

## **Method DAS-AM-01-058**

### **Sulfotepp impurities in Chlorpyrifos EC formulations**

#### A. ABSTRACT

Method DAS-AM-01-058 has been validated for the analysis of the relevant impurity sulfotepp in chlorpyrifos EC formulations. Ranges of Chlorpyrifos EC products containing 8 different surfactants were evaluated.

Linearity, system and method precision, accuracy, and interferences were assessed and found to be acceptable.

Sulfotepp is determined using gas chromatography with an HP-1 megabore column and FID detection. Quantification is by peak area measurements using an external standard calculation.

The linearity of the method has been determined in the range 6 to 202  $\mu\text{g/mL}$  and is demonstrated by an  $R^2$  of 0.998 for sulfotepp.

Method precision was determined by replicate analyses of one batch of chlorpyrifos EC. The precision at the 95 % confidence level was calculated as  $\pm 2.0 \%$ .

System precision was determined by replicate injection of a precision sample solution. The precision at the 95 % confidence level was calculated as  $\pm 2.9 \%$ .

Recoveries were assessed over the range 0.05 to 0.39 %w/w sulfotepp in the formulation. All recoveries for sulfotepp were within the limits 70-120% with an average recovery of 102.5%.

The method is applicable to the determination of sulfotepp at concentrations of approximately 0.05 to 0.39 % by weight in various chlorpyrifos formulations containing a range of surfactants.

## B. SUMMARY AND INTRODUCTION

### (1) Scope

A method has been validated for the determination of sulfotepp in chlorpyrifos EC formulations. Linearity, system precision, method precision, interferences, and accuracy were assessed. The procedure followed was based on Dow AgroSciences (NZ) Ltd, SOP/FORMS/04, "Validation of Methods of Analysis for Formulations".

### (2) Principles of the analytical procedure

Sulfotepp concentrations are determined using gas chromatography, with splitless injection, nitrogen as the carrier gas and flame ionisation detection. Quantitation was by peak area measurement using external standard calculations.

## C. MATERIALS AND METHODS

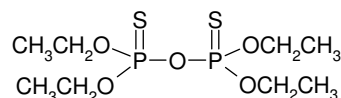
### (1) Equipment

- i) Balance: capable of weighing to 0.1 mg, Mettler AT261 or equivalent
- ii) Agilent 6890 gas chromatography system consisting of a split/splitless injector, flame ionisation detector (FID), autosampler, and HP Chemstation (Rev. A.06.03 [509]) chromatographic software. Supplied by Agilent Technologies, Wellington, New Zealand, or equivalent.
- iii) Column: 30m x 0.53mm i.d capillary column coated with 2.65 µm film thickness of HP-1 (cross-linked dimethylpolysiloxane)
- iv) Dispensors: capable of dispensing a volume of 24.4 mL, Brand Dispensette 5-50 mL, or equivalent
- v) Normal laboratory volumetric glassware
- vi) Disposable syringes: 3 ml volume, Luer lock, available from Biolab, Auckland, New Zealand, or equivalent
- vii) Syringe filters: 25 mm, 0.45 µm pore size, GF/0.45 µm GHP Membrane, available from Life Technologies, Auckland, New Zealand

### (2) Reagents and Standards:

#### (i) Reference Items:

Reference Item:	Sulfotepp analytical standard
Chemical Name:	O,O,O',O'-tetraethylthiodiphosphate
Molecular Structure:	



Molecular Weight:	322 g/mol
Reference Number:	TSN 101227

Assay: 98.6 %  
Recertification Date: 16<sup>th</sup> August 2005  
Storage Conditions: Freezer

