

Online sample cleanup on the Agilent 1290 Infinity LC using a built in 2-position/6-port valve

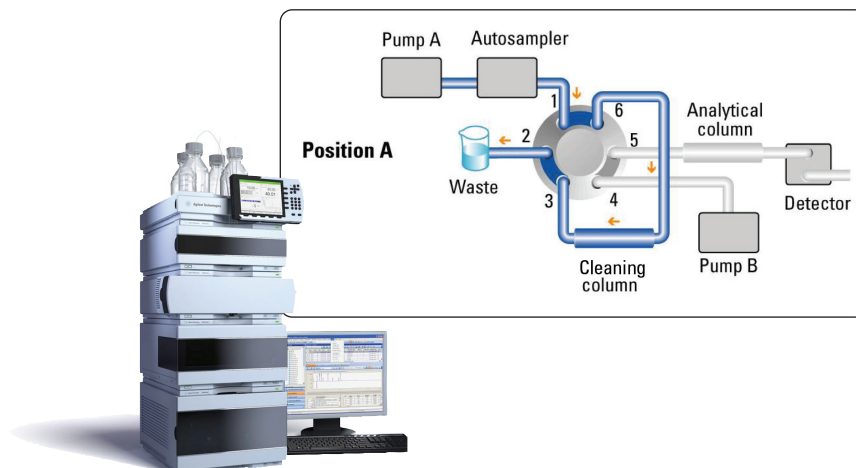
Analysis of Sudan red compounds in paprika powder

Application Note

Food

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Abstract

Sudan dyes are Azo-dyes and classified as Group 3 of the potential carcinogenic compounds based on findings of the IARC (International Agency for Research on Cancer). These compounds have been banned for food products in the EU, Japan, and the United States since 2004. In this Application Note it is shown how column switching using a 2-position/6-port valve can be used for online sample cleanup. The paprika extract was injected onto the cleaning column, and compounds eluting before and after the peaks of interest were discarded. Sudan red compounds in paprika powder were extracted and sensitively detected by selective ion monitoring (SIM) using an Agilent single quadrupole mass spectrometer. Online cleaning significantly increases sensitivity by almost 60% for Sudan 1 and 20% for Sudan 2. Sudan dyes can be detected down to approximately 100 µg/kg using the applied method.



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Introduction

Sudan dyes are Azo-dyes and classified as Group 3 of the potential carcinogenic compounds based on findings of the IARC (International Agency for Research on Cancer). These compounds have been banned for food products in the EU, Japan and the United States since 2004. If these compounds are detected in spices or products containing spices such as worcestershire sauce, the food products have to be recalled and destroyed¹.

In this Application Note we show the use of column switching with a 2-position/6-port valve for online sample cleanup. In addition, it is illustrated how Sudan red compounds in paprika powder can be sensitively detected by selective ion monitoring (SIM) using an Agilent 6140 single quadrupole LC/MS system.

Experimental

The instrument used was an Agilent 1290 Infinity LC system, equipped with the following modules:

- Two Agilent 1290 Infinity Binary Pumps with built-in degassing units
- Agilent 1290 Infinity Autosampler
An additional filter was installed between the injector valve and column preheating device to protect the cleaning column from clogging.
- Agilent 1290 Infinity Thermostatted Column Compartment
- Agilent 1290 Infinity DAD SL for 160-Hz operation
- 2-position/6-port valve for online sample cleaning

- Agilent 6140 single quadrupole LC/MS system
- Agilent ZORBAX RRHD Eclipse Plus C-18 columns, packed with 1.8- μm particles

Results and discussion

Sample preparation of Sudan dyes in spices such as chili or paprika powder is relatively simple and straightforward. The powder is extracted with acetonitrile in an ultrasonic bath. The liquid phase is then filtered and introduced onto an HPLC column. Nevertheless, the extract contains a substantial amount of coloring and non-coloring compounds (Figure 1).

