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IMPROVED PROCEDURES FOR HANDLING THIN-LAYER  
CHROMATOGRAPHIC SPOTS

R. AMOS

*Esso Research Centre, Abingdon, Berks. (Great Britain)*

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SUMMARY

This paper describes improved procedures for the isolation of substances separated from complex mixtures by thin-layer chromatography for subsequent spectroscopic examination. Avoidance of contamination by plastic tubing, adsorbents, solvents and unclean glassware, as well as the problem of physical handling of small quantities of material, were all taken into account in the development of the improved procedure.

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## INTRODUCTION

The combination of liquid chromatography, whether on thin layers<sup>1,2</sup> or in high-efficiency columns<sup>3</sup>, with infrared (IR) spectroscopy or mass spectrometry (MS) is a powerful analytical tool for separating and identifying additives in petroleum products. An early account of the use of TLC with IR spectroscopy was given by SNAVELY AND GRASSELLI<sup>4</sup>. NASH *et al.*<sup>5</sup> used micro potassium bromide discs to identify rotenone in a chromatogram of technical grade rotenone, but the isolation of pure eluted solute proved to be lengthy and cumbersome. MCCOY AND FIEBIG<sup>6</sup> describe the elution of 50–100  $\mu\text{g}$  amounts of compounds from TLC plates into the capillary section of modified Pasteur-type disposable pipettes, but sample handling problems arose in replacing the eluting solvent with carbon tetrachloride or carbon disulphide and running the IR spectra. RICE<sup>7</sup> reduced sample handling to an absolute minimum by marking the spot on the plate in the form of a "tear-drop" and removing the surrounding adsorbent. 10–20 mg powdered KBr was placed at the point of the "tear-drop" and the solute was eluted into the KBr with a suitable solvent. On trying out this technique, we found that considerable losses of solute occurred, particularly with colourless compounds. Furthermore, it was found to be suitable only for well resolved spots.

HEYNS AND GRUETZMACHER<sup>8</sup> were among the first to combine mass spectrometry with TLC. RIX *et al.*<sup>9</sup> recognised that sample contamination was a serious problem in TLC/MS. Without identifying specific sources, they overcame their difficulties by keeping the amounts of adsorbents, solvents and sample handling to an absolute minimum using the Pasteur-pipette capillary technique.

The efficiency of a chromatographic separation increases with decreasing sample size. Instrumentation is now available which will enable IR and mass spectra to be obtained on less than 10  $\mu\text{g}$  material. To take advantage of this sensitivity, not more than 0.5  $\mu\text{g}$  of an impurity can be tolerated.

This paper describes the development of an improved procedure for obtaining pure samples by identifying all possible sources of contamination, eliminating them where possible, and reducing sample handling to a minimum.

#### CONTAMINATION BY PLASTICISERS

##### *Experimental*

In our early work, 100-mg samples were applied to 20  $\times$  20 cm glass plates coated with Merck Silica Gel G, 750  $\mu$  thickness, using the sample applicator developed by RITTER AND MEYER<sup>10</sup>. After development of the chromatogram, the separated bands were collected in a Soxhlet extraction thimble using the vacuum device designed by the same authors. The apparatus used, marketed by Desaga, is shown in Fig. 1. Materials adsorbed on the silica gel were extracted with about 50 ml

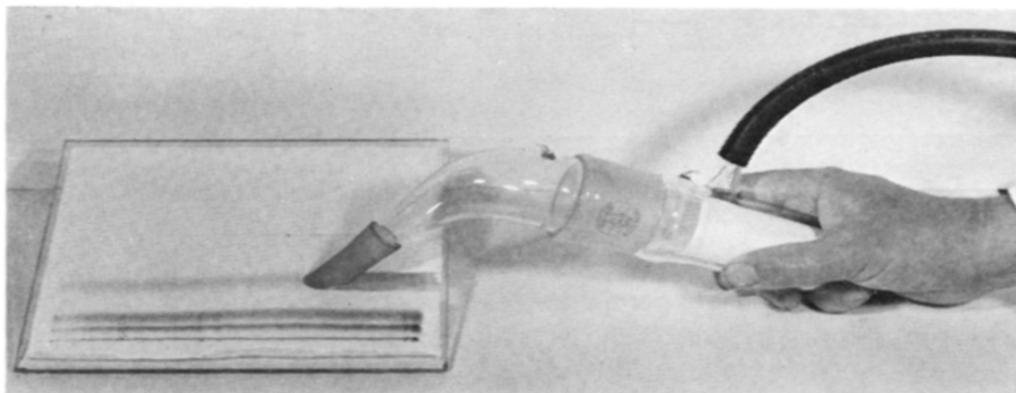


Fig. 1. Vacuum device for preparative layer chromatography.

ether, the solution was filtered through an  $\alpha$ -6 Metrical filter to remove silica gel fines, and the residue, after evaporating the ether, examined by IR spectroscopy in the form of KBr discs.