- (ii) Solvents  
Isooctane, Mallinckrodt HPLC grade, or equivalent.
- (3) Analytical Procedure:
- a) Preparation of calibration solutions:
- i) Prepare a sulfotepp stock solution by weighing (and recording to the nearest 0.1 mg) approximately 35 mg of sulfotepp analytical standard into a 10 mL volumetric flask. Dissolve in and dilute to volume with isooctane at 20°C. Sonication may be used to aid in dissolution.
  - ii) Prepare the calibration solution by transferring 60 µL of the stock solution into a 10 mL volumetric flask containing approximately 0.28 grams of chlorpyrifos Analytical Standard. Dissolve in and dilute to volume with isooctane at 20°C and analyse using the conditions given in Section 4.
- b) Preparation of sample solutions:
- i) Weigh and record (to the nearest 0.0001g) approximately 0.63 g of chlorpyrifos EC formulation into an appropriately sized sample glass vial.
  - ii) Add 10 mL of distilled water and 24.4 mL of isooctane to the sample using dispensers and shake vigorously for 20 minutes.
  - iii) Centrifuge at 2000 rpm for 5 minutes and filter an aliquot of the top organic layer of the prepared solution through a 0.45 µm syringe filter: 25 mm diameter with GF/0.45 µm GHP membrane directly into an autosampler vial.
  - iv) Analyze single aliquots of each solution using the conditions given in Section 4.
- (4) Instrumentation
- (i) Description
- An Agilent 6890 gas chromatography system consisting of a split/splitless injector, flame ionisation detector (FID), autosampler, and HP Chemstation (Rev.A.06.03 [509]) chromatographic software was used for chromatographic analyses during this method validation.
- (ii) Chromatographic Conditions
- The following chromatographic conditions were used for the method validations:

Instrument	HP 6890 Gas Chromatograph
Column:	HP-1, 30 m x 0.53 mm x 2.65µm film
Carrier gas	Nitrogen
Detector	Flame Ionisation Detector at 275 °C
Detector Gases - Air	300 mL/minute
- Hydrogen	30 mL/minute
- Nitrogen	25 mL/minute
Carrier flow	8 mL/min
Injection temperature	220°C
Oven temperature program	70 °C for 0.3 minutes, ramp 10 °C / minute to 110 °C, hold 0.1 minutes, ramp 12 °C / minute to 180 °C , hold 3 minutes, ramp 12 °C / minute to 260 °C, hold 5 minutes
Injection volume	1 µL
Equilibration time	3 minutes
Total run time	25 minutes
Typical Retention Time	
Sulfotepp	15.8 minutes
Quantitation	peak area

(iii) Analysis Procedures

Inject a single aliquot of the calibration solution and calculate the response factor using the equations given in section 8.

Inject a maximum of four sample solutions and calculate the weight % sulfotepp in the samples using the equations given in section 8.

(5) Interferences:

(i) Solvents

Isooctane and isooctane after water extraction were filtered through the 0.45 µm filter and showed no interference at the retention time of interest.

(ii) Labware

All glassware was washed using standard dishwashing liquid, rinsed with acetone and then dried. Glassware washed using this procedure was screened for interference as part of the isooctane check as described in (i) above.

(iii) Inerts Components

If the aqueous partition stage of sample preparation is not used then some surfactant inerts may show interfering peaks at or close to the retention time of sulfotepp on chromatographic system. The water extraction stage removed any surfactant interfering peaks and still yielded acceptable recoveries.

This method has been validated for Dow AgroSciences Chlorpyrifos EC formulations containing proprietary surfactants and is therefore only suitable for

these formulations without further validation of specificity. Contact Dow AgroSciences to confirm suitability.

(6) Confirmatory techniques:

Retention time comparisons between sulfotepp in standard and sample solutions was used as analyte confirmation for the method validation.

(7) Time Required for analysis:

A run time of 25 minutes is sufficient for the analysis of the compound of interest.

(8) Methods of Calculation

(i) Calculation of concentration (mg /mL) of sulfotepp in the calibration solution:

$$C(i, cal) = \frac{[W_i \times (P/100)]}{10\text{mL}} \times \frac{0.06\text{ mL}}{10\text{ mL}}$$

where

$C_{(i,cal)}$  = the concentration (mg/ml) of sulfotepp in the calibration solution

$W_i$  = weight (mg) of sulfotepp in the stock solution

$P$  = Purity of the impurity standard, expressed as a percentage

10 mL = the volume of stock solution prepared

0.06 mL = the volume of stock solution added to the calibration solution

10 mL = the volume of calibration solution prepared

(ii) Calculation of external standard response factor (RF) for sulfotepp:

$$RF(i) = \frac{C(i, cal)}{Area(i, cal)}$$

where

$RF_{(i)}$  = response factor of sulfotepp

$C_{(i,cal)}$  = the concentration (mg/ml) of sulfotepp in the calibration solution

$Area_{(i,cal)}$  = peak area obtained for sulfotepp in the calibration solution

(iii) Calculation of weight % concentration of sulfotepp in the samples:

$$\% w/w = \frac{(Area(sample) \times RF(i))}{(W(sample)/25\text{mL})} \times 100\%$$

where

$\% w/w$  = the weight percent of sulfotepp in the sample

$RF_{(i)}$  = response factor of the sulfotepp in calibration solution

$Area_{(sample)}$  = peak area obtained for sulfotepp in the sample solution

$W_{(sample)}$  = sample weight in mg

25 mL = the volume of sample solution prepared

## D. RESULTS AND DISCUSSION

Results reported here are calculated by spreadsheet and may vary slightly from those calculated by hand.

