



Automated Solid Phase Extraction for Sudan 1 Additive Screening

Introduction

Sudan I is a red dye that is used for colouring solvents, oils, waxes, petrol, shoes and floor polishes and has never been permitted in food in the UK or the rest of the EU.

Since 2003, when it was found in chili powder in France, EC emergency controls have required that all dried and crushed or ground chili and chili products imported into EU have a certificate to show that they have been tested and are free from Sudan dyes. Alternatively they must be tested at the point of import.

As well as being found in chili powder it has been found, together with a related dye Sudan IV in Palm Oil. Unlike permitted artificial food colours the Sudan dyes are oil soluble. This renders the established methods for testing foods for colours inappropriate.

In February 2005 over 850 different product lines were withdrawn from sale following the discovery of Sudan I in a sauce which had been used as an ingredient in numerous food products.

This application focuses on the sample preparation and clean up to remove interferences by Solid Phase Extraction so traditional GCMS, HPLC or LCMS analysis can be performed. The laboratory that developed this assay is UKAS accredited and reached detection levels of 1ug/litre.

Typical Food Sample Clean-Up for Extraction of Additives

- A **10.0g** sample of food is weighed into a test tube and **50ml** of Hexane is added
- The sample is shaken, allowed to settle and approximately **6.0ml** is transferred to a vial which is put into a RapidTrace
- This Hexane extract is then put through a **3ml Silica packed SPE cartridge 200mg** (Argonaut Isolute) which retains the components of interest and lets the fatty interferences go to waste
- Elution is achieved using **Methanol** to give approximately **1.5 ml** of eluent ready for final evaporation to dryness followed by reconstitution and derivitisation with BSFTA 70ul for analysis by GCMS.
- HPLC Analysis can be done directly on the extract when evaporated to approximately 100ul.

Instrumentation Used for Sample Preparation



The RapidTrace for Automated SPE



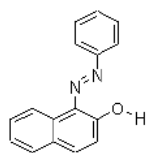
TurboVap LV for Automated Sample Evaporation

The RapidTrace Method

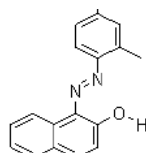
The solvent lines of the RapidTrace are first purged with the solvents (8 max). The organic, Chlorinated, aqueous and cannula waste solvent lines are separate. The rack has two rows of 10 test tubes. The first row for the samples has 13mm x 100mm (7mL) tubes and the second row for fraction collection has 12mm x 75mm, (4mL) tubes.

STEP	SOURCE	DESTINATION	VOLUME (ML)	FLOW (ML/MIN)
Condition	Hexane	Organic Waste	3	5
Load	Sample	Organic Waste	5.8	1
Rinse	Hexane	Organic Waste	6	2
Dry	Nitrogen	5 minute		
Collect	Methanol	Fraction 1	1.5	1.5
Purge Cannula	Methanol	Organic Waste	3	2

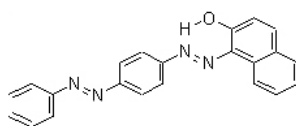
A Typical Range of components Screened Using the Sudan Method



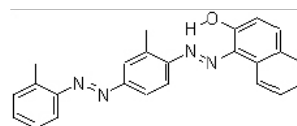
Sudan 1



Sudan II



Sudan III



Sudan IV

Common Reagent Table for All Methods

LINE NO.	REAGENT NAME	SIP SPEED (ML/MIN)
1	Methanol	30
2	Hexane	30
WASTE NAME	ABBREVIATION	
Organic Waste	OrgW	
Cannula Waste	Cannula	
Air Push = 2ml	Air Push Multiplier = 2	

Detection Limit Units — Conversion Table

STATED VALUES	CONCENTRATION UNITS	DETECTION LIMITS
1000 mg/L	1000 PPM	1 mg/mL
1 mg/L	1 PPM	1 ug/mL
1 ug/L	1 PPB	

Acknowledgements

Caliper Life Sciences would like to thank Richard Sykes, Martin Bowden and their team at the West Yorkshire Analytical Services Laboratory in Leeds, UK for their assistance with this application note.



Corporate Headquarters
68 Elm Street
Hopkinton, MA 01748-1668

Tel: 508 436 9500
Fax: 508 435 3439
Email: cust.support@caliperLS.com

www.caliperLS.com

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