

## **METHOD 145B**

### **COLLABORATIVE TRIAL 145 OF A METHOD FOR THE DETECTION AND DETERMINATION OF SUDAN I IN CHILLI PRODUCTS BY HPLC**

Note: This procedure will be validated in the FSA collaborative trial programme to assess methods of analysis of interest or of particular concern. It was developed in the West Yorkshire Analytical Services Laboratory as a relatively “simple” method for the determination of Sudan I in chilli powder and products containing chilli powder.

# THE DETECTION AND DETERMINATION OF SUDAN I IN CHILLI PRODUCTS BY HPLC

## 1. SCOPE AND FIELD OF APPLICATION

- 1.1 The method describes the determination of Sudan I in chilli powders and products containing chilli powder.
- 1.2 Sudan I is an oil-soluble, mono azo dyestuff which is carcinogenic and is not permitted for use in food.

## 2. DEFINITION

The Sudan I content means the content of Sudan I as extracted and determined by this method.

## 3. PRINCIPLE

Sudan I is extracted from the prepared sample with methanol. After filtering, any Sudan I is detected and determined by HPLC using rapid scanning or diode-array UV/vis detection.

## 4. REAGENTS

- 4.1 GPR, AR and HPLC grade reagents are suitable unless otherwise stated. Water should be deionised, distilled or of similar quality.
- 4.2 Sudan I: 97% purity is available from Aldrich
  - 4.2.1 Sudan I stock solution: Weigh 0.1000g Sudan I (4.2) and transfer to a 100mL volumetric flask with methanol (4.3). Dissolve and make to volume with methanol. Mix well. This solution has a concentration of 1000mg/L.
  - 4.2.2 Sudan I working solutions: Prepare as appropriate, the following working solutions by transferring the stated volumes of Sudan I stock solution (4.2.1) to a 100mL volumetric flask. Dilute to volume with methanol. Mix well.

<b>Volume of Stock solution (4.2.1)</b>	<b>concentration</b>
100 µl	1.0 mg/l
0.5 ml	5.0 mg/l
1.0 ml	10.0 mg/l
2.0 ml	20.0 mg/l
5.0 ml	50.0 mg/l
10.0 ml	100 mg/l

Once prepared the solutions should be protected from the light as far as is practicable e.g., by wrapping in aluminium type foil.

- 4.3 Potassium dihydrogen phosphate
- 4.4 Tetrabutylammonium bromide
- 4.5 Methanol: HPLC grade.
- 4.6 Mobile phase for HPLC: up as follows:

A 0.6804g potassium dihydrogen phosphate made up to 400mL with water.

B 2.0240g tetrabutylammonium bromide made up to 1600mL with HPLC methanol.

Mix solutions A and B and filter through a 0.45 micron filter.

## **5. APPARATUS**

- 5.1 Normal laboratory glassware and apparatus.
- 5.2 12 ml screw top vials for holding sample extracts
- 5.3 Filter papers: GF/A 70mm diameter.
- 5.4 0.2 micron x 13mm syringe filters – Chromacol 13-MF-02 (T) are suitable
- 5.5 2ml autosampler vials
- 5.6 HPLC with scanning UV/vis or diode array detector (see Appendix 1).
- 5.7 Laboratory homogeniser.
- 5.8 50ml measuring cylinders
- 5.9 Timing device.

## **6. PROCEDURE**

- 6.1 Preparation of test sample
  - 6.1.1 Dry chilli products (e.g. chilli powder or crushed chilli) need only to be mixed thoroughly. No attempt should be made to macerate chilli powder in high speed blenders due to the potential formation of irritating aerosols.
  - 6.1.2 Wet chilli-containing food products (e.g. relishes, chutneys) should be rendered as homogenous as possible in a suitable laboratory homogeniser (5.7)
  - 6.1.3 Keep the prepared sample in an airtight, opaque plastic container and store it in such a way that deterioration and change in composition are avoided.

- 6.1.4 Analyse the prepared sample as soon as possible after homogenisation. Immediately prior to analysis the prepared sample should be mixed to ensure homogeneity.
- 6.2 Weigh 5g to the nearest 0.01g prepared sample into a 50mL measuring cylinder (5.8)
- 6.3 Add 50.0mL methanol (4.5) to the test portion.
- 6.4 Shake for 30 seconds
- 6.5 Allow to stand for 30 minutes shaking occasionally.
- 6.6 Filter the supernatant through a filter paper (5.3) into a suitable vial (5.2).
- 6.7 The filtered samples are passed through disposable syringe filters (5.4) and collected in 2ml autosampler vials (5.5)
- 6.8 Protect all sample extracts from light as far as possible by storing in the dark or by using aluminium foil.
- 6.9 Set up the HPLC system (Appendix 1) and allow to stabilize for at least 1 hour.

