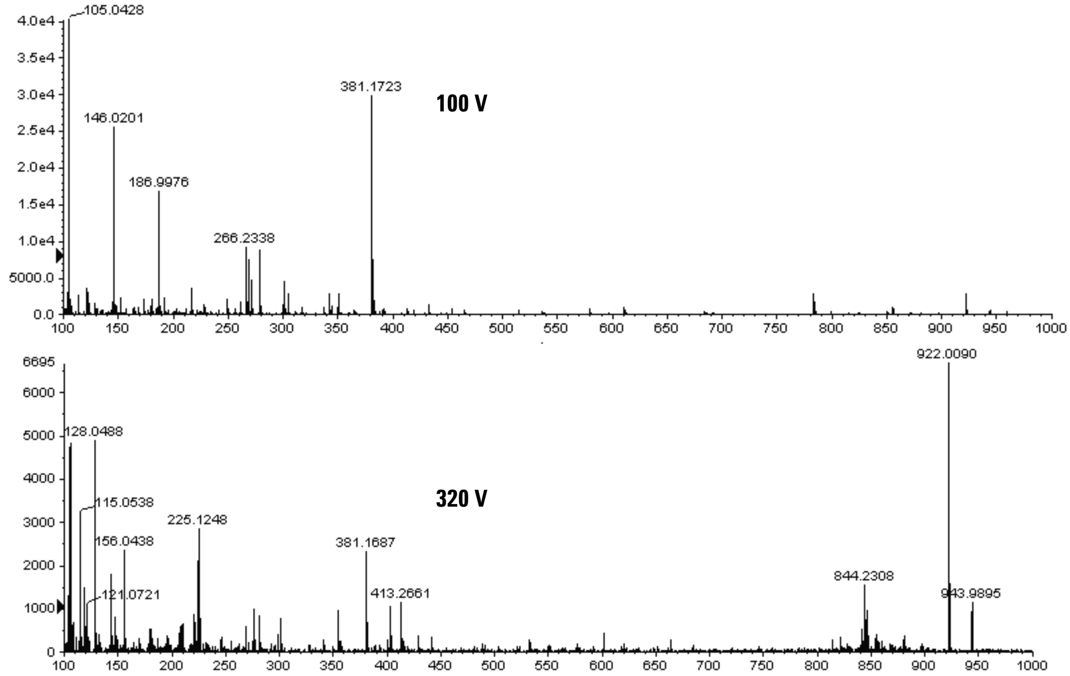
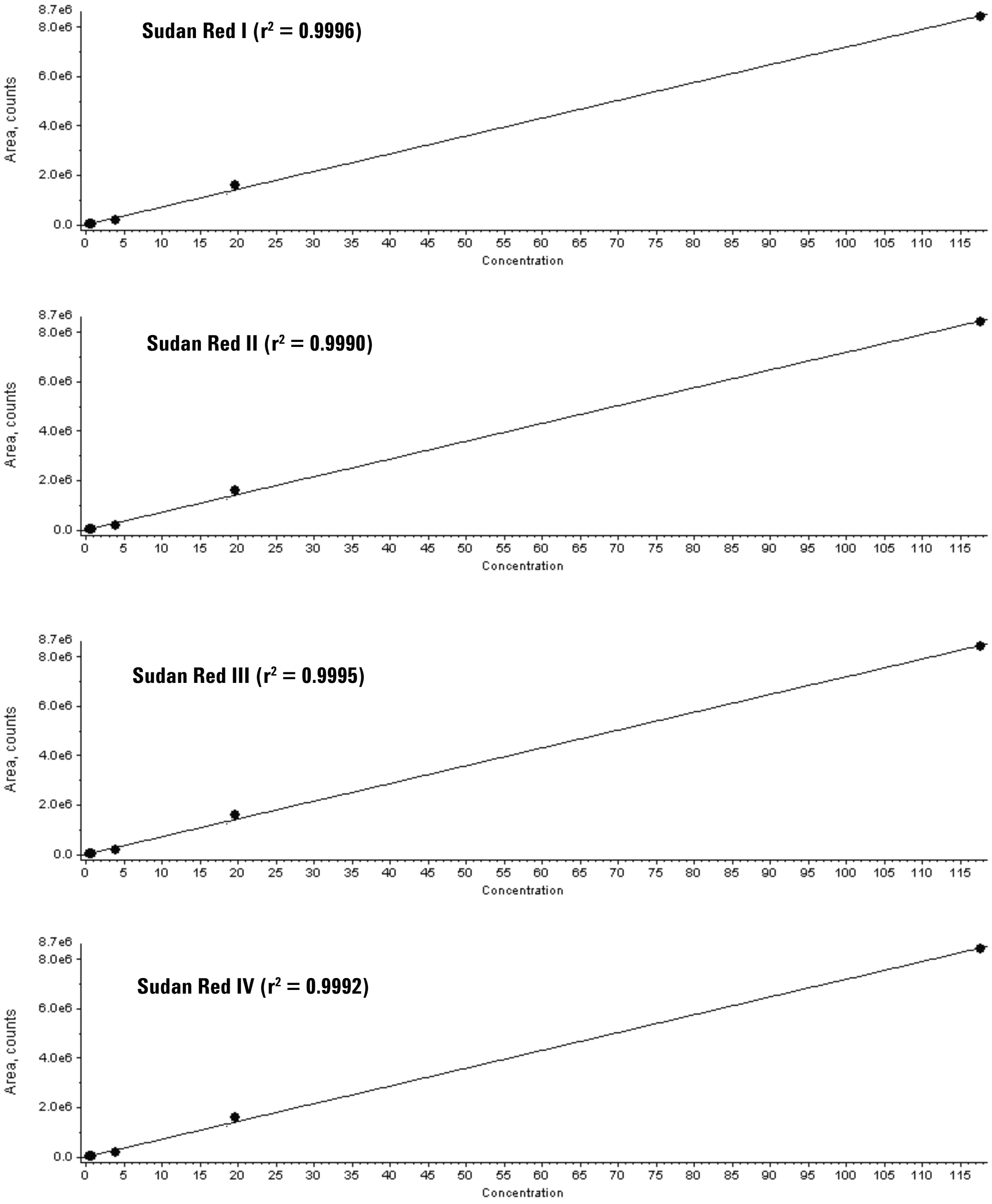


