

# Analysis of Long Chain Sultones by Thin Layer Chromatography

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## ABSTRACT AND SUMMARY

The separation and semiquantitative estimation of nine C<sub>14</sub> sultones by thin layer chromatography (TLC) on silica gel is reported. Vapor phase charring gives detection limits of several ppm in olefin sulfonate, ethoxylated alcohol sulfates, and formulated products. Isopropyl ether is the solvent of choice, and two-dimensional TLC with this and a chlorinated solvent mixture gives an exclusive sultone area, as well as added sensitivity. Simplified preparative TLC on plates with preadsorbent is described for confirmation by independent analytical methods.

## INTRODUCTION

The products of SO<sub>3</sub> sulfonation of alpha olefins are alkene sulfonates and sultones (1). The latter are reduced to very low level by an alkaline hydrolysis. The final manufacturing step is a hypochlorite bleach which may produce traces of chloro and unsaturated sultones, which have been indicted as skin sensitizers (2). We report here a thin layer chromatographic (TLC) method; a complimentary gas chromatographic (GC) method is reported separately (3) as is the identification of these sultones (4).

The preliminary step before TLC is the isolation of the neutral oils from the alpha olefin sulfonate (AOS) by petroleum ether extraction (5). These neutral oils contain, in addition to small amounts of the sought-for sultones, relatively large amounts of hydrocarbons (mainly isomerized olefins) and long chain alcohols.

## EXPERIMENTAL PROCEDURES

### Adsorbents

Analytical work was carried out with Quantum Industries' Q6 hard surface silica gel plates, 200 x 200 x 1/4 mm. They were divided into 1 cm lanes with a Schoeffel Instrument's scorer; the two edge lanes on each side were not used. A stop line was scored 8 cm above the 1 1/2 cm high origin. No prewashing of the plates was needed.

For preparative TLC, Quantum Industries' unique PLQ 1000 plates, 200 x 200 x 1 mm, were used. These have a 3 cm preadsorbent layer and the sample is applied to the upper 2 cm. All of the sample advances with the solvent front through the preadsorbent layer, resulting in a narrow band of sample at the origin.

### Solvents

Baker reagent grade diisopropyl ether was stored over 1/2% aqueous sodium sulfite solution to eliminate the hazard of peroxide formation. Extreme caution was also observed because of the very low flash point (-15 F) of this solvent. Petroleum ether, a 30-60 C cut, was freshly redistilled before use. Other solvents used were Baker, either reagent grade or the best where reagent grade was not available.

### Developing Chambers

N chambers, (8 1/2 x 3 x 10 1/2 in.) supplied by

Brinkman Instruments, were lined on three sides by filter paper. The isopropyl ether developing solvent was replenished as required; all mixed solvents were prepared fresh.

### Apparatus

The vapor phase charring equipment described by Martin and Allen (6) was used. Charring was carried out with 20% fuming sulphuric acid at 150 C (block temperature) for 10 min.

### Extraction

ASTM D 1568-63 method for neutral oils (5) was followed in principle, but scaled up. A 25 g sample (38% sulfonate in water) was dissolved in 400 ml ethanol:water 1:1 and extracted six times with 100 ml portions of redistilled 30-60 C petroleum ether and the extract evaporated to dryness in a tared flask.

### Chromatography

The neutral oil was diluted to 10% w/v with hexane. A total of about 400 µg sample was put down by applying two spots about 3 mm apart with a 1 µl MICROCAP, then repeating once after allowing the spots to dry. Appropriate standards for identification and quantitation were spotted in the same way. These materials were isolated from AOS by preparative column chromatography and by preparative high pressure liquid chromatography (HPLC). The identification is described elsewhere (4). The range of standards spotted was 0.4 to 4.0 µg. Activation of the plates was not required. They were developed twice in isopropyl ether, allowing the plate to air dry before the second development. Then the plates were again air dried and the final traces of solvent removed by blowing with a hot air dryer.

### Two-Dimensional Chromatography

A single 5 µl spot (about 500 µg) of sample was applied 1 1/2 cm from each edge in the lower left corner of the plate. Stop lines were scored at right angles to each other 8 cm above and 8 cm to the right of this. Sample and appropriate standards were applied outside these stop lines on origin lines 1 1/2 cm above the bottom and 1 1/2 cm from the left edge of the plate. The plate was developed twice in isopropyl ether in the upright position allowing the solvent to dry between developments. The plate was then turned through a right angle, so the second origin line was at the bottom of the plate, and developed twice in 60:40 n-butyl chloride:methylene chloride.