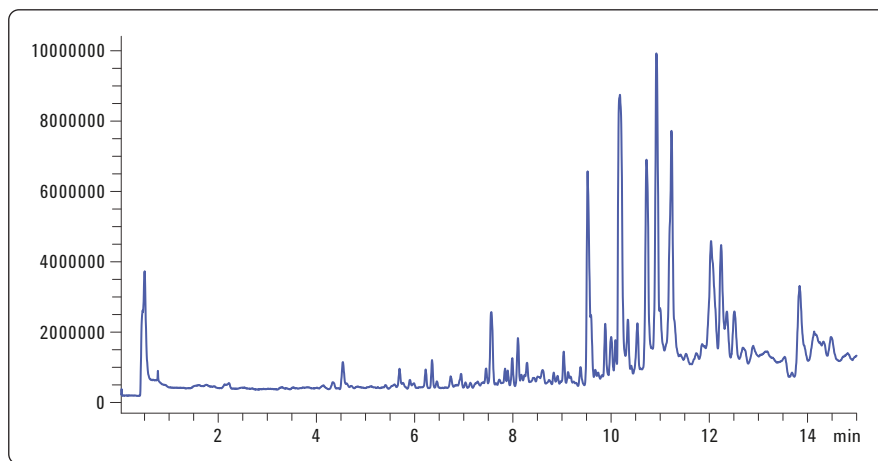


Figure 1
Paprika powder extract analyzed using MSD in scan mode.

Chromatographic conditions

Columns:

Analytical column: Agilent ZORBAX RRHD Eclipse Plus C-18, 2.1 mm \times 100 mm, 1.8 μm

Cleaning column: Agilent ZORBAX RRHT Eclipse Plus C-8, 2.1 mm \times 30 mm, 1.8 μm

Mobile phases: A = water, B = acetonitrile

For the analytical pump the water was modified with 400 μL TFA

Flow analytical pump: 0.5 mL/min; stop time 15 min; post time 3 min

Flow cleaning pump: 0.3 mL/min; stop time no limit

Gradient for analytical pump: At 0 min 5% B, at 5 min 95% B

Gradient cleaning pump: At 0 min 5% B

Column oven: 40 $^{\circ}\text{C}$ on both sides, valve switch = next run

Valve switch: At 0.2 min position B

DAD: 220/20 nm, Ref = off, 450/20, PW >0.0012 min, 20 Hz, slit width 4 nm

Injector: 1 μL injection volume, needle wash for 6 s

Online sample cleanup is performed by a precolumn, or cleaning column, which is accessed by valve-switching between the analytical and the cleaning columns. This process eliminates severe contamination of the analytical column from oils and other residues present in spices. The online cleaning procedure uses two columns, a 2-position/6-port valve, and a second pump to provide flow through both columns simultaneously. The workflow used for cleaning the paprika powder extract online is as follows (Figure 2):

1. The sample is introduced to the shorter cleaning column (valve position A). All compounds, eluting within 1 minute are wasted.
2. The valve is switched after one minute to operate the cleaning column and analytical column in series (valve position B). The analytical gradient starts and the peaks of interest elute.
3. The valve is switched again to the waste position and all remaining compounds are discarded (valve position A). The acetonitrile percentage for the cleaning column is increased to 95% to flush out all remaining compounds. Then the column is equilibrated to the start conditions.
4. Equilibration of the analytical column to the start conditions takes place after the analytical run.

Figure 3 illustrates a scheme of the two valve positions and two different flow directions.

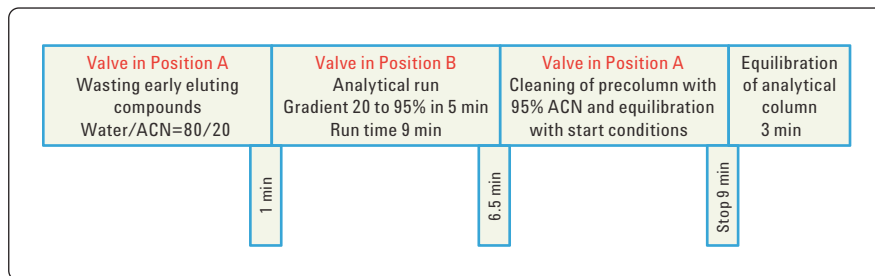


Figure 2
Workflow of online sample cleaning.

