A Chromatographic-Colorimetric Method for Determining Low Residues of Piperonyl Butoxide in Flour

A sensitive chromatographic-colorimetric procedure for determining piperonyl butoxide residues in flour has been developed. The residue is extracted with pentane and chromatographed on a column of Florisil, with two successive eluting solutions of ethyl acetate in pentane.

Previously published procedures for determining piperonyl butoxide residues (Jones *et al.*, 1952; Williams and Sweeney, 1956) worked well for some formulations and for paper over the range of 430 to 538 m^2 (40 to 50 mg per ft^2). However, these procedures lacked the sensitivity required for determination of low residues in flour or in other products containing oils, waxes, or other interfering substances.

To determine piperonyl butoxide residues in flour packed in pyrethrins-piperonyl butoxide-treated multiwall bags, a procedure was needed which would detect trace amounts of piperonyl butoxide in the flour.

PROCEDURE

Extraction. Extract 200 g of flour with 400 ml of pentane by tumbler at 10 rpm for 3 hr or by wrist-action shaker for 30 min; then filter using paper, 24 cm E&D #513 or equivalent.

Chromatography. Florisil used as an adsorbent for chromatography is 60- to 100-mesh, activated at 640° C. To prepare the Florisil for use in the procedure, dry it in an oven at 130° C for 5 hr, cool it, add 0.5% water, mix it for one-half hour, and let it stand overnight.

To chromatograph the flour sample, add Florisil to a depth of 10 cm to a 20- × 400-mm chromatographic tube containing coarse porosity, fritted disk.

Dry pentane by shaking it with anhydrous sodium sulfate. Add enough pentane to saturate the adsorbent and transfer the sample aliquot (a maximum of 200 ml) to the column containing the adsorbent. After the sample has entered the adsorbent, rinse down the sides of the column with pentane using a wash bottle to be sure that all of the sample is on the adsorbent. During the chromatography, tap the column at intervals to remove air bubbles. Elute the column successively with three 50-ml portions of 4% ethyl acetate in pentane (dried with Na_2SO_4), and rinse the tip of the column with pentane. Then discard the eluate which will contain most of the oils and waxes.

Elute the column with three successive 50-ml portions of 15% ethyl acetate in pentane (dried with Na_2SO_4). Add a solid glass bead and carefully concentrate the eluate to dryness under a stream of air on a hot-water bath. Dissolve the residue in about 10 ml pentane, quantitatively transfer the solution to a 25- × 300-mm test tube, and analyze it for piperonyl butoxide content by the colorimetric procedure.

Colorimetry. COLOR REAGENT. Dissolve 0.025 g of purified tannic acid in 20 ml of acetic acid and make to 100-ml volume with orthophosphoric acid (85%). Allow the air bubbles to escape before using reagent.

The colorimetric procedure alone can be used for samples with piperonyl butoxide content of more than 6.0 ppm. Samples with content of less than 6.0 ppm must first be taken through the cleanup process just discussed. The colorimetric procedure (based on the procedure by Jones *et al.*, 1952) is as follows. For samples above 6.0 ppm, transfer aliquots to test tubes, place test tubes in a basket, and carefully evaporate the solvent to dryness. Place the tubes horizontally on top of the water bath until all traces of solvent have been removed. Add 1 ml of Deo-Base (deodorized kerosene) and whirl to dissolve the residue. Add the color reagent (Jones *et al.*, 1952) slowly from a burette, shake for 2 min, then place in the water bath (95–100° C) for 5 min (Jones *et al.*, 1952). Pour the samples while hot into matched colorimeter tubes, immediately centrifuge, then allow to cool. When cool, read on spectrophotometer at 630 mμ. Set the spectrophotometer with a blank containing 5 ml of color reagent and 1 ml of Deo-Base, and take through the color-development procedure.

Calculations. After reading the samples, prepare the required standards. Weigh 0.05 g of pyrethrins (20%) and 0.1 g of piperonyl butoxide (purified analytical grade) into a 50-ml volumetric flask and make to volume with hexane (pure grade). For calculation of samples to be taken through the colorimetric procedure only (piperonyl butoxide content higher than 6.0 ppm), add the standard aliquots to untreated sample extracts (internal standards) of weights identical to the samples analyzed (Table I). For calculation of chromatographed samples, add aliquots containing 40 to 60 μg of piperonyl butoxide to untreated flour extracts of weights approximately the same as those of the chromatographed samples, and take these standards through the entire chromatographic-colorimetric procedure (Table I).

$$\text{ppm} = \frac{\mu\text{g in standard}}{\text{Optical density of standard}} = \frac{\mu\text{g in sample}}{\text{Optical density of sample}}$$

Table I. Recovery of Piperonyl Butoxide from Flour

Added ppm	Absorbance	Calculated from Standard μg	Flour Sample g	Found ppm	% Recovery
		No Cleanup			
7.1	0.23	67.6	10	6.8	96
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25.4	0.29	67.4	2.5	27.0	106
25.4	0.29	67.4	2.5	27.0	106
50.8	0.37	78.7	1.5	52.5	103
50.8	0.37	78.7	1.5	52.5	103
		Chromatographic Cleanup			
0.20	0.18	36	186	0.19	95
0.20	0.15	30	160	0.19	95
0.40	0.19	42	100	0.42	105
0.65	0.28	62	100	0.62	95
1.22	0.27	60	50	1.20	98
2.00	0.23	50	25	2.00	100

Standards for Samples without Cleanup

Added ppm	Absorbance	μg	Untreated Sample g	Absorbance Per 100 μg
6.1	0.21	60.9	10	0.34
24.4	0.26	60.9	2.5	0.43
32.5	0.35	81.2	2.5	0.43
54.1	0.38	81.2	1.5	0.47
		Standards for Samples with Cleanup		
0.20	0.10	20	100	0.50
0.40	0.18	40	100	0.45
1.20	0.27	60	50	0.45
2.40	0.28	60	25	0.46

RESULTS AND DISCUSSION

In the colorimetric procedure the amount of Deo-Base used in the Jones *et al.* procedure (1952) is increased from 0.1 ml to 1.0 ml. After the colors develop, the increase in Deo-Base dissolves and separates the oils present from the acid phase. This separation is necessary for distinct blue colors to form.

The described procedure is used for residues in flour above 6.0 ppm without a cleanup. Smaller residues require a cleanup on Florisil. To determine whether the cleanup procedure is necessary, a maximum sample aliquot of 10 g is run through the colorimetric procedure. If no blue color is obtained, or if the color is very light, the cleanup procedure is necessary, and a larger aliquot is taken for analysis. The sensitivity of the procedure after cleanup is 0.2 ppm or 20 μg in flour. Satisfactory recoveries were obtained on all samples analyzed (Table I).

Internal standards must be used for calculations, as the intensity of the blue color varies inversely with sample weights.

The chromatographic procedure can also be used to determine residues in paper and other low-fat commodities. For high-fat commodities, such as shelled nuts, an acetonitrile-pentane cleanup is used previous to the described chromatographic cleanup.

The color reagent must be made fresh daily, as it is hygroscopic.

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