### (1) Accuracy

Accuracy was assessed by analysis of eleven recovery samples over the formulation concentration range 0.05 % to 0.39 %. All recoveries were within the limits of 70-120 %. The combined average recovery was 102.5 % for the eleven preparations. Recovery data is listed in Table III.

Three recovery samples were prepared using an inert mix of EC (1) and chlorpyrifos analytical standard, TSN 100227, fortified with a solution of sulfotepp in isooctane at levels ranging from 0.05 % to 0.16 %. Five recovery samples were prepared using an inert mix of 480EC(1) and chlorpyrifos technical fortified with a solution of sulfotepp in isooctane at levels ranging from 0.21 % to 0.39 %. Three recovery samples were prepared using an inert mix of 480EC (2) (containing Surfactants known to give interference peaks) and chlorpyrifos analytical standard fortified with a solution of sulfotepp in isooctane at levels ranging from 0.05 % to 0.15 %. The samples were prepared for analysis according to the sample preparation details in section C above.

### (2) Precision

System precision was measured by injection of the same sample ten times. The measured system precision was calculated using the Students T-Test at the 95% confidence level as  $\pm 2.9$  %. System precision data is listed in Table I.

Method precision was measured by preparation and injection of ten samples on one day, and five samples on the following day using the conditions described in section C above. The measured method precision was calculated using the Students T test at the 95% confidence level as  $\pm 2.0$  %. Method precision data is listed in Table II.

The Students T-Test is listed below for analysis of 15 samples:

Precision = 2.15 x Relative Standard Deviation

### (3) Linearity

The detector response was found to be linear for sulfotepp in the following concentration ranges:

	Linear Range
Sulfotepp	6 $\mu\text{g/mL}$ to 202 $\mu\text{g/mL}$

Linearity samples were prepared by dilution of a stock calibration solution prepared in isooctane to contain approximately 200  $\mu\text{g/mL}$  sulfotepp. Linearity data for sulfotepp is presented in Figure 1.

(4) Limits of Detection and Quantification

The validated limit of quantification for this method was 0.05 % for sulfotepp in the formulation. This limit is determined from the linearity range analysed, in conjunction with accuracy and precision results discussed in this section.

(5) Limitations

Only the range of Dow AgroSciences inerts has been screened for interferenceS using this method. Caution needs to be taken when analysing products where the inerts are unknown and may produce interferant peaks. In these cases, and where the sulfotepp level is determined to have exceeded the specification maximum, it is recommended that a confirmatory technique is used, e.g. GC-MSD.

Where surfactant inerts are known, and can be checked for intereference peaks, then the need for a water extraction clean-up stage can be eliminated.

## E. CONCLUSIONS

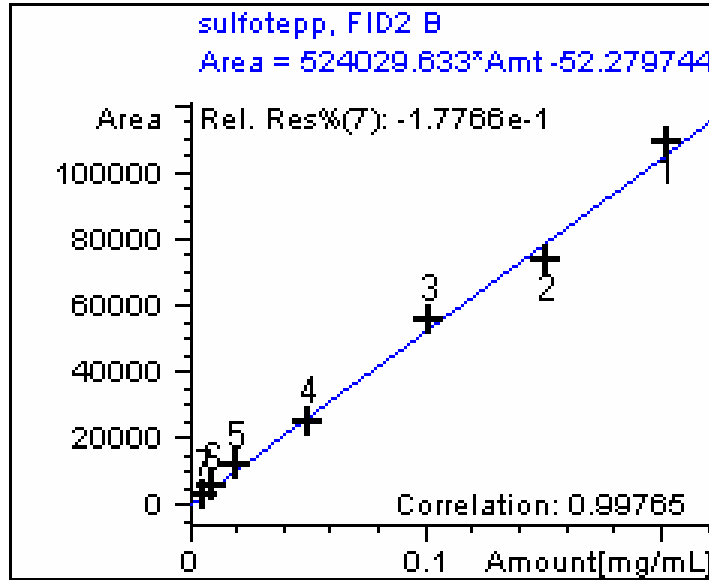
A gas chromatographic method using peak area quantitation and external standard calculation was validated for the analysis of sulfotepp in chlorpyrifos EC formulations containing proprietary inert components.. Linearity, system and method precision, accuracy, and interferences were assessed and found to be acceptable.