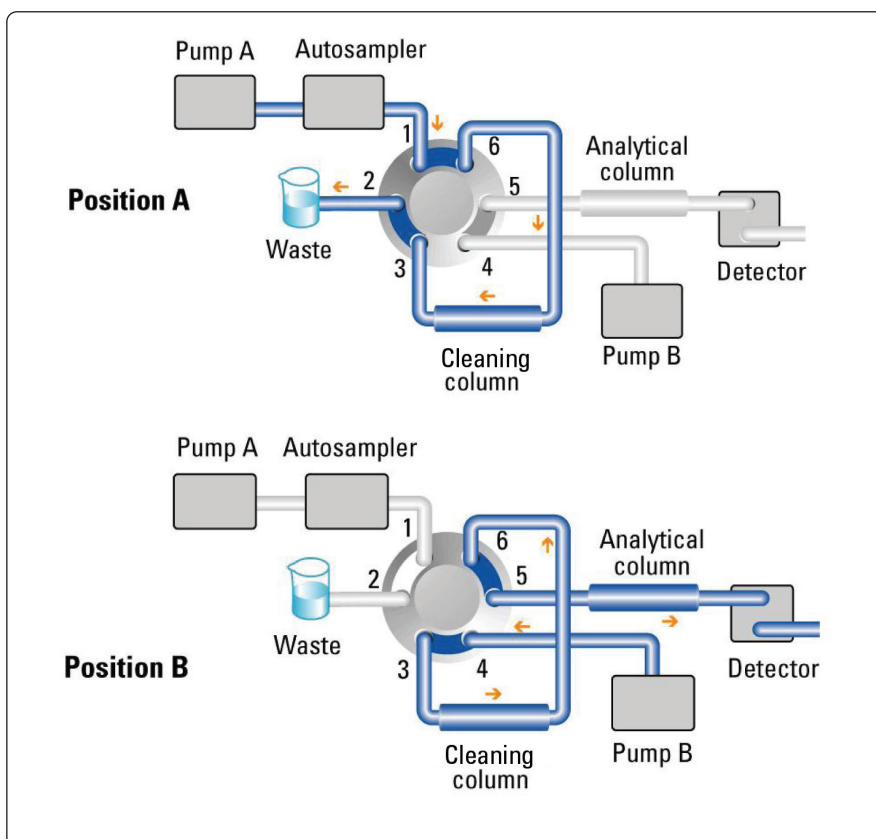


Figure 3
Scheme of the two valve positions.

Sample preparation of paprika powder

The following procedure was used for sample preparation:

1. A 2.5 g amount of paprika powder was extracted with 20 mL of acetonitrile and left for 45 minutes in the ultrasonic bath.
2. The liquid phase was filtered through a 0.45- μ m Econofilter from Agilent.
3. A 1 mL amount of filtered liquid was spiked with 50 ng Sudan 2, 30 ng Sudan orange G and 40 ng Sudan.
4. A 5 μ L amount of the prepared extract was injected.
5. A 5 μ L sample contained 50 pg/ μ L of Sudan 2. This procedure can detect approximately 100 μ g/kg in a Paprika powder sample.

Determination of Sudan red compounds

A standard was injected according to the above workflow. The resulting chromatograms are shown in Figure 4.