Figure 5. Sudan Red I, II, III, and IV at different in-source CID voltages (continued).

TOF Linearity

The components analyzed by TOF were tested for linearity as part of this study. Figure 6 shows the linearity of these four compounds. The regression values, r^2, of over 0.99 were seen for each of these compounds.


Sudan Red I ($r^2 = 0.9996$)

Sudan Red II ($r^2 = 0.9990$)

Sudan Red III ($r^2 = 0.9995$)

Sudan Red IV ($r^2 = 0.9992$)

Figure 6. Linearity of the four Sudan Red dyes.

LOD and LOQ

Table 2 summarizes the limits of detection (LODs) and limits of quantitation (LOQs) for each of the components analyzed by this method. As shown in these results, this method is designed to provide a broad screening tool for the analysis of banned colorants.

Table 2. LOD and LOQ for Components by LC/MSD TOF

Component	LOD (ppb) (s/n = 3)	LOQ (ppb) (s/n = 20)
Sudan Red I	3.6	24.4
Sudan Red II	2.4	18.7
Sudan Red III	17.6	126.4
Sudan Red IV	20.4	132.2

An example of the determination for LOD and LOQ is shown for the Sudan Red I dye in Figures 7 and 8, respectively. The upper chromatogram of each figure is the TIC, while the lower chromatogram corresponds to an EIC of *m/z* 249.1022 with a window of 10 ppm. The LOD is calculated based on a signal-to-noise (S/N) value of 3. Figure 7 corresponds to an on-column injection of 4.4 ppb and has a S/N value of 3.7. Therefore, the LOD is calculated as 4.4 ppb * (3/3.7) = 3.6 ppb, which is listed in Table 2. Figure 8 corresponds to an on-column injection of 33.7 ppb. The LOQ is calculated based on a S/N value of 20. Therefore, the LOQ is calculated as 33.7 ppb * (20/27.6) = 24.4 ppb, which is also shown in Table 2. Similar reasoning is used for the other dyes.