The statistical methods used were means, standard deviations, relative standard deviations, and t-test.

F TABLES AND FIGURES

(1) Linearity

Figure 1: Detector Linearity of Sulfotepp by FID





(2) Precision

**Table I – System Precision Data**

<b>Injection Number</b>	<b>Analysis Result (% w/w)</b>
Inj 1	0.113
Inj 2	0.111
Inj 3	0.114
Inj 4	0.113
Inj 5	0.112
Inj 6	0.111
Inj 7	0.115
Inj 8	0.114
Inj 9	0.114
Inj 10	0.115
Average	0.113
Std Dev	0.0015
RSD (%) <sup>1</sup>	1.3
Precision <sup>2</sup>	± 2.9

1. Relative standard deviation (RSD) calculated by dividing the standard deviation by the average and multiplying by 100.
2. Precision calculated by multiplying the RSD by 2.26 (2.26 the students T-test for analysis of 10 samples at the 95% confidence level)

**Table II. Method Precision Data**

<b>Sample Number</b>	<b>Analysis Result (% w/w)</b>
Day 1	
Sample 1	0.113
Sample 2	0.113
Sample 3	0.113
Sample 4	0.112
Sample 5	0.113
Sample 6*	0.113
Sample 7*	0.113
Sample 8	0.114
Sample 9	0.115
Sample 10	0.112
Day 2	
Sample 11	0.112
Sample 12	0.112
Sample 13	0.112
Sample 14	0.111
Sample 15	0.111
Average	0.113
Std Dev <sup>1</sup>	0.0011
RSD (%) <sup>2</sup>	0.94
Precision <sup>3</sup>	± 2.0

1. Standard Deviation calculated using the (n-1) standard deviation.
2. Relative standard deviation (RSD) calculated by dividing the standard deviation by the average and multiplying by 100.
3. Precision calculated by multiplying the RSD by 2.15 (2.15 the students T-test for analysis of 15 samples at the 95% confidence level)

Table III. Method Recovery Data

Sample ID	Theoretical Concentration (% w/w)	Measured Concentration (% w/w)	Recovery (%)
R4A (EC1)	0.0534 <sup>1</sup>	0.0579	108.4
R5A (EC1)	0.1053 <sup>1</sup>	0.1057	100.4
R6A (EC1)	0.1556 <sup>1</sup>	0.1540	99.0
R7 (EC1)	0.2098 <sup>2</sup>	0.2059	98.1
R8 (EC1)	0.2392 <sup>2</sup>	0.2402	100.4
R9 (EC1)	0.3298 <sup>2</sup>	0.3284	99.6
R10 (EC1)	0.3591 <sup>2</sup>	0.3571	99.4
R11 (EC1)	0.3896 <sup>2</sup>	0.3913	100.4
R14 (EC2)	0.0510 <sup>1</sup>	0.0581	113.9
R15 (EC2)	0.1022 <sup>1</sup>	0.1045	102.3
R16 ( EC2)	0.1541 <sup>1</sup>	0.1622	105.3
		Average	102.5
		Std Dev	4.9
		RSD (%)	4.7

1. Theoretical concentration calculated by dividing mass of sulfotepp added to sample by the total sample weight of inerts, chlorpyrifos analytical standard and sulfotepp.
2. Theoretical concentration calculated by dividing mass of sulfotepp added to sample plus the mass of sulfotepp in the aliquot of chlorpyrifos technical weighed, by the total sample weight of inerts, chlorpyrifos technical and sulfotepp.

## H ANALYTICAL METHOD SUMMARY

### **Sulfotepp in Chlorpyrifos EC Formulations DAS-AM-01-058**

#### **1. Standard Preparation**

i) Stock solution:

Accurately weigh, to 0.1mg, approximately 0.035 g of sulfotepp analytical standard into a 10 mL volumetric flask. Dilute to volume with isooctane at 20 °C.

ii) Calibration Standard:

Pipette 60 uL of stock solution into a 10 mL volumetric flask containing approximately 0.28 g of chlorpyrifos analytical standard and dilute to volume with isooctane at 20 °C. Mix thoroughly.