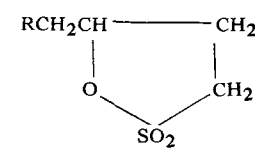
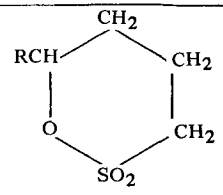
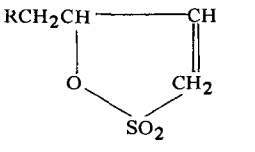
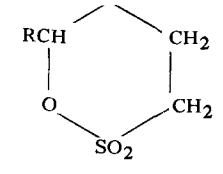
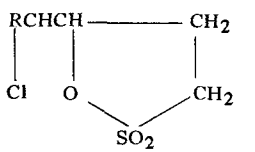
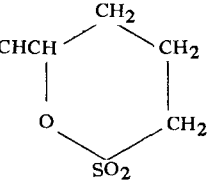
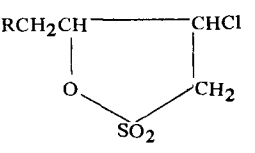
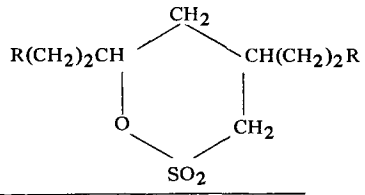
### Visualization and Quantitation

Excellent sensitivity was obtained using Martin and Allen's charring technique (6). The plate, free of developing solvents, was placed face up on the heated block maintained at 150 C. The Pyrex lid, smeared with 20% fuming sulphuric acid, was quickly placed over the plate. This rested on a second glass plate that ensured no acid would run onto the edges of the plate. Within 10 min, satisfactory black spots were obtained.

Quantitation was carried out by matching the intensity of the charred sample sultone spots with those of C<sub>14</sub> saturated delta sultone standards. Those sample spots that fell between standards were estimated. Samples containing spots appreciably above the 4 µg highest standard were rechromatographed after diluting the sample.

TABLE I

## Structures of Sultones

Gamma Sultone ( $\gamma$ sultone)		Delta Sultone ( $\delta$ sultone)	
1,2-Unsaturated Gamma Sultone (1,2-unsaturated $\gamma$ sultone)		3-Chloro Delta Sultone (3-Cl $\delta$ sultone)	
4-Chloro Gamma Sultone (4-Cl $\gamma$ sultone)		5-Chloro Delta Sultone (2 Diastereomers) (5-Cl $\delta_1$ , and 5-Cl $\delta_2$ sultones)	
2-Chloro Gamma Sultone (2-Cl $\gamma$ sultone)		Dimer Sultone (ref. 2)	

R = C<sub>10</sub> (or C<sub>12</sub>)  
R' = C<sub>9</sub> (or C<sub>10</sub>)

A reagent specific for alkylating agents, 4-(4'-nitrobenzyl)-pyridine (NBP) was used for visualization (A. Dillarstone, private communication). It was sprayed and heated according to the following schedule:

- Spray heavily with 2% NBP in acetone.
- Immediately spray lightly with freshly prepared 1:1 acetone:20% aqueous ammonium carbonate.
- Heat 1 hr at 115 C.
- Spray with 1:1 ethanol:20% aqueous sodium hydroxide.
- Cover immediately with a glass plate to retard fading of the purple spots. Evaluate and mark the position of any faint spots.

NBP visualization was not used for quantitation. The purple color fades rapidly for faint spots and can be temporarily restored by respraying with the ethanol-aq NaOH solution. However, the relative intensities have been observed to change on respraying.

#### Preparative TLC

The neutral oil, dissolved in chloroform, was applied without special precaution to the upper 2 cm of 3 cm pre-adsorbent strip on a PLQ 1000 preparative plate (7). A 1 ml syringe with a 20 gauge needle was suitable for quickly delivering the desired amount of sample. After the solvent was evaporated off with a warm air dryer, the plate was scored 10 cm above the preadsorbent to limit development.

To determine which solvent system to use first, a pair of two-dimensional pilot experiments was run on normal 1/4 mm Q6 plates. Either 60:40 n-butyl chloride:methylene chloride or 30:70 dioxane:hexane was used in the first direction, and isopropyl ether in the second. The more suitable first dimension solvent system was chosen, based on how well the sultone of interest was separated.