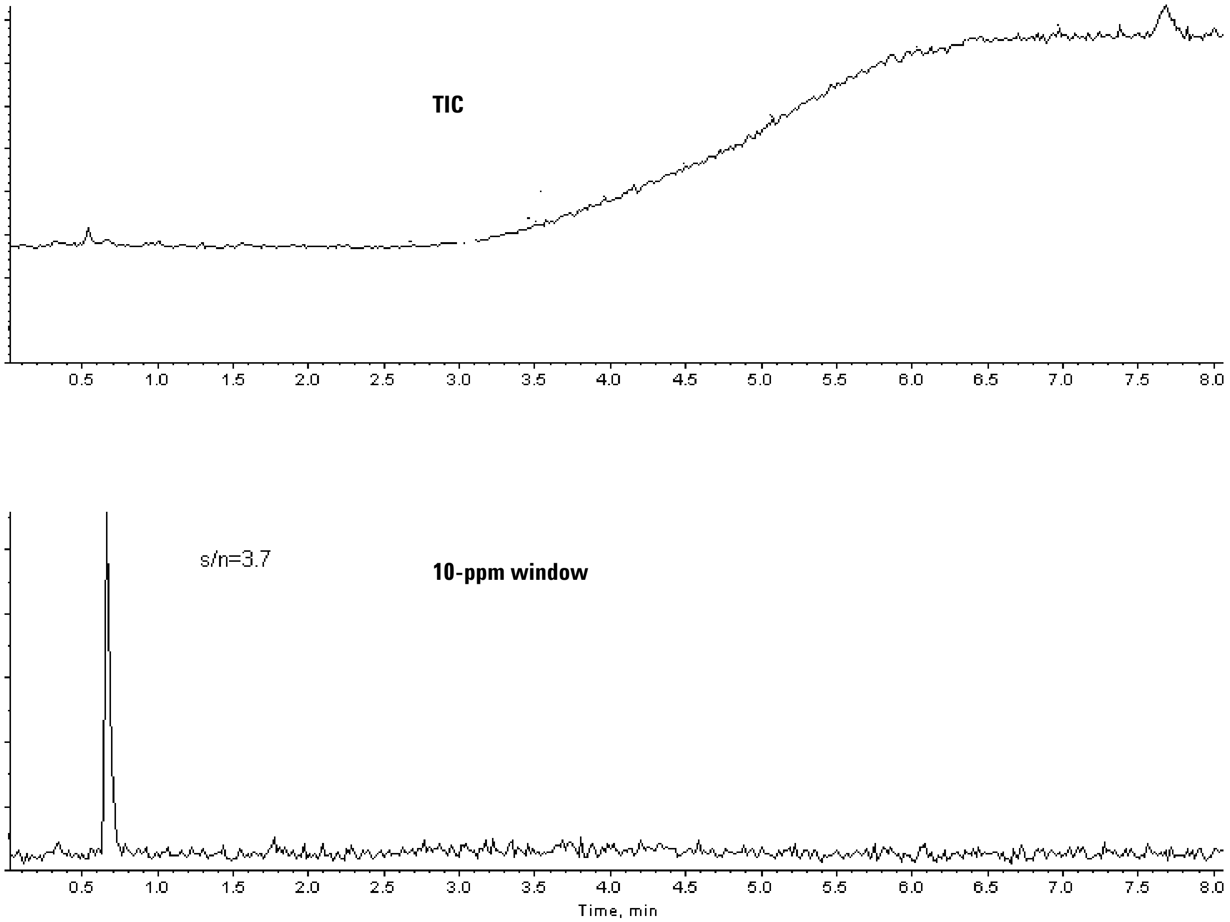


Figure 7. LOD of the Sudan Red I.