## **Calibration**

Linearity has been previously established over the range of working standard solutions (4.2.2) and it is therefore not necessary to perform a full calibration on each occasion. A full linearity check, however, must be performed when new working standard solutions are prepared.

- 6.9 Sequentially inject an appropriate volume (20 $\mu$ L) of test portion extract (6.7) and the same volume of suitable working standard solutions (4.2.2). Inject sample extracts in duplicate and bracket with standard solutions. A small amount of carry-over has been noted when a high concentration solution is followed by a solution with low concentration. It is therefore recommended that the 20 mg/l standard be used routinely and sample extracts diluted if necessary. Some chilli powders may contain very high levels of Sudan I. Any samples or standard solutions injected immediately after such extracts may be affected by carry-over and may need to be re-injected.
- 6.10 Identify the compound of interest in the extract chromatogram by virtue of its retention time and by comparison of its wavelength scan with reference to the working standard solution of a similar concentration (see Appendix 2 for a typical chromatogram and wavelength scan).
- 6.11 Record the areas of any identified peaks at 480nm from the test portion extract chromatograms (6.9).
- 6.12 Record the areas of the peak of interest at 480nm from the working standard solution chromatograms (6.9).

## 7. CALCULATION

$$\text{Concentration (mg/kg)} = \frac{A}{B} \times Y \times \frac{50}{W} \times D$$

Where

A = mean area of any identified peak in the test portion extract.

B = mean area of peak of interest in the working standard solution.

Y = concentration (mg/l) of the suitable working standard solution

W = weight of sample

D = the dilution factor (if any) (6.9)

## 8. EXPRESSION OF RESULTS

Record the result to the nearest 1 mg/kg.

## 9. ANALYTICAL QUALITY ASSURANCE

### 9.1 Performance Characteristics (Typical)

9.1.1 Limit of detection: to be assessed in the light of the collaborative trial.

#### 9.1.2 Bias

WRm: 102.6 (tentative)

WRs: to be determined

#### 9.1.3 Precision

Wp: to be determined

Wp(relative): to be determined

## APPENDIX I: EXAMPLE OF CHROMATOGRAPHIC AND EXPERIMENTAL CONDITIONS

Instrument: Isocratic HPLC system including rapid scanning or diode array UV/VIS detector, injector and electronic data handling system.

Column: Waters spherisorb 5 $\mu$ m ODS 4.6 X 150 mm with suitable guard column

Injection

Volume: 20 $\mu$ L

Mobile

Phase: A 0.6804g potassium dihydrogen phosphate made up to 400mL with water.  
B 2.0240g tetrabutylammonium bromide made up to 1600mL with HPLC methanol.

Mix solutions A and B and filter through a 0.45 micron filter.

Mobile phase

flow rate: 1mL min<sup>-1</sup>

Detector: Scan 400-600 nm and monitor at 480 nm

Data

Collection: Suitable integrator or PC based data collection system.