##### *Results and discussion*

Spectra obtained in this manner invariably contained strong carbonyl bands in the 1700  $\text{cm}^{-1}$  region which were not present in the spectra of the original samples. Examination by MS showed that contamination was due to the presence of acetyl tributyl citrate. A systematic search for the source of this contaminant was carried out by soaking plastic washers from the sample applicator, caps from the sample vials, extraction thimbles and Metrical filter overnight in ether. No contamination occurred from the thimbles or filters but the plastic washers and caps gave a small (1%) residue, the spectra of which resembled that of polyethylene. On removing the piece of plastic tubing from the vacuum suction device, a dramatic drop in the carbonyl absorption was observed. This was surprising since only a portion of dry ad-

sorbent was ever in contact with this tubing, and then only momentarily whilst being sucked off the plate. Subsequent solvent extraction of this tubing showed that it contained 43% acetyl tributyl citrate. Using the vacuum suction device without the plastic tubing caused scratching of the glass plates and this method was soon abandoned in favour of removing the silica gel layer with the aid of a spatula.

Although a big improvement in the quality of the spectra occurred from this point in time, extraneous bands and high backgrounds were still observable. It was therefore considered worthwhile to see whether impurities in the adsorbent or solvent made any significant contribution.

#### ORGANIC IMPURITIES IN SILICA GELS

##### *Experimental*

50-g portions of a number of commercial adsorbents were agitated on a mechanical shaker for 30 min with 150 ml solvent. The mixture was filtered through a Gelman  $\alpha$ -6 Metrical filter and the solvent removed by evaporation. The amounts of residue obtained are given in Table I.

TABLE I

SOLVENT EXTRACTABLE IMPURITIES IN TLC GRADE SILICA GELS  
mg impurities per 100 g adsorbent.

<i>Source</i>	<i>Acetone solubles</i>	<i>Ethanol solubles</i>
Merck Silica Gel G	19.2	42.2
H	3.6	28.4
HF <sub>254</sub>	4.4	34.0
HF <sub>254+304</sub>	4.6	31.8
HF <sub>254</sub> silanised	15.4	88.8
PF <sub>254</sub> silanised	7.6	63.0
HR pure	1.0	28.4
F <sub>254</sub>	38.8	92.2
Whatman Chromedia	11.4	23.4
Woelm Silica Gel	38.2	374.0
B.D.H. Silica Gel	10.6	73.8
M. & B. Chromalay	23.2	37.6
Blank on solvent and filter	<0.1	<0.1

##### *Results and discussion*

The acetone solubles were dark brown oils and their IR spectra were rather diffuse. That of Silica Gel G for example shown in Fig. 2a contained maxima due to C-H absorptions at 2940, 1460 and 1360  $\text{cm}^{-1}$ , together with maxima in the 1700  $\text{cm}^{-1}$  carbonyl-absorbing region. The spectrum of the acetone extract from a pre-coated plate is shown in Fig. 2b, which clearly indicates that these plates are unsuitable for combined TLC/IR spectroscopy. Presumably the spectrum is partly due to the binding material used in the manufacture of these plates.