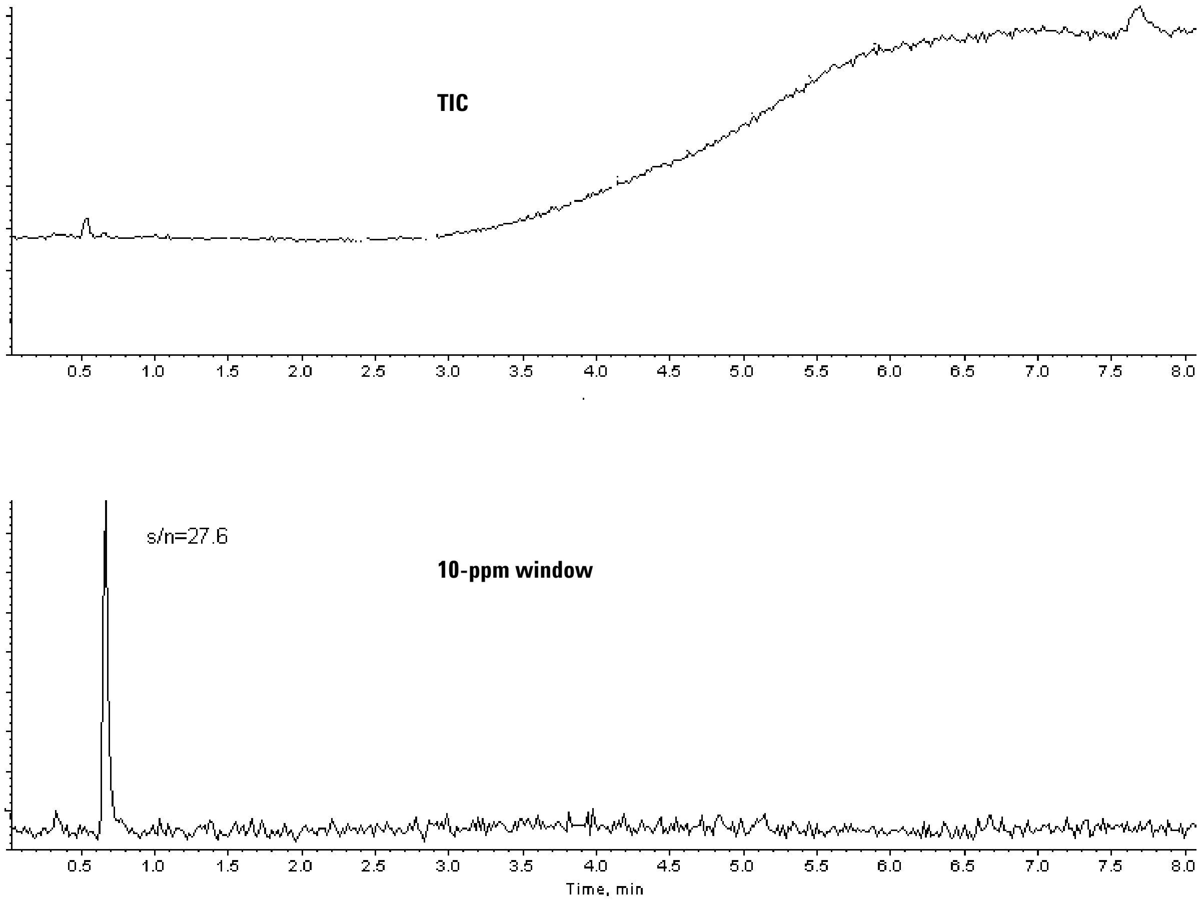


Figure 8. LOQ of the Sudan Red I.

Analysis of Sample

The instrument and software provides the user with the ability to create a database for all components they wish to automatically screen for. The minimum requirement for this database is the empirical formula and name for the component of interest, although the inclusion of a retention time will assist with confidence in the confirmation and reduce analysis time.

Sample Preparation

The sample analyzed in this work is Indian curry powder, but it should be noted that similar results were found in the analysis of Indian, Chinese, and South Korea chili powders. About 5 g of the Indian chili powder is weighed to within 10 mg in a ground Erlenmeyer flask with 100 mL of acetonitrile added using a graduated test tube. Sonicate for 1 hour and filter on filter paper in a ground Erlenmeyer flask. This method is identical to the one recommended by the EU.

Screening and Recovery Results

The prepared sample was spiked with 0.4 g/mL Sudan Red solution. The TIC for this sample is shown in Figure 9. However, also shown is the EIC of the sum of the four *m/z* values for each of the Sudan Red dyes.

