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THIN-LAYER CHROMATOGRAPHIC METHOD FOR DETERMINING ULTRAVIOLET ABSORBERS IN PARAFFIN WAX

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SUMMARY

A specific quantitative thin-layer chromatographic method has been developed for determining ultraviolet absorbers in paraffin wax. This paper describes the extraction procedure used for isolating the ultraviolet absorber from the wax, the thin-layer chromatographic separation of similar ultraviolet absorbers and the quantitative determination of the ultraviolet absorber to detect, in our case, 0.01 % ultraviolet absorber by use of the double beam scanning densitometer. The four ultraviolet absorbers studied in this investigation were: 2-hydroxy-4-methoxybenzophenone, 2-hydroxy-4-*n*-octoxybenzophenone, 2-hydroxy-4-*n*-decyloxybenzophenone, and 2-hydroxy-4-*n*-dodecyloxybenzophenone.

INTRODUCTION

Paraffin wax is used extensively as paper coating. The coating is light stabilized by the addition of small amounts of ultraviolet absorbers. At present, these ultraviolet absorbers cannot be identified and determined directly for two main reasons: (a) the absorber content is in the 0.005–0.01 % range, thus large samples of wax would have to be taken which would lead to solubility problems, and (b) if a thin-layer chromatographic approach was taken the direct application of the wax would hinder the migration of the spot, thus making the interpretation of the resulting chromatogram difficult. This work is concerned with the development of a method for isolating the ultraviolet absorber from the wax, thin-layer chromatographic identification of the absorber, and the quantitative determination of the absorber content.

APPARATUS

Silica Gel G Uniplates, 8 in. × 8 in., reversed phase (5 % Dow Corning Silicone), obtained from Analtech, Inc., 100 Justison Street, Wilmington, Del. The plates were used as received from the supplier after a short equilibration time in the development solvent vapors.

20 μl capillary pipet obtained from Scientific Products.

Chromatographic chamber obtained from A. H. Thomas Company, internal diameter 10 3/8 × 23/4 in. × 10 1/4 in. with a glass cover. The chamber contained an 8 in. × 8 in. sheet of Whatman No. E-17 filter paper to maintain a saturated solvent atmosphere during development.

Chromatographic sprayer obtained from Ace Scientific Company (Cat. No. 11-3055).

Joyce-Loebl Chromoscan—with 0–1.0 optical density wedge, the C-cam, 1005 aperture, 5-gain, VS lamp, 465 blue filter, and a 1–2 gear ratio. This instrument is distributed by National Instrument Laboratories, Inc., 1230 Parkland Drive, Rockville, Md. 20852.

Reagents

Extraction reagent. Dissolve 28 g of KOH pellets in a 500-ml volumetric flask with a 50:50 (by vol.) mixture of ethanol and distilled water. Dilute to volume.

Chromatographic spray solution[1,2]. Dissolve 0.10 g of nitrobenzenediazonium fluoborate (Eastman Organic Chemicals P-7078) in a 100-ml volumetric flask with distilled water. Dilute to volume. Continuous shaking will dissolve all solid material. Transfer to a chromatographic sprayer.

Development solvent. Add 3 vol. of ethanol to 1 vol. of distilled water.

Synthetic standard solution. Accurately weigh, to the nearest 0.1 mg, 0.10 to 0.11 g of the U.V. absorber to be determined into a 200-ml volumetric flask and dilute to volume with carbon tetrachloride (this represents a 0.01 % synthetic standard based on a sample weight of 50 g/10 ml). The following equation can be used to calculate the percent ultraviolet absorber in synthetic standards.

$$\% \text{ U.V. absorber in the synthetic standard} = \frac{(5)\ (W_A)}{(W_B)} = P$$

where W_A = the sample weight of the absorber and W_B = the sample weight of the wax.

Extraction procedure

Liquefy a 50-g sample (W_B) of the paraffin wax to be analysed in a 400-ml Pyrex glass beaker on a hot plate. Add 200 ml of reagent grade hexane to the liquefied sample, stir, and quantitatively transfer to a 500-ml long-stem separatory funnel. Add 100 ml of the extraction reagent to the funnel, shake the contents vigorously for 2 min (intermittently opening to release pressure buildup), allow the phases to separate, and draw off the lower aqueous phase into a 400-ml Pyrex glass beaker. Repeat this extraction 3 additional times with 50-ml aliquots of the extraction reagent.

