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PP #1F1139. Method of tryout for Karb (RH-315) on lettuce.

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The Rohm and Haas Company's residue met od described in their RAR memorandum No. 581, November 15, 1968 entitled: Determination of RH-315 Residues in Lettuce, Alfalfa and Milk was evaluated in our laboratory using lettuce samples fortified at 1 and 4 ppm.

The petitioner has used a GLC system equipped with an electron capture detector in the pulsed mode. Instead of this, we used a GLC system equipped with electron capture detector in the DC mode--the type available in all FDA laboratories. Since Aerosorb 30 is no longer available, we used Gas Chrom Q-80/100 mesh as the solid support in the column packing.

The results of the trial indicate that the petitioner's method is satisfactory for enforcement purposes. The control values for crop blanks were less than 0.01 ppm apparent Karb. The recoveries from the fortified samples were 80% and 85% at 1 ppm and 87.5% and 92.5% at 4 ppm. No interfering peaks were observed in the blank samples.

Summary of the Method

The chopped lettuce is refluxed with methanol and 18N sulfuric acid for 16 hours.

The reaction product, methyl 3,5-dichlorobenzoate is then steam distilled. The ester is extracted from the steam distillate with petroleum ether and further cleaned-up by passing it through a deactivated Florisil column. Twenty percent benzene in hexane is used to elute the ester from the column. The eluate, without any concentration, is used for the GLC analysis.

Gas Chromatography

The petitioner has used a Victoreen Series 4000 Gas Chromatograph equipped with an electron capture detector (Ni^{63}) in the pulsed mode. Since most of the FDA District Laboratories are equipped with Barber Colman 5000 Series Gas Chromatographs fitted with the electron capture detector (Tritium source) in the D.C. mode, we therefore used this type of equipment for the determinative step.

Our operating parameters were as under:

Column:	6' x 4 mm id glass U-tube packed with 10% OV-17 on 80/100 mesh Gas Chrom Q.
Temperatures:	Column oven, 180°C; injector, 240°C; Detector, 203°C.
Gas Flows:	Nitrogen 60 ml/min
Electrometer Sensitivity:	1×10^{-9} amps full scale into 1 5 mv recorder.
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With these parameters the peak for methyl 3,5-dichlorobenzoate appeared at the retention time of two minutes. The voltage was adjusted to give one half full scale deflection for 1.0 ng of the ester. The response was linear in the tested range of 0.2-1.5 ng of the ester. The volume of the final samples and standards injected into the GLC system was held constant at 5 microliters.

Details of the Trial

Before undertaking the analysis of fortified samples, the elution pattern of methyl 3,5-dichlorobenzoate from the Florisil was checked. We used PR Grade Florisil 60/100 mesh, instead of 100/200 mesh used by the petitioner. The Florisil was activated at 150°C for 24 hours, cooled and then 5% water was added (by weight) and the material was mixed thoroughly and stored in a glass stoppered bottle. The chromatographic column was prepared as described in the petition and 50γ of methyl 3,5-dichlorobenzoate dissolved in 25 mls of petroleum ether was applied to the column. Twenty-five mls of the 20% benzene in hexane eluate was collected and diluted to 250 mls. Five µl of this dilution were injected into the GLC system and compared to known standards. Quantitative recoveries were obtained and the Florisil was thereby considered to be suitable for chromatography.

Love

Lettuce Samples

Duplicate 25 g of chopped samples in round bottom flasks were fortified at 2 and 4 ppm levels of Kerb (dissolved in methanol). The samples were refluxed for ca. 16 hours as required and ca. 75 ml of steam distillate was collected. The distillate was cleaned up as recommended in the method.

Five microliter aliquots from the final 25 ml of 20% benzene in hexane eluate of check samples were readied for injection directly into the GLC system. However, due to the high fortification levels of 2 and 4 ppm, the final fortified samples were diluted 10 fold to 250 ml before the samples could be injected into the GLC system. No interfering peaks were observed in any of the check and fortified samples and the background was low. The peak heights of the fortified samples were measured and the quantity of the ester calculated from a standard curve run concurrently. The factor of 1.25 (the M.W. of Kerb is 258.13; methyl 3,5-dichlorobenzoate, 204) was used to calculate the quantity of Kerb actually present in the sample. The results of the trial were as under:

Recoveries of Kerb from Lettuce

Sample No.	ppm Kerb Added	ppm Kerb Found	% Recovery
1.	none	.01*	--
2.	none	.01*	--
3.	2.0	1.6	80
4.	2.0	1.7	85
5.	4.0	3.3	82.5
6.	4.0	3.7	92.5

* No peak actually found in check samples.

Conclusions

The residue method submitted by Rohm and Haas Company can be used for enforcement by the FDA District Laboratories with the following changes: (a) Any Gas Chromatograph equipped with electron capture detection in the DC mode may be used instead of the Victoreen Series 4000 Gas Chromatograph equipped with electron capture detection in the pulsed mode prescribed by the petitioner.

(b) Gas Chrom Q - 80/100 mesh may be substituted for Aero pack 30.

(c). Florisil PR Grade 60/100 mesh may be used instead of Florisil 100/200 mesh.

(d) The final samples may have to be diluted before injection into the gas chromatograph, if the residue levels are high (e.g., 2 ppm or more).

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