Other

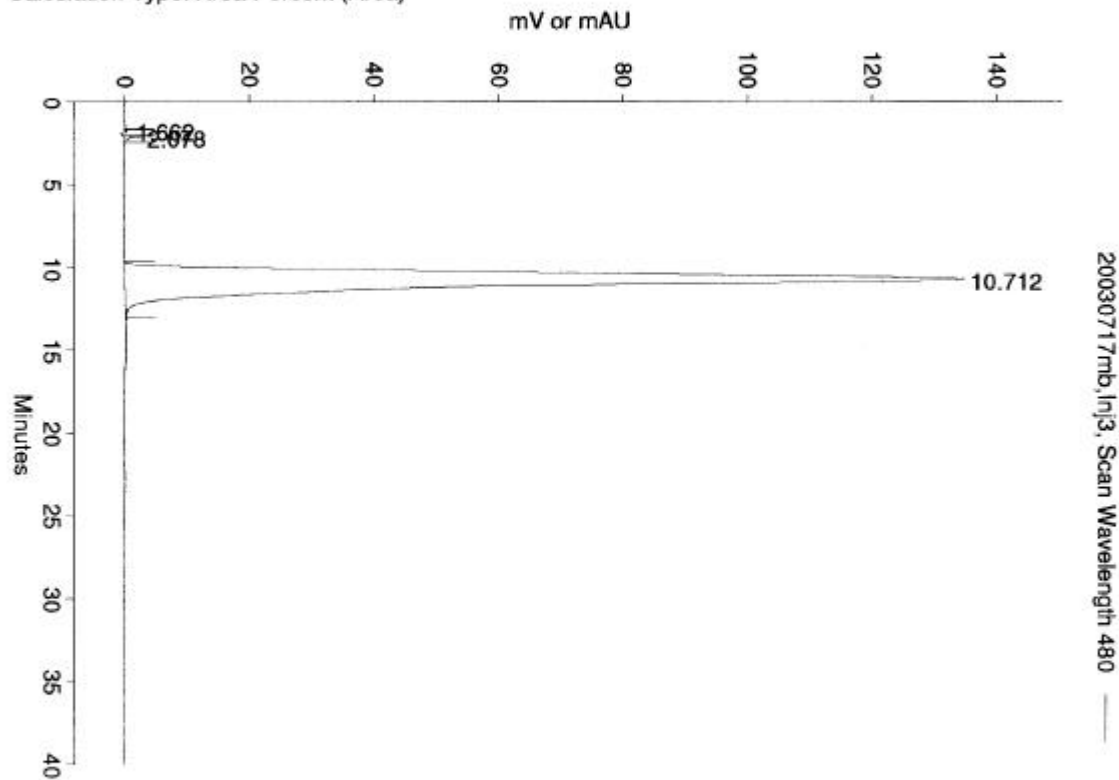
Details: After use the system must be flushed by pumping degassed methanol for at least 20 minutes.

Mode: Acquired Data  
 Original Results: C:\TSP\SYSTEM1\Data\20030717mb.RMS

Reported On: 05-09-03 09:33:39

Analysis Report

Signal 2: Scan Wavelength 480  
 Calculation Type: Area Percent (Area)