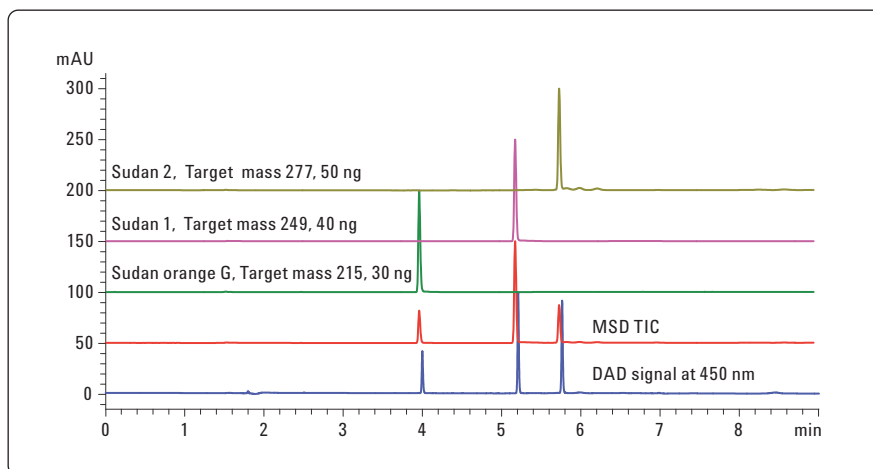


Figure 4
Overlay of DAD signal, MSD TIC and extracted ion chromatograms of a standard sample.

Chromatographic conditions

Columns:	
Analytical column:	Agilent ZORBAX RRHD Eclipse Plus C-18, 2.1 mm \times 100 mm, 1.8 μ m,
Cleaning column:	Agilent ZORBAX RRHT Eclipse Plus C-8, 2.1 mm \times 30 mm, 1.8 μ m
Mobile phases:	A= water, B= acetonitrile Analytical pump Water +400 μ L TFA
Flow analytical pump:	0.5 mL/min; stop time 9 min; post time 3 min
Flow cleaning pump:	0.5 mL/min; stop time no limit
Gradient for analytical pump:	At 0 min 20% B; at 1 min 20% B; at 5 min 95% B
Gradient cleaning pump:	At 0 min 20% B; at 6.5 min 20% B; at 6.6 min 95% B; at 8.1min 20% B
Column oven:	40 $^{\circ}$ C on both sides, valve switch = next run
Valve switching:	At 0 minutes position A; at 1 min position B; at 6.5 min position A
DAD:	220/20 nm, Ref = off, 450/20, PW >0.0012 min, 20 Hz, slit width 4 nm
Injector:	1 μ L injection volume, needle wash for 6 sec
MSD:	Peak width 0.03 min, Positive SIM parameters for Mass 215, 249 and 277, fragmentor = 100, Actual dwell 45 Gas temp = 350, drying gas = 12 L/min, Neb Pres = 35 psig, V_{cap} positive = 3000V

The Agilent 6140 single quadrupole MS was used in SIM mode to analyze the Sudan red compounds with highest sensitivity. The target masses were 215, 249 and 277.

The detection conditions were evaluated by injecting 5 μL of the standard sample. The resulting chromatograms are shown in Figure 5. The max pressure achieved was approximately 825 bar.

It can be seen that the extract contains a certain amount of compounds detected at the selected parameters. Sufficient resolution using a 100 mm length column can confidently detect the Sudan dyes. A 5 μL injection contained 50 $\text{pg}/\mu\text{L}$ of Sudan 2, 30 $\text{pg}/\mu\text{L}$ Sudan orange G and 40 $\text{pg}/\mu\text{L}$ Sudan 1. Sudan dyes in a paprika powder sample can be detected down to approximately