Add 30 ml of conc. HCl to the combined extracts, and check to ensure that the solution is acid to universal indicator paper (deep red). Quantitatively transfer the solution to a 500-ml long-stem separatory funnel and add 150 ml of distilled water. Add 50 ml of reagent grade carbon tetrachloride to the funnel, shake vigorously for 2 min, allow phases to separate, and draw off lower organic phase into a 400-ml Pyrex glass beaker. Repeat this extraction 5 additional times. Concentrate the combined carbon tetrachloride extracts to a 30–40 ml volume on a hot plate (add a

few glass beads to prevent bumping), quantitatively transfer to a 50-ml Pyrex glass beaker and slowly concentrate to 7–8 ml, quantitatively transfer to a 10-ml volumetric flask, allow to cool, and dilute to volume with carbon tetrachloride.

Chromatographic procedure

Two 20-μl aliquots of both the sample and the synthetic standards were applied to the thin-layer plate, the spots were dried with a heat gun, and the chromatogram eluted for 30–40 min with the development solvent. The resulting chromatogram was dried, sprayed with the chromatographic spray solution, redried and scanned, using the Joyce–Loebl Chromoscan. The area of each zone can be calculated by triangulation (area calculated by multiplying the height times the width at half height) or by use of the integrator of the instrument. The quantitative results can be calculated from the following ratio.

$$\% \text{ U.V. absorber in the paraffin wax} = \frac{A_B \times P}{A_A}$$

where A_A = the average numerical count or area recorded for the synthetic standard. A_B = the average numerical count or area recorded for the sample tested. P = the percent U.V. absorber present in the synthetic standard.

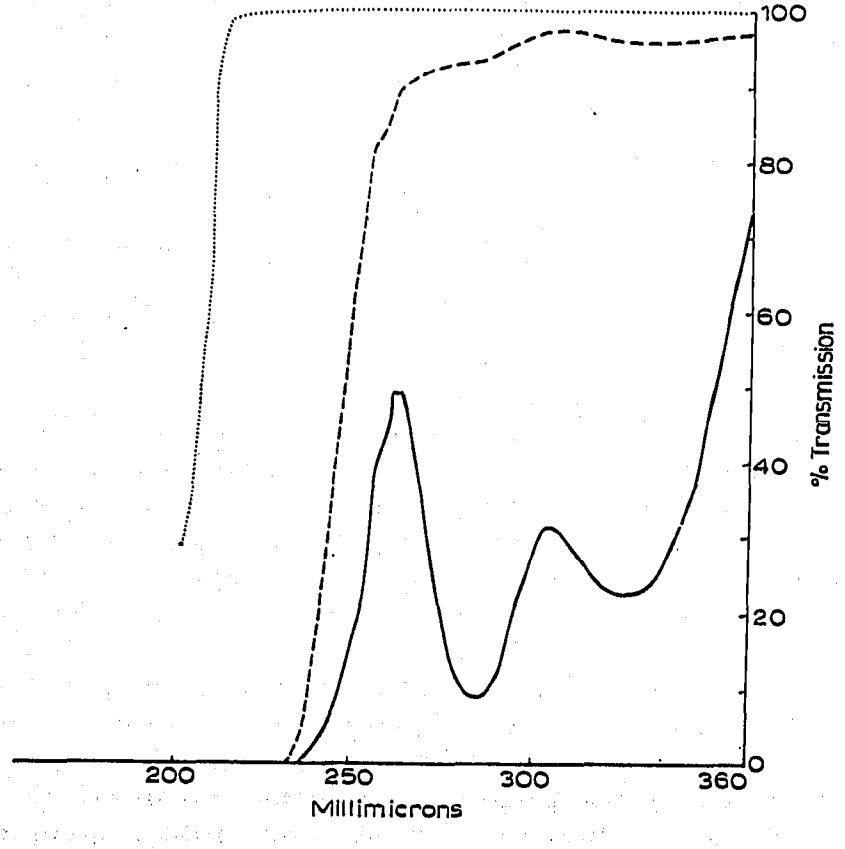

Fig. 1. U.V. spectrum of extracted hexane. Beckman DK-2A spectrophotometer. 1-cm silica cells. Reference: hexane. Concentration: 5 mg/250 ml. ·····, hexane; - - - - -, extracted hexane (originally containing 5 mg U.V. absorber; ———, synthetic standard (5 mg).