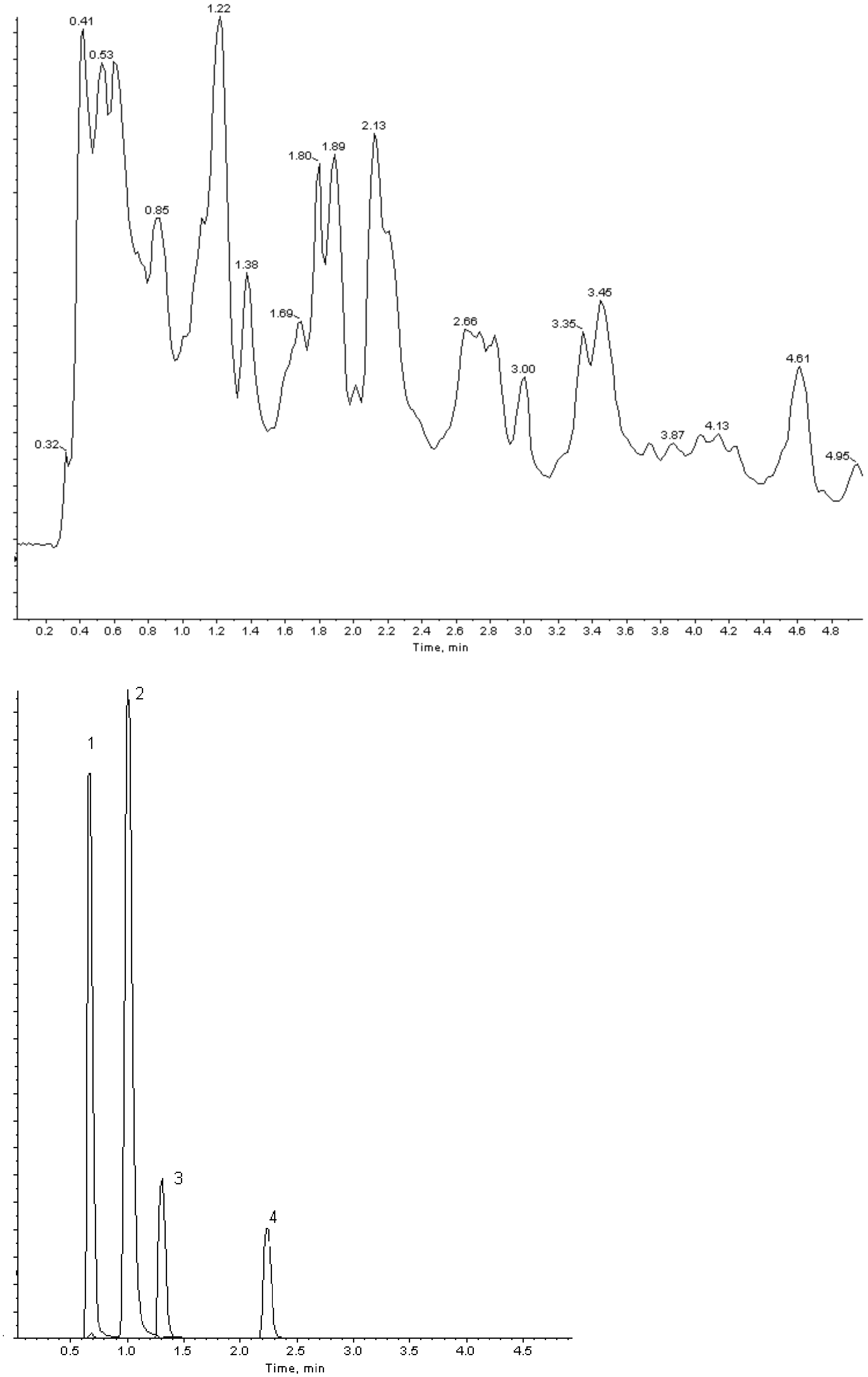


Figure 9. Real sample spectrum (upper); EIC of the 4-Sudan Red colorants (lower).