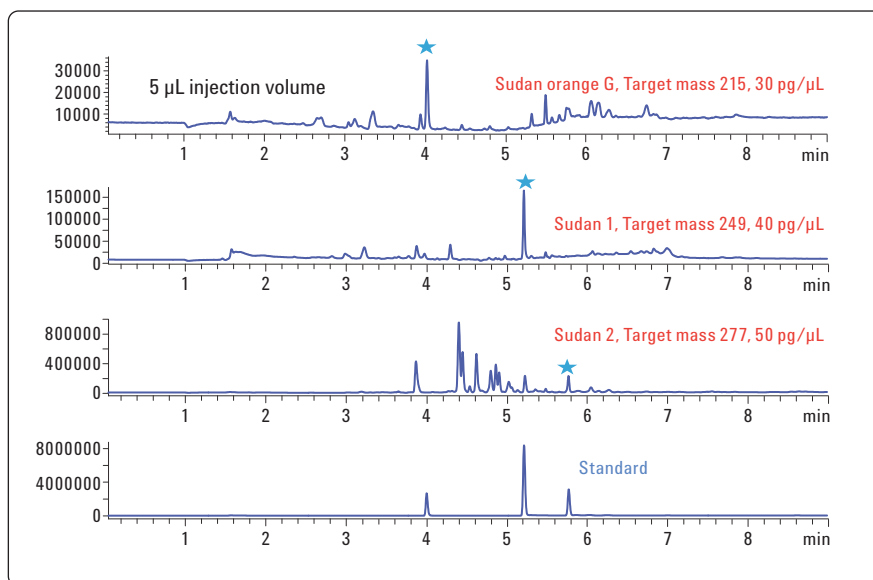


Figure 5
Overlay of standard and sample extract chromatograms using Agilent 6140 single quadrupole MS in SIM mode.

Chromatographic conditions

Columns:

Analytical column: Agilent ZORBAX RRHD Eclipse Plus C-18, 2.1 mm \times 100 mm, 1.8 μm ,

Cleaning column: Agilent ZORBAX RRHT Eclipse Plus C-8, 2.1 mm \times 30 mm, 1.8 μm

Mobile phases: A= water, B= acetonitrile

Analytical pump Water +400 μL TFA

Flow analytical pump: 0.5 mL/min; stop time 9 min; post time 3 min

Flow cleaning pump: 0.5 mL/min; stop time no limit

Gradient for analytical pump: At 0 min 20% B; at 1 min 20% B; at 5 min 95% B

Gradient cleaning pump: At 0 min 20% B; at 6.5 min 20% B; at 6.6 min 95% B; at 8.1 min 20% B

Column oven: 40 $^{\circ}\text{C}$ on both sides, valve switch = next run

Valve switching: At 0 minutes position A, at 1 min position B at 6.5 min position A

DAD: 220/20 nm, Ref = off, 450/20, PW >0.0012 min, 20 Hz, slit width 4 nm

Injector: 1 (standard) or 5 μL (spiked extract) injection volume, needle wash for 6 sec

MSD:

Peak width 0.03 min; Positive SIM parameters for Mass 215, 249 and 277, fragmentor = 100; Actual dwell 45; Gas temp = 350, drying gas = 12 L/min; Neb Pres = 35 psig; V_{cap} positive = 3000 V

100 µg/kg.

Influence on abundance with and without online cleaning

The Sudan standard and the spiked matrix was injected without online cleaning on a 100 mm × 2.1 mm Agilent ZORBAX RRHD Eclipse Plus C-18 column to measure the effect of cleaning on abundance results. The resulting standard chromatogram is shown (Figure 6). The injection volume for the Sudan standard was set to 3 µL and the spiked extract to 5 µL, to be comparable with the injection volume used with online cleaning.

The spiked matrix was injected under the same chromatographic conditions as the standards without online cleaning. The influence on the abundance of the peaks of interest is significant for Sudan 1 and 2 (Figures 7 and 8). For Sudan orange, the same abundance with and without cleaning was observed (Figure 9). At the retention time of Sudan orange, the matrix is relatively clean, whereas many co-eluting peaks occur for Sudan 1 and 3 (Figure 1).