Discussion of the extraction procedure

Since commercial paraffin wax does not contain U.V. absorbers, synthetic samples of 2-hydroxy-4-*n*-octoxybenzophenone in paraffin wax were used throughout this investigation. For reasons already discussed, it was necessary for the extraction procedure to be incorporated into the procedure. Theoretically, the extraction of the absorber appears straightforward, for example, extraction from an organic solvent as the sodium salt, acidification, and reextraction into an organic solvent. Initial attempts at extracting the absorber from the organic solution with NaOH failed. Initially, the failure was believed to be caused by the soaping or sudsing action caused by NaOH; however, the same results were encountered, although the sudsing effect was reduced, when a lower strength NaOH was used. The long chain octyl group's lack of affinity for the aqueous layer is the present assumption for the inability to form the sodium salt. In order to test this theory, the extraction was made with normal KOH made up in a 50:50 mixture of ethanol and distilled water (this would give more affinity for the aqueous phase). Fig. 1 shows the U.V. analysis of the extracted organic phase *versus* a synthetic standard. Three extractions with the KOH solution extracted 95 % of the U.V. absorber from the hexane solution.

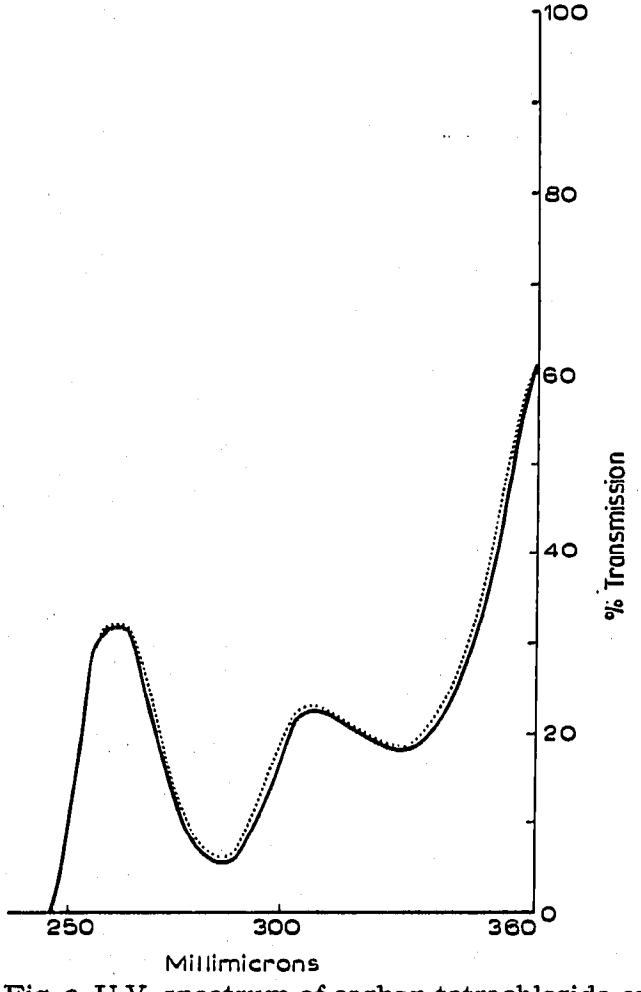

Fig. 2. U.V. spectrum of carbon tetrachloride extracts. Beckman Model DK-2A spectrophotometer. 1-cm silica cells. Reference: carbon tetrachloride. Concentration: 5 mg/250 ml. ———, synthetic standard (5 mg); ·····, carbon tetrachloride extracts (should contain 5 mg of U.V. absorber.

After acidification, a similar extraction problem was encountered; the absorber was not reextracted into an organic phase. It is believed that the ethanol concentration was holding the absorber in the aqueous phase, hindering migration into the carbon tetrachloride phase. This problem was remedied by the addition of excess distilled water to the system prior to the carbon tetrachloride extraction. U.V. analysis (Fig. 2) of a synthetic standard *versus* the combined carbon tetrachloride (5 extractions) extracts showed that 98 % of the U.V. absorber had been extracted.

Discussion on the thin-layer chromatographic procedure

STAHL[3], RANDERATH[4] and BOBBITT[5], discuss at great lengths the amount of work that has been done in the past on antioxidant and phenolic type compounds using various substrates and solvent systems. The more common solvent systems published in these books did not help resolve the problem, nor separate the four U.V. absorbers in question (since these experiments add little or no light on the solution of the problem, they are not included in this paper). Based upon a thin-layer work of COPIUS-PEEREBOOM[6] on the separation of phenolic compounds on Silica Gel G using a solvent system of petroleum ether, benzene and acetic acid, a similar approach was made. The components in the best separation are separated only by an R_F value of 0.03 by the systems listed and quite inadequate for our purposes. The next approach