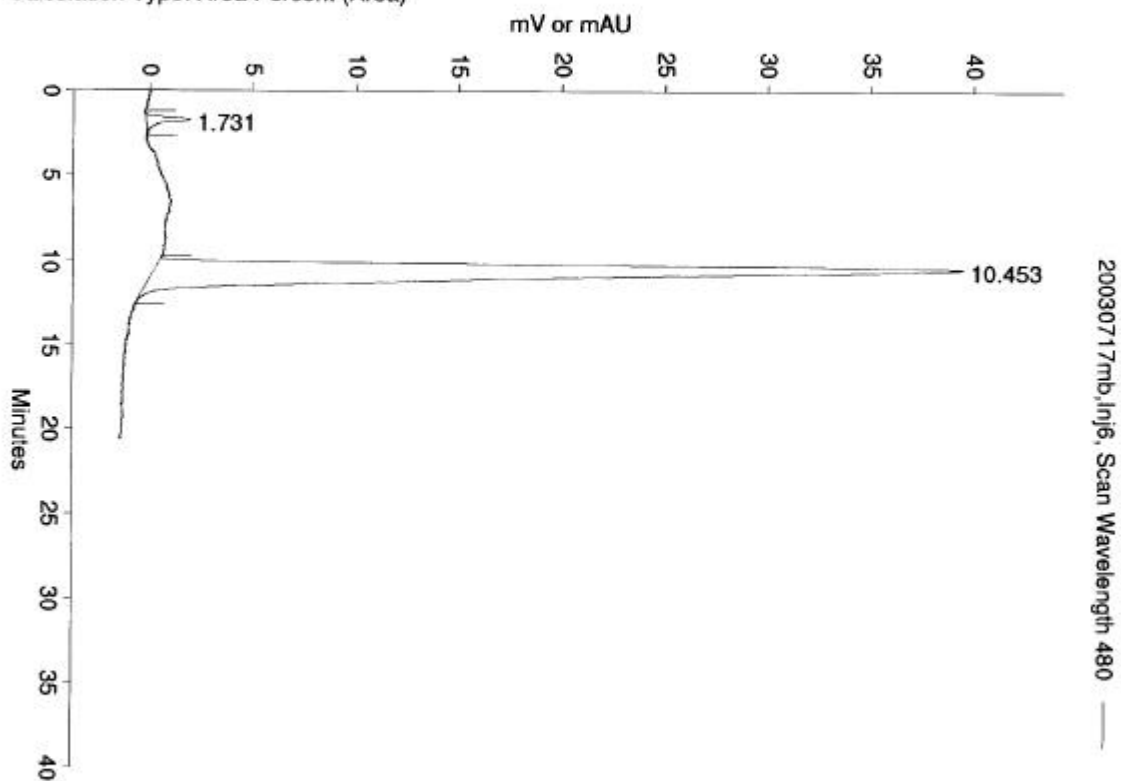


Component	RT(min)	Area	Height	Area%	Peak Type
Unident0001	1.662	1849	785	0.02	Modified
Unident0002	2.078	15387	2595	0.20	Modified
Unident0003	10.712	7505665	134789	99.77	Resolved
Totals		7522901	138169	100.00	

Sudan I standard 100 mg/l

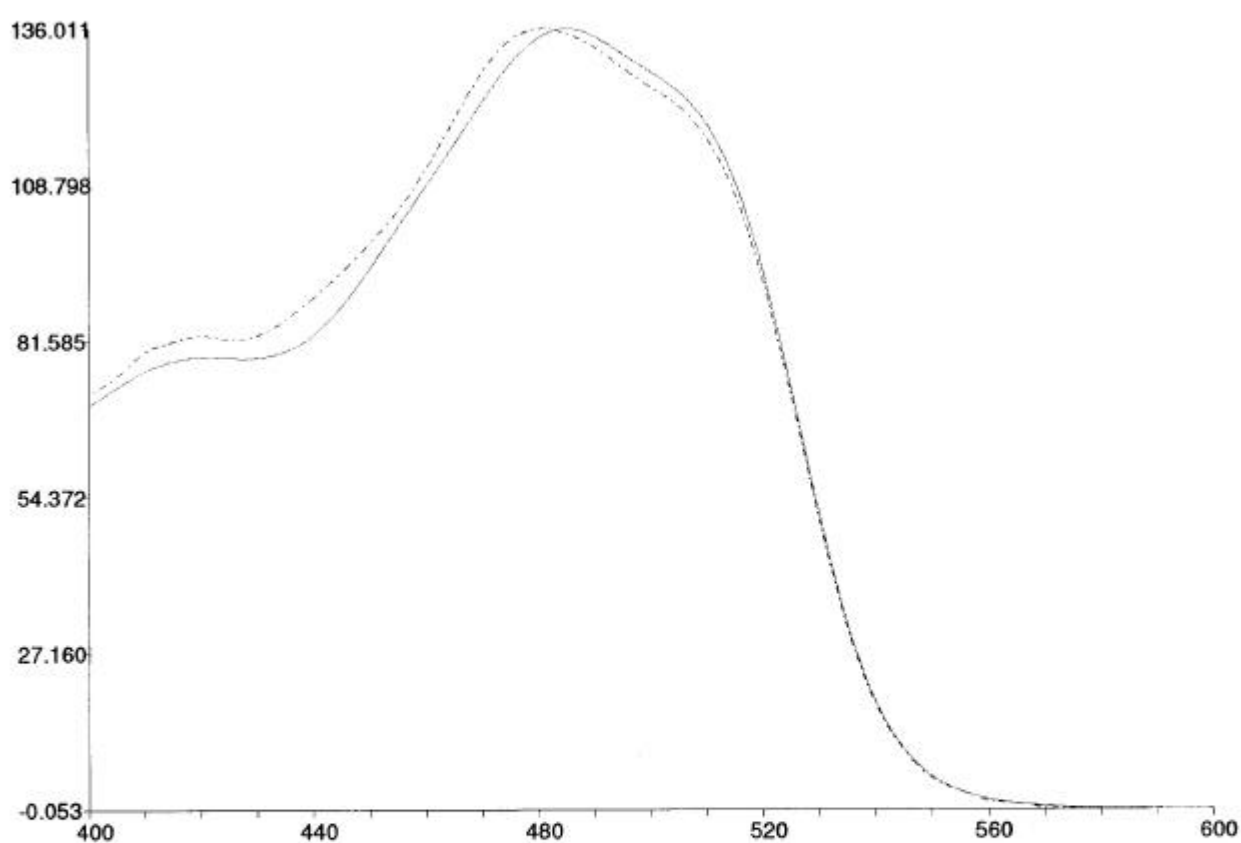
### Analysis Report

Signal 2: Scan Wavelength 480  
Calculation Type: Area Percent (Area)