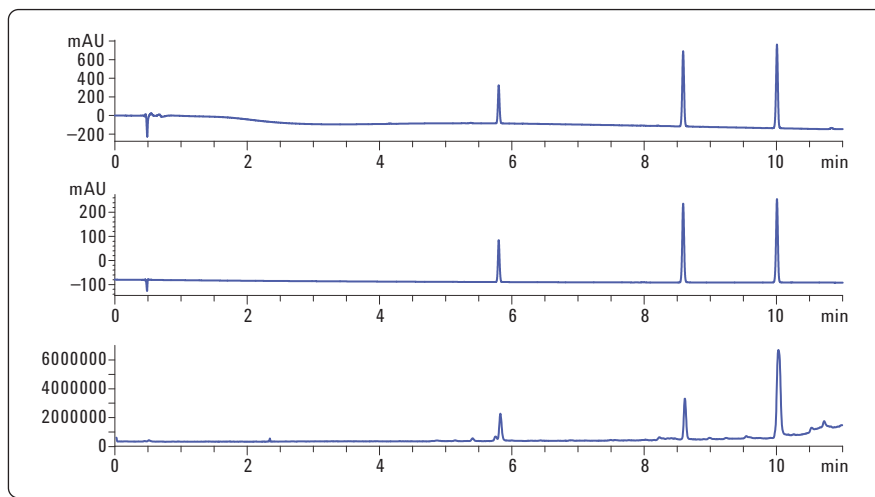


Figure 6
Analysis of Sudan standard without online cleaning.

Chromatographic conditions

Columns:	Agilent ZORBAX HD Eclipse Plus C-18, 2.1 mm × 100 mm, 1.8 µm,
Mobile phases:	A = Water +400 µL/l TFA, B = acetonitrile
Flow :	0.5 mL/min; stop time 15 min; post time 3 min
Gradient:	At 0 min 15% B; at 10 min 90% B
Column oven:	40 °C on both sides, valve switch = next run
DAD:	220/20 nm, Ref = off, 450/20, PW >0.0012 min, 20 Hz, slit width 4 nm
Injector:	3 (standard) or 5 µL (spiked extract) injection volume, needle wash for 6 sec
MSD:	Peak width 0.03 min; Positive SIM parameters for Mass 215, 249 and 277, fragmentor = 100; Actual dwell 45; Gas temp = 350, drying gas = 12 L/min; Neb Pres = 35 psig; V _{cap} positive = 3000 V

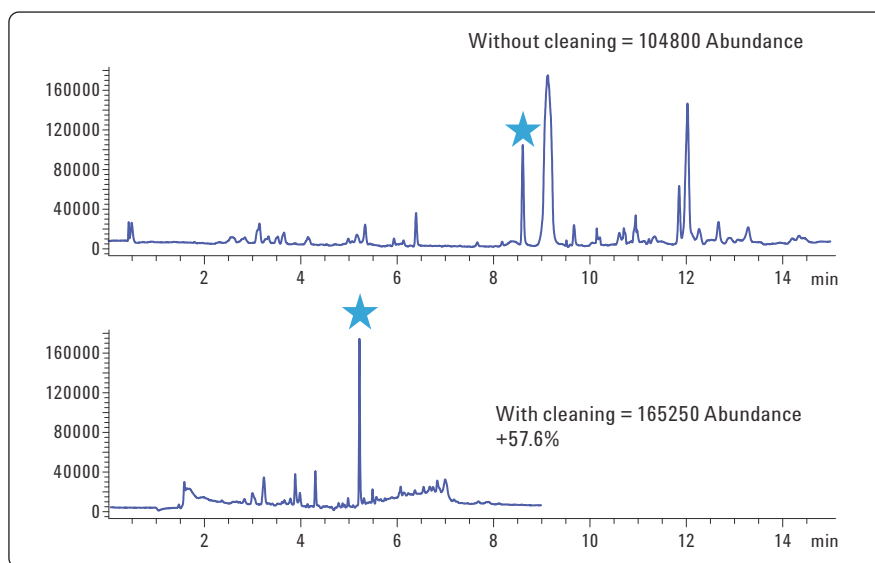


Figure 7
Influence of cleaning on abundance for Sudan 1.

Cleaning of the matrix before and after elution of the peaks of interest is advantageous if a more sensitive compound analysis is needed. Up to nearly 60% more abundance could be obtained for Sudan 1.