Table 3 illustrates the excellent quantitation accuracy of the spiked levels as compared to what is expected from the calibration curves. The levels of 0.4 and 0.2 ppm are below the 0.5-ppm cutoffs regulated by the EU but are well quantitated at the 0.4-ppm level with accuracies all close to 100%. At both levels the %RSDs of peak areas are still below 10%.

Table 3. Method Accuracy and Reproducibility for the Four Target Colorants Spiked in Indian Chili

Formula	Compound name	Mass	Peak RT (min)	Peak area	CAS number
$C_{16}H_{12}N_2O$	Sudan Red I	248.09496	0.65	6.6932E5	842-07-9
Species	**Abundance (cts)**	**Ion mass**	**Measured mass**	**Error (mDa)**	**Error (ppm)**
$[M+H]^+$	2.5354 e5	249.10224	249.10249	0.26	1.0
Formula	**Compound name**	**Mass**	**Peak RT (min)**	**Peak area**	**CAS number**
$C_{18}H_{16}N_2O$	Sudan Red II	276.12626	1.01	8.8136E5	3118-97-6
Species	**Abundance (cts)**	**Ion mass**	**Measured mass**	**Error (mDa)**	**Error (ppm)**
$[M+H]^+$	2.9543E5	277.13354	277.13372	0.18	0.7
Formula	**Compound name**	**Mass**	**Peak RT (min)**	**Peak area**	**CAS number**
$C_{22}H_{16}N_4O$	Sudan Red III	352.13176	1.30	3.2776E5	85-86-9
Species	**Abundance (cts)**	**Ion mass**	**Measured mass**	**Error (mDa)**	**Error (ppm)**
$[M+H]^+$	8.5544E4	353.13904	353.13931	0.27	0.7646
Formula	**Compound name**	**Mass**	**Peak RT (min)**	**Peak area**	**CAS number**
$C_{24}H_{20}N_4O$	Sudan Red IV	380.44821	2.24	2.7679E5	85-83-6
Species	**Abundance (cts)**	**Ion mass**	**Measured mass**	**Error (mDa)**	**Error (ppm)**
$[M+H]^+$	7.9608E4	381.17095	381.17098	0.03	0.0787

Reproducibility

	Sudan Red I		Sudan Red II		Sudan Red III		Sudan Red IV	
Standard (ppm)	**RSD (%)**	**Accuracy (%, avg)**	**RSD (%)**	**Accuracy (%, avg)**	**RSD (%)**	**Accuracy (%, avg)**	**RSD (%)**	**Accuracy (%, avg)**
0.2	6.04	97.31	5.76	97.61	7.11	82.3	8.87	87.4
0.4	6.98	101.95	5.64	100.27	8.04	103.12	8.26	104.32
0.8	4.61	104.75	6.12	103.78	8.37	96.23	8.43	102.72
1.6	5.17	102.53	5.99	105.92	7.56	92.79	7.73	95.78
2	6.12	96.77	4.74	94.72	7.31	101.27	6.20	100.79

Conclusions

An overwhelming advantage of using TOF MS for the trace-level detection of any component is the confirmation that is provided through accurate mass. The data shown here demonstrates the ability of LC/MSD TOF to confirm with accurate mass measurement, and quantitate with selective, narrow mass window. High-mass resolution and accuracy (in every spectrum) provides the selectivity that reduces chemical noise for quantitation and confirmation. Also, this method can easily include new components such as other banned or limited use dyes. Furthermore, this application shows the capability of the Agilent LC/MSD TOF as a single tool for both screening and confirmatory analysis, with quantitative information, often at levels below those currently analyzed for today. Using the TOF can save more than 50% the time required as compared to many other methods used today. It provides a strong tool for the large quantity samples for screening.

References

1. ASTA White paper on Sudan and Related Dyes, a publication of the American Spice Trade Association (http://www.astaspice.org/pubs/ASTAWhitePaper.cfm), copyright 2005
2. Solid-phase extraction procedure adapted from People's Republic of China approved national standards method of analysis for sudan dyes, 2005, Standardization Administration of China (http://www.sac.gov.cn/english/home.asp), Standard Number GB/T 19681-2995.

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