The concentrations of ethanol-extractable materials were very much higher because of the increased solubility of silica and inorganic additives. Polar solvents such as alcohols are clearly unsuitable for the extraction of TLC spots for IR or MS

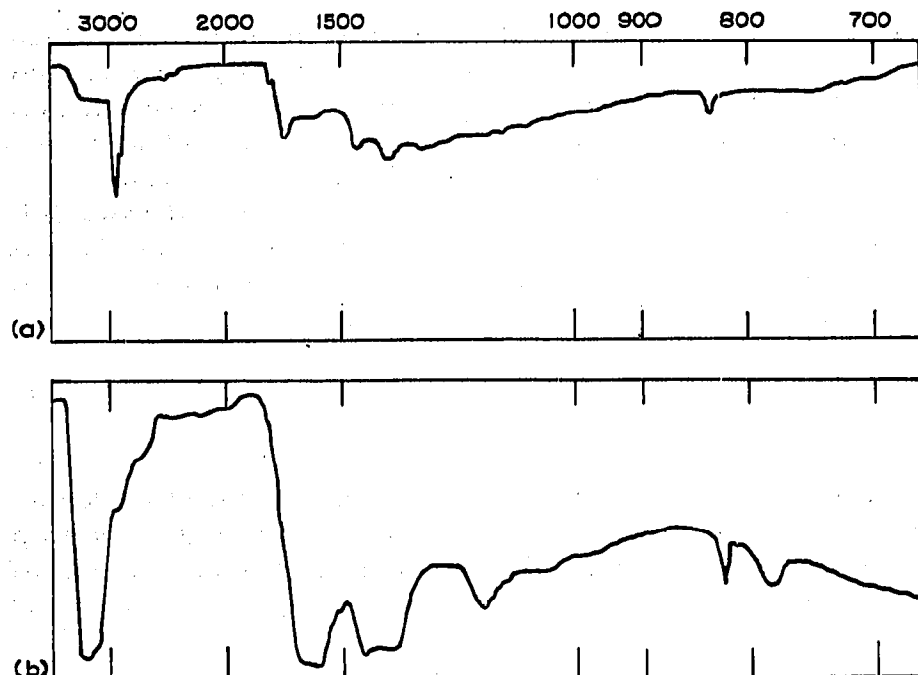


Fig. 2. IR spectrum of acetone extract of (a) Silica Gel G and (b) Silica Gel F<sub>254</sub>.

examination. As a result of this investigation Silica Gel H or HR was used for all further work.

#### INVOLATILE MATTER IN SOLVENTS

##### *Experimental*

To obtain a spectrum of 10  $\mu\text{g}$  material eluted from a TLC spot means that not more than 0.5  $\mu\text{g}$  of a contaminant can be tolerated, *i.e.* not more than 0.5 ml of solvent containing 1 mg/l non-volatile residue can be used to elute the spot. A number of commercial solvents were tested to see how closely they met this requirement. Three general grades were tested: (i) laboratory reagent grade, (ii) analytical reagent grade, and (iii) high-purity solvents, *i.e.* doubly redistilled in glass and described as suitable for spectroscopy. All glassware used was new and rinsed with acetone before use. Solvent samples of 500 ml from freshly opened bottles were placed in 600-ml beakers and evaporated down on a boiling-water bath under a gentle stream of nitrogen. When the volume had been reduced to less than 10 ml, the solvent was transferred to a tared 10-ml beaker. The large beakers were rinsed with 2 ml acetone and the washings added to the 10-ml beakers. Evaporation was continued until a constant weight of residue had been obtained.

##### *Results and discussion*

The results are given in Tables II–IV. Although all the solvents tested were within the specifications quoted by the suppliers, it can be seen that many analytical reagent grade solvents are unsuitable for TLC/spectroscopic work, *i.e.* they contain non-volatile residues greater than 1 mg/l.

TABLE II

## NON-VOLATILE RESIDUE OF LABORATORY GRADE SOLVENTS

When several results are reported they represent either different batches or different sources.

<i>Solvent</i>	<i>Residue (mg/l)</i>
<i>n</i> -Heptane (IP grade)	0.8
Toluene (sulphur free)	2.2
	2.3
	8.5
Acetone	0.4
Butanone	139.6

TABLE III

## NON-VOLATILE RESIDUE OF ANALYTICAL REAGENT GRADE SOLVENTS

When several results are reported they represent either different batches or different sources.