The preparative plate was then double-developed in the solvent of choice along with a reference plate (50 x 200 x 1/4 mm LQD plate spotted with the same sample and the

appropriate known sultones). The 50 x 200 plate was then charred in the normal manner and served to indicate where the preparative plate should be scraped to remove the sultone of interest. The scraped silica was transferred to a small glass column and leached with about 25 ml of methylene chloride. This was evaporated to about 1 ml, and then applied in the same manner as before to a second PLQ 1000 plate and a second 50 x 200 LQD plate. They were developed twice for 10 cm in isopropyl ether and the sultones of interest were located and eluted as before. The separated sultones were examined by other analytical techniques for identification; IR and GC-MS were most frequently used.

#### RESULTS AND DISCUSSION

The structures of the chlorinated, unsaturated, and saturated sultones discussed in this report are shown in Table I. The identification of these sultones is discussed elsewhere (4).

We have investigated a variety of solvent systems for the separation of sultones. The most useful was found to be isopropyl ether, and the separation of nine sultones is shown in Figure 1. One advantage of this solvent is that the separation is little affected by humidity, so it was not found necessary to activate the hard surface silica plates (Quantum Industries Q6) which have good resolution and fast development. We did find it useful to develop twice in the same solvent for improved separation. There was a barely distinguishable increase in R<sub>f</sub> (about 0.01 unit) for C<sub>2</sub> increase in carbon length for the saturated delta sultone.

The other useful solvent systems are shown in Table II; all except the last two were double-developed.

The ethyl ether and ethyl acetate solvents had been reported (A. Dillarstone, private communication) as used for separation of sultones by HPLC, using silica gel or nitrile columns and refractive index detection. We found these two solvent mixtures similar to isopropyl ether, but

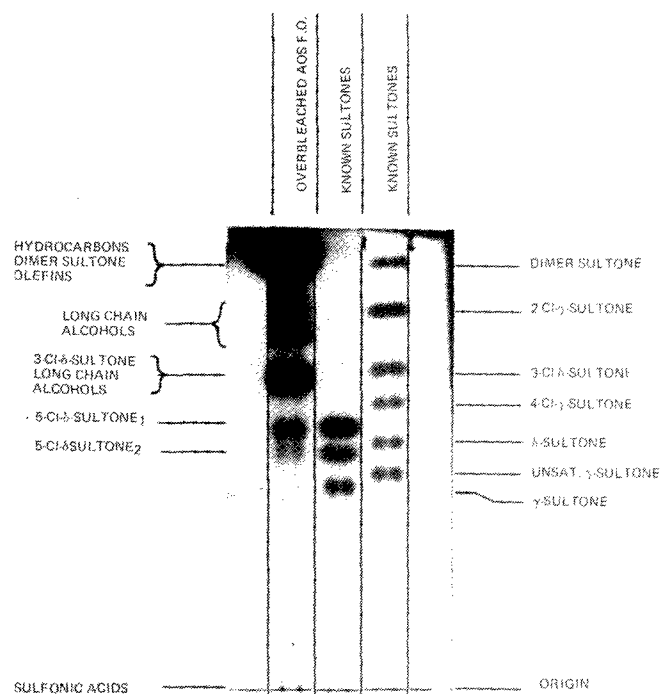


FIG. 1. Chromatography of neutral oil extracted from overbleached alpha olefin sulfonate, and of known sultones. Plate: Quantum Q6 silica. Developing solvent: isopropyl ether, developed twice. Visualization: vapor phase SO<sub>3</sub> charring. Sample: 400 μg of neutral oil, dissolved in hexane.

gave poorer resolution of some sultones. Tert-butyl alcohol gave a reversal of order of some sultones.

Solvent mixtures 4-6 were useful for resolving alcohols, which obscure the 3-chloro delta sultone and bracket the 2-chloro gamma sultone (Fig. 1). The pyridine and dioxane move the sultones less, so that the alcohols preceded all the sultones (except the dimer sultone). The pyridine solvent tends to give tailing spots. The chlorinated solvent mixture moves the alcohols less, so the sultones all have higher R<sub>f</sub> values than any alcohols. In all three solvents several of the sultone spots overlap.

The last two solvents are specifically useful for separating several less polar components found near the top of the plate in the region of the dimer sultone.