Conclusion

In this Application Note it is shown how column switching using a 2-position/6-port valve can be employed for online sample cleanup. The paprika extract was injected onto the cleaning column, and compounds eluting before and after the peaks of interest were discarded. Online cleaning can significantly increase sensitivity, close to 60% for Sudan 1 and 20% for Sudan 2. Sudan red compounds in paprika powder were extracted and were detected by SIM mode using an Agilent 6140 single quadrupole LC/MS system. Approximately 100 µg/kg Sudan dyes in paprika powder can be detected.

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

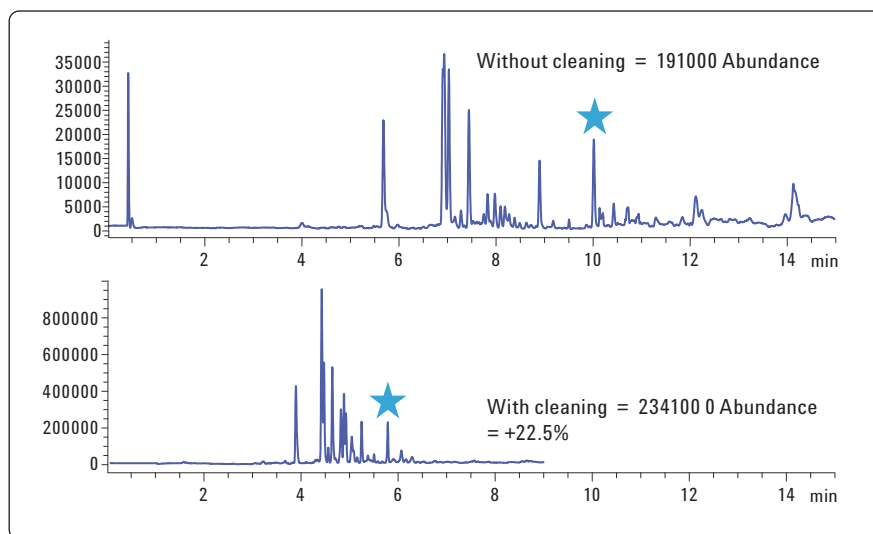


Figure 8
Influence of cleaning on abundance of Sudan 2.

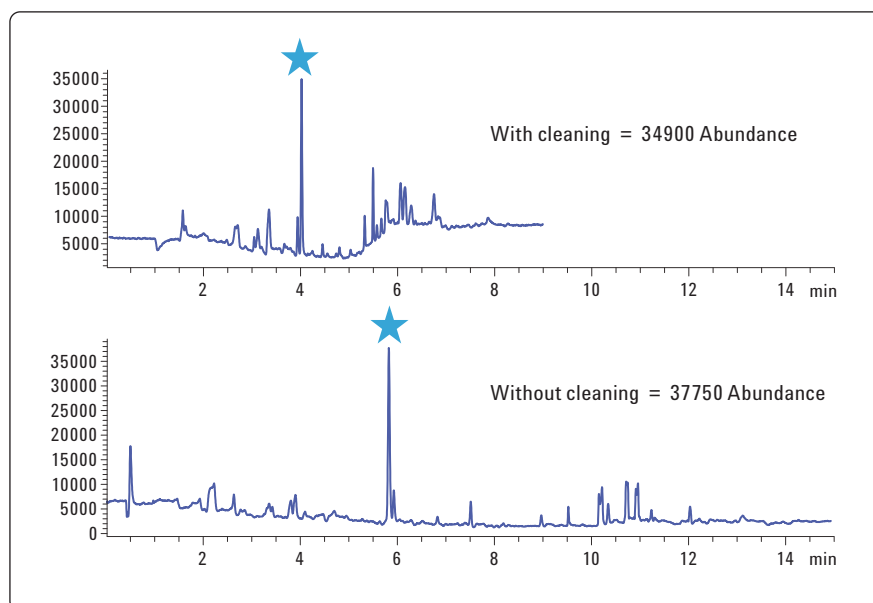


Figure 9
Influence of cleaning on abundance for Sudan orange.

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© Agilent Technologies, Inc., 2010
February 1, 2010
Publication Number 5990-5255EN



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