<i>Solvent</i>	<i>Residue (mg/l)</i>
Toluene	0.14
	44.1
	10.3
	65.4
	30.6
Carbon tetrachloride	0.8
	2.3
Diethyl ether	3.0
Chloroform	173.8
	165.2
	14.6
	2.4
	4.8
	1.2
Ethyl acetate	2.7
	3.8
Acetone	<0.01
Methanol	0.4

TABLE IV

## NON-VOLATILE RESIDUE OF HIGH-PURITY SOLVENTS

<i>Solvent</i>	<i>Residue (mg/l)</i>
Cyclohexane	3.9
Cyclohexane	1.9
Carbon tetrachloride	0.04
Toluene	4.5
Chloroform <sup>a</sup>	0.6
Ethyl acetate	1.6
Diethyl ether <sup>b</sup>	2.0
Methanol	0.8
Methanol	1.2

<sup>a</sup> Stabilised with 1-2% ethanol.<sup>b</sup> Stabilised with 2 p.p.m. of *n*-propyl gallate.

The IR spectra of the residues from a number of solvents were examined. There was no evidence to show material was being leached out from screw caps (polyesters), but inside the caps, covering the lips of the bottles, were discs of aluminium foil attached to a wad of cork by adhesive. This adhesive was found to be soluble in most organic solvents and would appear to be responsible for the high residues in some batches of toluene. Both the adhesive and toluene residues had spectra resembling an aliphatic hydrocarbon wax.

All batches of analytical reagent grade chloroform were contaminated, some heavily, by phthalate esters. It seems possible that the solvent had come into contact with plastic tubing during the period between the manufacturers' final distillation stage and the bottling process.

The presence of inhibitor (*n*-propyl gallate) could be detected in the ether residues and this solvent, though commonly recommended in the literature, is not suitable for eluting TLC spots prior to IR or MS examination. We have found that acetone is an effective substitute provided that it does not react with the solute (*e.g.* amines).

The lips of bottles of high purity grade solvents were covered with plastic discs, from which nothing measurable could be extracted. Such solvents were generally found to be fairly satisfactory for TLC/spectroscopic work but are relatively expensive. When these are not available, a simple distillation of analytical reagent grade solvents is effective in reducing the non-volatile matter of the first 70% distilled to acceptable levels. Table V shows the results obtained on three solvents, initially of unsatisfactory quality.

TABLE V

EFFECT OF A ONE-PLATE DISTILLATION  
70% distilled.

Solvent	Residue (mg/l)		
	Original	Overheads	Bottoms
Acetone	0.6	0.4	0.8
Chloroform	2.4	0.7	4.0
Toluene	44.1	0.04	57.6

#### CLEANLINESS OF LABORATORY GLASSWARE

A petroleum laboratory has its own special problems in maintaining a supply of spectroscopically clean glassware. Two persistent sources of contamination are from silicone grease and surface-active polymers of high molecular weight. The work reported below was carried out to find out which cleaning method was most effective. The success of the cleaning method was assessed by running an IR spectrum on 20  $\mu$ g of zinc diisobutyl dithiophosphate (ZDDP) which was introduced, as a dilute solution, into a number of vessels which had previously been contaminated and cleaned.

#### Experimental

5-ml pear-shaped flasks were filled with a dispersion of a surfactant polymer and silicone grease in toluene, evaporated to dryness and allowed to weather for six

weeks. Each flask was then rinsed with toluene to remove the bulk material and then soaked for 24 h in either hot caustic soda, chromic or nitric acids, or in 2% solutions of five commercial detergents. After cleaning, the flasks were rinsed ten times with distilled water and dried in an oven at 110° for 30 min. 20 µg ZDDP dissolved in 2 ml high-purity acetone were added to each flask and the solution evaporated to dryness. The contents of each flask were extracted with 1 ml acetone and transferred to ground KBr powder which was pressed into a disc 1.5 mm in diameter. The IR spectra were recorded using the Perkin-Elmer Model 521 and the beam condenser accessory.

### *Results and discussion*

Visual inspection of the vessels showed that overnight soaking (toluene, chloroform, acetone, methanol) was inadequate. Of the chemical cleansing solutions, chromic acid was the least effective, the spectrum of the ZDDP being completely swamped by bands due to the presence of silicone grease. Similar results were given by the nitric acid and hot caustic soda.

Merely soaking the dirty vessels for 24 h in 2% aqueous solutions of each of the five detergents did not completely remove all traces of contamination. On the other hand, immersing the flasks in three of the detergents, "Extran", "R.B.S. 25", and "Decon 75", in an ultrasonic bath for 30 min successfully cleaned the flasks and good spectra on 20 µg of ZDDP were obtained. The two other detergents, "Lab-brite" and "D.D.N. 150", were less satisfactory, even when using the ultrasonic bath.