**Quantitation**

Table III documents the accuracy of C<sub>14</sub> delta saturated sultone analysis. Known amounts were added to sulfonate slurry and the method was applied.

For a number of samples, we have used gas chromatography to verify our TLC results. The results, in the range 2-18% of saturated delta sultone, differed by an average of 1.25% and a maximum of 3%. Gas chromatography tended to give the higher result.

**Visualization**

Charring is the best means of visualization and the vapor-phase charring technique of Martin and Allen (6) gives far better resolution than spraying with 50% sulfuric acid solution. The limit of detection of a single sultone spot is about 0.2 μg and all sultones studied gave a similar response; thus equal quantities of different sultones gave spots similar in appearance.

We found 400 μg to be the largest amount of free oil (applied as two 200 μg spots as described in the procedure) that could be usefully applied before the background became streaked in the sultone area, making detection of trace amounts uncertain. Thus the effective detection limit was 0.4 μg (two 0.2 μg spots) in 400 μg. A sulfonate con-

TABLE II  
Alternative Solvents to Isopropyl Ether

1.	70:30 v/v Ethyl ether:hexane
2.	10:90 v/v Ethyl acetate:toluene
3.	15:85 v/v Tert butanol:hexane
4.	5:95 v/v Pyridine:hexane
5.	30:70 v/v Dioxane:hexane
6.	40:60 v/v Methylene chloride:n-butyl chloride
7.	n-Butyl chloride
8.	Toluene

TABLE III  
Recovery Study of C<sub>14</sub> Delta Sultone

Percent added	Percent found
0.00	0.2
0.14	0.1
0.28	0.2
0.69	0.5
1.45	1.0
2.72	2.7
6.85	6.4

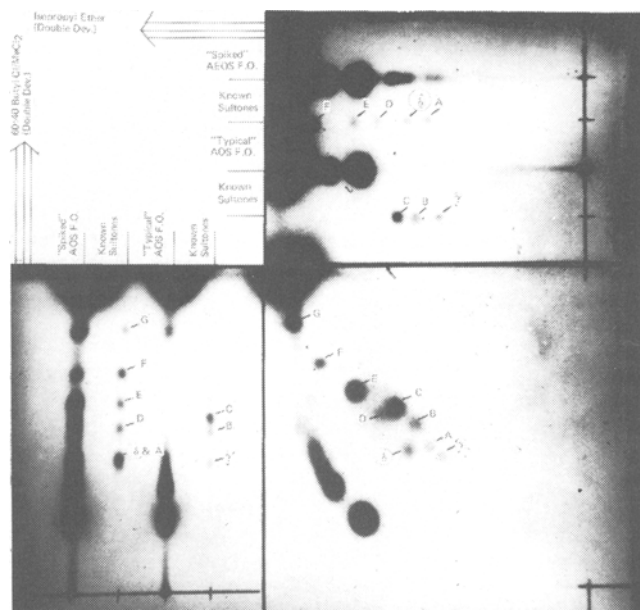


FIG. 2. Two-dimensional chromatography of neutral oil extracted from alpha olefin sulfonate, spiked with known sultones. Plate: Quantum Q6 silica. Developing solvent 1: isopropyl ether, developed twice. Developing solvent 2, at right angles: 60:40 butyl chloride:methylene chloride, developed twice. Visualization: vapor phase SO<sub>3</sub> charring. Sample: 500 μg of neutral oil, spiked with known sultones and dissolved in hexane. Sultones: A, 1,2-unsaturated γ; B, 5-Cl δ<sub>1</sub>; C, 5-Cl δ<sub>2</sub> diastereomer; D, 4-Cl γ; E, 3-Cl δ; F, 2-Cl γ; G, dimer sultone; δ and γ, saturated sultones.

taining 1% neutral oil, formulated into a product as 20%, would give a detection limit of 2 ppm for each sultone.

It has been reported (A. Dillarstone, private communication) that NBP is specific for visualizing alkylating agents. We found NBP does visualize all the sultones, but it also visualizes spots from neutral oils where we have found no sultones. NBP visualizes sultones to varying extents so that, unlike with charring, every sultone should be spotted for quantitation. The method is more complex, gives lower sensitivity, and faint spots rapidly fade. We, therefore, have used it solely as a confirmatory procedure for sultones.