TABLE I

R_F VALUES OF U.V. ABSORBERS ON REVERSED PHASE PLATES*

Developing solvent	*R_F values*			
	A	*B*	*C*	*D*
Methanol	0.78	0.72	0.68	0.67
Methanol–water (9:1)	0.74	0.54	0.49	0.34
Methanol–water (7:3)		no separation		
Isopropyl alcohol–water (9:1)		frontal movement		
Butanol–water–methanol (9:1:1)		frontal movement		
Ethanol–water (9:1)	0.86	0.78	0.74	0.66
Ethanol–water (4:1)	0.81	0.73	0.69	0.57
Ethanol–water (7:3)	0.86	0.49	0.40	0.25
Ethanol–water (3:1)	0.83	0.60	0.52	0.45
Ethanol–water (3:2)		no separation		

* Silica Gel G, reversed phase (5 % Dow Corning silicone). The U.V. absorbers represented are: *A*, 2-hydroxy-4-methoxybenzophenone; *B*, 2-hydroxy-4-*n*-octoxybenzophenone; *C*, 2-hydroxy-4-*n*-decyloxybenzophenone; *D*, 2-hydroxy-4-*n*-dodecyloxybenzophenone.

TABLE II

EXPERIMENTAL RESULTS

Sample	*% Ultraviolet absorber**	
	Added	*Found*
Synthetic sample	0.01	0.0093, 0.0098

* 2-Hydroxy-4-*n*-octoxybenzophenone was used as the representative U.V. absorber throughout recovery investigations.

was to investigate the possible use of reverse phase plates. The theory behind the reverse phase plate would be the higher the molecular weight, the more the solubility into the silicone, partitioned by the aqueous alcohol system (Table I). As is shown in Table II and Fig. 3, the experimental results coincide with the theoretical assumption made on the separation of the U.V. absorbers by molecular weight.

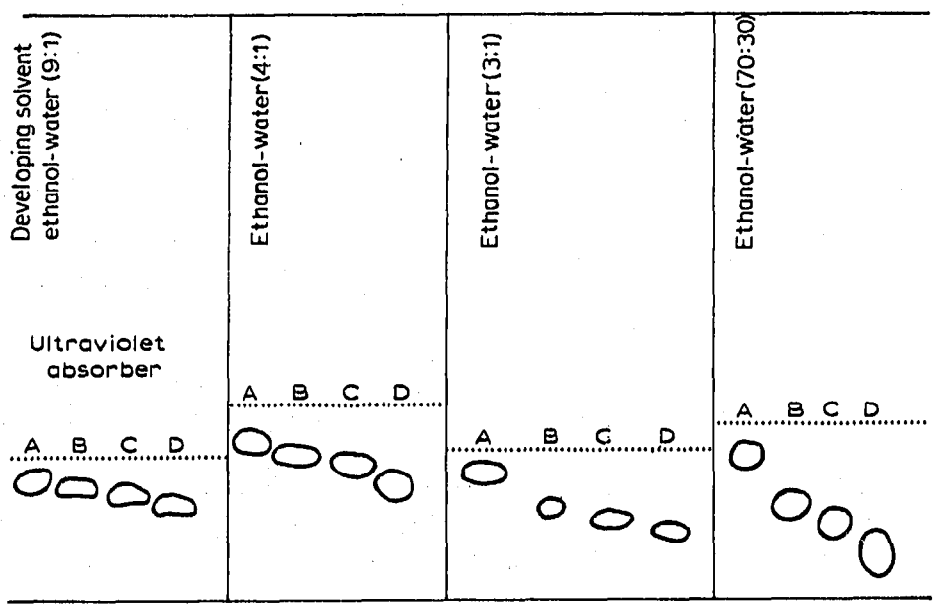

Fig. 3. Thin-layer chromatographic separation of U.V. absorber. Silica Gel G, reversed phase, 5% Dow Corning silicone. A, 2-hydroxy-4-methoxybenzophenone; B, 2-hydroxy-4-octoxybenzophenone; C, 2-hydroxy-4-*n*-decyloxybenzophenone; D, 2-hydroxy-4-*n*-dodecyloxybenzophenone.

RESULTS

Two synthetic standards were prepared to contain 0.01% 2-hydroxy-4-*n*-octoxybenzophenone. The procedure for separation and quantitatively determining the U.V. absorber was followed, the results of which are shown in Table II. As can be seen from the results shown in Table II, recovery of the U.V. absorber is excellent.

ACKNOWLEDGEMENT

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