### FINAL TLC/IR PROCEDURE

The work described in the previous sections has shown that contamination from plasticisers, adsorbents, solvents and dirty glassware is a serious problem in obtaining spectra on fractions separated by high-efficiency liquid chromatography, whether in columns or on thin layers. Instrumentation is now available for obtaining meaningful spectra on 10 µg material or less, but contamination must be kept to well below 0.5 µg.

We have achieved this by:

(a) Using the minimum amount of the purest adsorbent available, *e.g.* Silica Gel HR (Merck).

(b) Eluting with the minimum amount of a solvent (*i.e.* less than 1 ml), containing less than 1 p.p.m. of non-volatile residue. Redistillation may be necessary but analytical reagent grade acetone out of freshly opened bottles has been found to be suitable.

(c) Keeping sample handling down to the absolute minimum. All glassware is cleaned with an efficient detergent in an ultrasonic bath. Contact of all materials and samples with plastics is avoided.

The procedure we now successfully use is as follows: 40 µl of a 5% solution of the sample is applied as a short band (2 cm in length) to a 5 × 20 cm TLC plate coated with Silica Gel HR, 750 µ thickness. 2 µl of a 1% solution of the same sample is usually chromatographed alongside and used as a reference. The chromatogram is obtained in the usual way using solvents containing less than 1 p.p.m. of non-volatile residue and the 2-µl sample is sprayed with iodine solution so that the positions of the

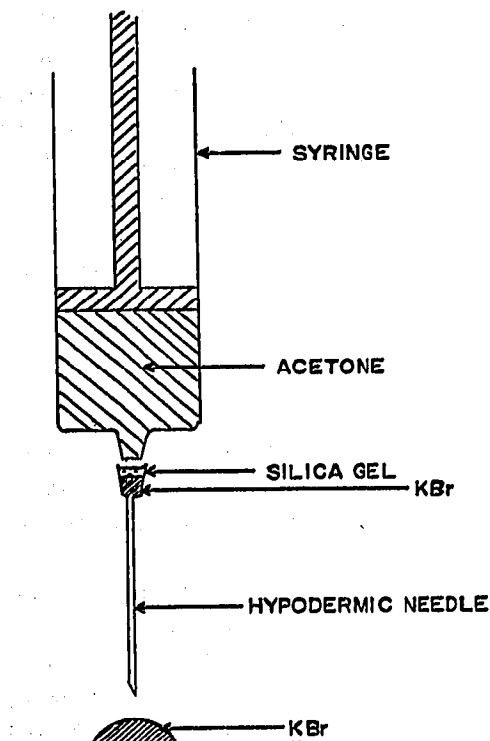


Fig. 3. Apparatus for transferring sample spot from adsorbent to potassium bromide.

separated bands in the larger sample can be located. The band of solute under examination is scraped off with a microspatula and placed on top of a small amount of KBr powder tamped down the cone joint of an 18-gauge hypodermic needle (see Fig. 3). The KBr serves as a filter to prevent silica gel fines from passing through the needle. A 1-ml glass syringe is filled with pure acetone, connected to the needle and the solute is eluted drop by drop onto a pile of 10 mg ground and dried KBr powder. Each drop is allowed to evaporate completely before the addition of the next drop. About 20 drops are usually sufficient to transfer the whole of the adsorbed solute from the silica gel to the KBr. The KBr powder and solute are then mixed with a microspatula and pressed into a disc 1.5 mm in diameter. Excellent spectra are obtainable on 5–30  $\mu\text{g}$  solute on the Perkin-Elmer 521 IR spectrophotometer with its microsampling assembly.

Alternatively the solute can be eluted directly onto the insertion probe of the mass spectrometer.

#### APPLICATIONS

The improved procedure is now used routinely with excellent results. Typical applications include the identification of zinc dialkyl dithiophosphates in lubricating oils and phenolic antioxidants in turbine oils. Fig. 4 compares spectra of zinc diisobutyl dithiophosphate obtained by the old and new procedures. Extraneous bands due to plasticiser and silica gel can be clearly seen to have been eliminated. Similarly, Fig. 5 compares spectra of 2,6-di-*tert.*-butyl-4-methylphenol isolated by the two