**Two-Dimensional TLC**

There are a large number of neutral oil components in AOS, and a large sample is spotted to attain the needed

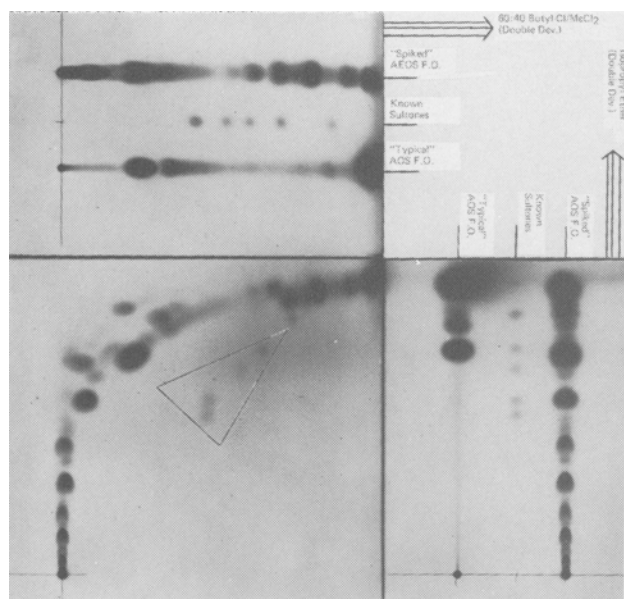


FIG. 3. Two-dimensional chromatography of neutral oil extracted from ethoxylated alcohol sulfate, spiked with known sulfones and showing the triangular "SULTONE AREA." Plate: Quantum Q6 silica. Developing solvent 1: isopropyl ether, developed twice. Developing solvent 2, at right angles: 60:40 butyl chloride:methylene chloride, developed twice. Visualization: vapor phase  $\text{SO}_3$  charring. Sample: 500  $\mu\text{g}$  of neutral oil, spiked with known sulfones and dissolved in hexane.

sensitivity. Ethoxylated alcohol sulfate raw materials and formulated products containing both these and AOS have been examined. In order to deal with the added interferences which obscure part of the sultone area, and to reach the highest sensitivities, we have used two-dimensional TLC. We were able to increase the sample to 500  $\mu\text{g}$ , put down as a single spot, and detect 0.2  $\mu\text{g}$  (single spot) of each sultone in 500  $\mu\text{g}$  free oil.

A combination of isopropyl ether in the first dimension with a chlorinated solvent mixture in the second was able to resolve both 3-chloro delta sultone and the dimer sultone, which are not separated by the ether alone, plus all the other sulfones discussed. A separation of known sulfones, added to an AOS free oil, is shown in Figure 2. The sensitivity was increased by a factor of 2-4, because even with very large samples the spots are clearly resolved. When developed in one dimension, a very large sample gives a background streak and very faint spots cannot be seen.

For highest sensitivity a small sample of neutral oil was first passed through a mini-column of silica to separate it

into four fractions. The first, was a hydrocarbon and dimer sultone fraction (hexane eluate); the second alcohols, 2-chloro gamma and some 3-chloro delta sultone (10% ether in hexane); the third all the other sulfones (ether eluate), while sulfonates remained on the column.

In the two-dimensional chromatogram we discovered that there is a triangular area that is exclusive to sulfones in the samples that we have examined. This is shown in Figure 3.

This sulfone area has been particularly useful to us as a general test for the presence or absence of sulfones, especially as ethoxylated alcohols and alcohol sulfates interfere with one-dimensional TLC. This is dramatically illustrated by Figure 3, which is a sample of ethoxylated alcohol sulfate, spiked with known sulfones. A confirmation of our conclusion about the exclusive sulfone area was the samples of paraffin sulfonate, a similar product which cannot form sulfones, was completely free of spots in that "sulfone area." Only the dimer sulfone and the 2-chloro gamma lie outside this area.

### Preparative TLC

The complex nature of the free oils created difficulties in isolation of sulfones by preparative TLC, particularly from formulation products. We found it necessary to chromatograph twice or even three times, using different solvent systems (isopropyl ether, methylene chloride-butyl chloride, dioxane-hexane). This gave a separation quite similar to two-dimensional TLC, but isolating far larger amounts.

We found Quantum's PLQ 1000 1 mm plates to be especially convenient for this work (A. Dillarstone, private communication). These have a unique pre-adsorbent layer which can be heavily loaded with sample in a very short time without a special applicator, yet retain good resolution.

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