Calculate the exact concentrations of impurities as detailed in section 4 (i).

#### **2. Sample Preparation**

i) *Formulations where surfactant inerts can be checked to confirm no interferences*

Accurately weigh, to the nearest 0.1mg, approximately 0.63 g of chlorpyrifos EC formulation technical into an 11 dram or other appropriately sized, screw top vial.

Add 24.4 mL of isocotane to the sample using a dispenser and shake to mix.

Filter an aliquot of the prepared solution through a 0.45 um syringe filter, 25 mm diameter with GF/0.45 um GHP Membrane.

ii) *Formulations containing interferant surfactants (or where the the surfactants are unknown)*

Accurately weigh, to the nearest 0.1mg, approximately 0.63 g of chlorpyrifos EC formulation technical into an 11 dram or other appropriately sized, screw top vial.

Add 10 mL of distilled water and 24.4 mL of isocotane to the sample using dispensers and shake vigorously for 20 minutes.

Centrifuge at 2000 rpm for 5 minute and filter an aliquot of the top layer of the prepared solution through a 0.45 um syringe filter, 25 mm diameter with GF/0.45 um GHP Membrane.

### 3. Instrumentation and Conditions

Instrument	HP 6890 Gas Chromatograph
Column:	HP-1, 30 m x 0.53 mm x 2.65µm film
Carrier gas	Nitrogen
Detector	Flame Ionisation Detector at 275 °C
Detector Gases - Air	300 mL/minute
- Hydrogen	30 mL/minute
- Nitrogen	25 mL/minute
Carrier flow	8 mL/min
Injection temperature	220°C
Oven temperature program	70 °C for 0.3 minutes, ramp 10 °C / minute to 110 °C, hold 0.1 minutes, ramp 12 °C / minute to 180 °C , hold 3 minutes, ramp 12 °C / minute to 260 °C, hold 5 minutes
Injection volume	1 µL
Equilibration time	3 minutes
Total run time	25 minutes
Typical Retention Time	
Sulfotepp	15.8 minutes
Quantitation	peak area

### 4. Calibration

- (i) Calculation of concentration (mg /mL) of sulfotepp in the calibration solution:

$$C_{(i,cal)} = \frac{[W_i \times (P/100)]}{10\text{mL}} \times \frac{0.06 \text{ mL}}{10 \text{ mL}}$$

where

$C_{(i,cal)}$ =	the concentration (mg/ml) of sulfotepp in the calibration solution
$W_i$ =	weight (mg) of sulfotepp in the stock solution
$P$ =	Purity of the impurity standard, expressed as a percentage
10 mL =	the volume of stock solution prepared
0.06 mL =	the volume of stock solution added to the calibration solution
10 mL =	the volume of calibration solution prepared

(ii) Calculation of external standard response factor (RF) for sulfotepp:

$$RF(i) = \frac{C(i, cal)}{Area(i, cal)}$$

where

$RF_{(i)}$  = response factor of sulfotepp  
 $C_{(i, cal)}$  = the concentration (mg/ml) of sulfotepp in the calibration solution  
 $Area_{(i, cal)}$  = peak area obtained for sulfotepp in the calibration solution

## 5. Sample Analysis

The response factor of the standard injection before the samples is calculated and then applied to the samples. **A maximum of four samples may be run and quantitated against each standard injection.**

Calculation of weight % concentration of sulfotepp in the samples:

$$\% w/w = \frac{(Area(sample) \times RF(i))}{(W(sample)/25mL)} \times 100\%$$

where

$\% w/w$  = the weight percent of sulfotepp in the sample  
 $RF_{(i)}$  = response factor of the sulfotepp in calibration solution  
 $Area_{(sample)}$  = peak area obtained for sulfotepp in the sample solution  
 $W_{(sample)}$  = sample weight in mg  
25 mL = the volume of sample solution prepared

## 6. Method Precision

Data obtained using this procedure indicates relative method precision values at the 95% confidence level (Table II) of.

Analyte	Precision
Sulfotepp	$\pm 2.0 \%$

This data was obtained from analysis of a sample ten times on day one (including five sample preparations using the water extraction step) and five times on day two.