Component	RT(min)	Area	Height	Area%	Peak Type
Unident0001	1.731	45952	2144	2.23	Modified
Unident0002	10.453	2012105	39226	97.77	Modified
Totals		2058057	41370	100.00	

Extract of chilli powder containing 1320 mg/kg Sudan I



Spectra Clipboard

2: C:\TSP\SYSTEM1\Data\20030717mb\20030717mb,Inj3.AQR (BR)

— Scan at 10.688 min

1: C:\TSP\SYSTEM1\Data\20030717mb\20030717mb,Inj6.AQR (BR)

- - - Scan at 10.500 min

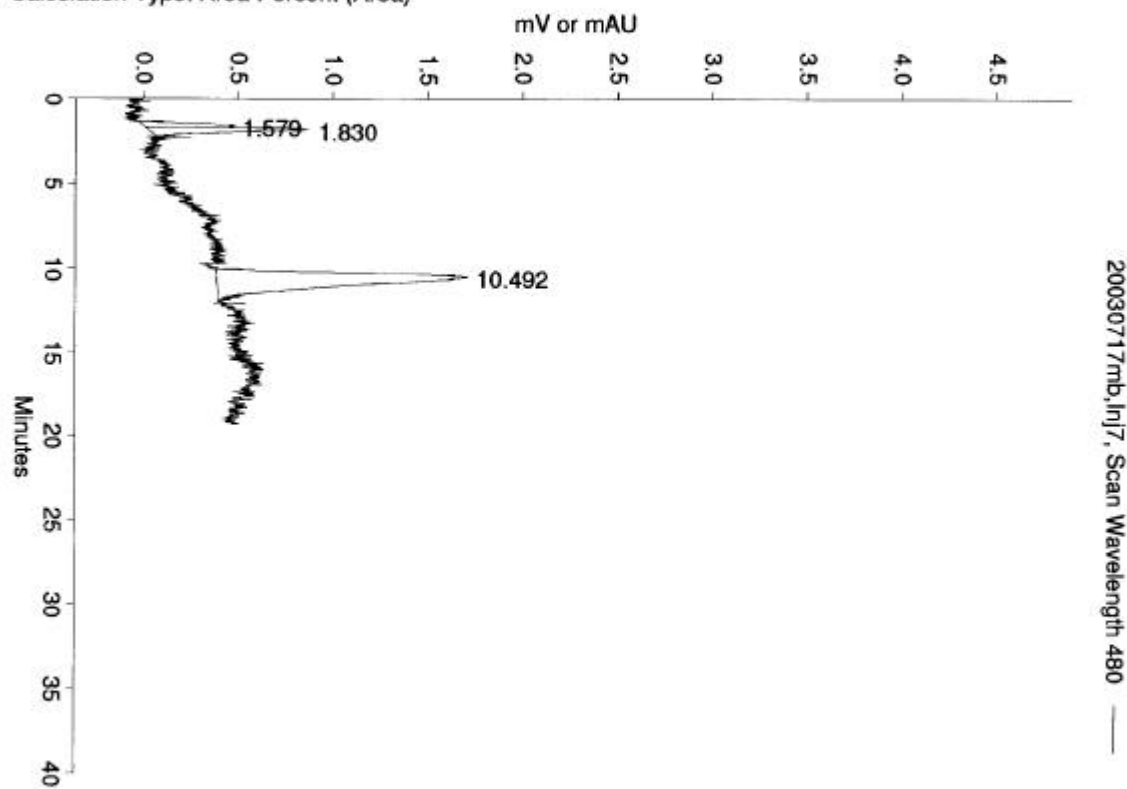
Comparison of wavelength scans

Mode: Acquired Data  
 Original Results: C:\TSP\SYSTEM1\Data\20030717mb.RMS

Reported On: 05-09-03 09:44:34

Analysis Report

Signal 2: Scan Wavelength 480  
 Calculation Type: Area Percent (Area)



Component	RT(min)	Area	Height	Area%	Peak Type
Unident0001	1.579	6159	486	7.12	Fused
Unident0002	1.830	13524	859	15.63	Fused
Unident0003	10.492	66838	1332	77.25	Resolved
Totals		86521	2677	100.00	

Extract of chutney product containing 8 mg/kg Sudan I