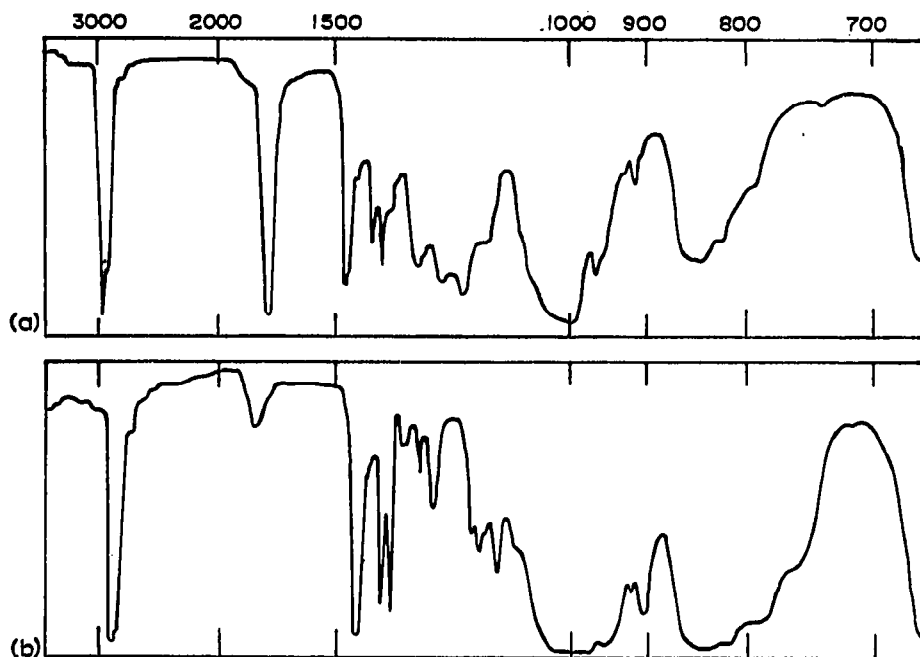


Fig. 4. IR spectra of zinc diisobutyl dithiophosphate using (a) old and (b) new procedures.

methods. It can be seen that the old method (Fig. 5a) failed to produce a recognisable spectrum of this relatively volatile antioxidant, but the new approach gave a good spectrum (Fig. 5b), free from interferences, from which the compound could be recognised.

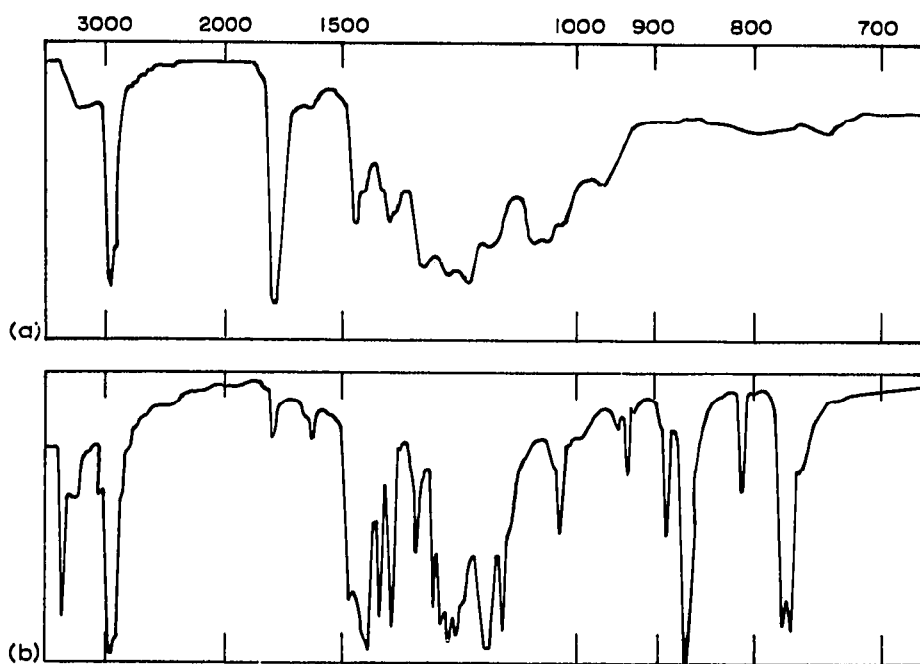


Fig. 5. IR spectra of 2,6-di-*tert.*-butyl-4-methylphenol using (a) old and (b) new procedures.

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