**7. Accuracy**

Analysis of sulfotepp added to samples of chlorpyrifos analytical standard or chlorpyrifos technical and inerts of 480EC(1) or 480EC(2), gave the following mean recovery level (Table III):

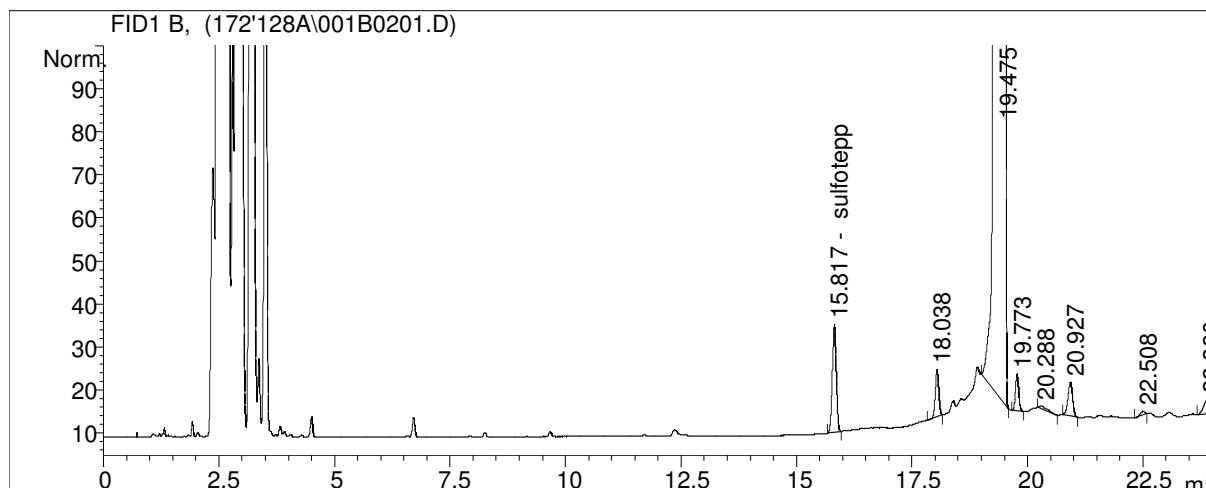
Concentration Range	Accuracy
0.05–0.39 %w/w	102.5 %

**8. Linearity**

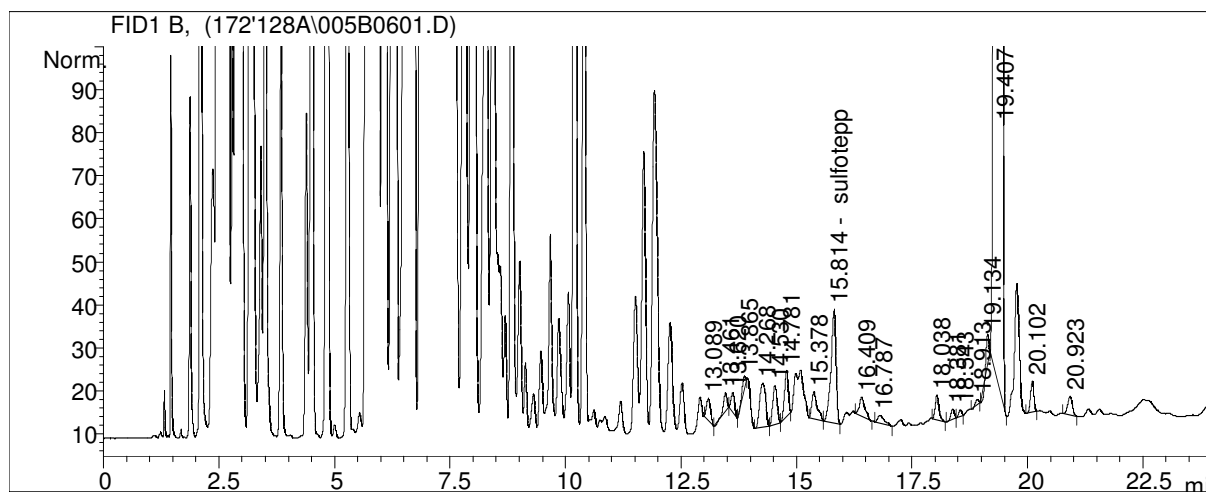
The detector response was found to be linear for sulfotepp in the following concentration range:

	Linear Range
Sulfotepp	6 -202 ug/mL

**Figure 1. Chromatogram of Standard Solution**



**Figure 2. Chromatogram of Formulation without Water Extraction Step**



**Figure 3. Chromatogram of Formulation with water extraction step included.**

