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PP #1F1024. Method tryout for Daconil 2787 and DAC 3701 - Peanuts and Broccoli.

Mr. Bart J. Puma
Analytical Methods Section
Chemistry Branch
Pesticides Tolerances Division

The Diamond Shamrock Corporation's method entitled: "Analytical method for Determination of Daconil 2787 and DAC 3701 Residues" was evaluated in our laboratory on peanuts and broccoli. Since the original method did not include the steps for extraction and cleanup of oily crops, we used the modification submitted by the petitioner on September 14, 1970 for peanuts.

Information submitted by the petitioner indicates that a Dohrmann Gas Chromatograph (Model G-100) equipped with a microcoulometric titration cell (T-100) can be used for the routine analysis of Daconil 2787 and methylated DAC 3701. A Beckman (Model GC-4) gas chromatograph equipped with an electron capture detector could be used as well. Previously we had conducted a successful tryout with Daconil 2787 on potatoes and cabbage (PP #7F0599) using a Barber Colman Model 5000 Gas Chromatograph equipped with an electron capture detector. Our previous experience had shown that Daconil 2787 could be determined with a very high degree of sensitivity by means of an electron capture detector.

When 100 grams peanut (nutmeat) samples were carried through the extraction and cleanup procedure submitted by the petitioner, we noted that ca. 0.4-0.5 ml of oil was left in the final sample for quantitation of Daconil 2787. If we had followed the instructions given by the petitioner under "Gas Chromatography of Dacnnil 2787 and Methylated DAC 3701" on page vi of the method, at the fortification level of 0.3 ppm, with the sample size of 100 g of peanuts, the final volume should be made to 0.3 ml.

and this is less than the quantity of oil actually found in the sample. In other words we would be injecting oil in the GLC system fitted with a Dohrmann Microcoulometric titration cell and fouling the whole system.

Due to the high degree of sensitivity of both the compounds to the electron capture detector, the final volumes of the crop samples are very high--50 to 300 ml in the case of peanuts and 100 to 2500 ml in the case of broccoli. Only 5 microliter aliquot injections of both the crops are needed to give us a peak of ca. 60 to 80% full scale for both compounds.

Some modifications was also made in the extraction step of peanuts to simplify the filtration step. Recoveries of (a) DAC 2787 from the fortified samples of peanuts ranged from 83 to 98% and from samples of broccoli ranged from 88 to 102%. (b) DAC 3701 from fortified samples of peanuts ranged from 90 to 110% and from samples of broccoli from 80-100%. The apparent residue values for the crop blanks were <0.02 ppm.

The method is satisfactory for determining residues of DAC 2787 and DAC 3701 at the levels indicated in peanuts and broccoli.

Details of Trial

Gas Chromatography: We used a Barber Colman Model 5000 GLC system under PAM I Section 311.2 conditions with the following parameters:

Column:	glass 6' x 4.5 mm id packed with 10% DC-200 on Chromosorb W/HP 80/100 mesh.
Temperatures:	
column:	200°C
Injector:	230°C
Nitrogen Flow:	120 ml/min
Detector:	Tritium source, concentric type, operated in electron capture mode.
Detector voltage:	DC voltage at which 1 ng of heptachlor epoxide causes 50% full scale deflection at 1×10^{-9} AFS, Attn. 1.
Recorder:	5 mv
Electrometer sensitivity:	1×10^{-9} AFS, Attenuator 1.

At these parameters:

0.77 ng Daconil 2787 gives 50% FSD at RRT_A of 0.52
0.66 ng DAC 4824 (equivalent to 0.625 ng of DAC 3701) gives 50% FSD at RRT_A of 0.58.

A plot of peak heights vs. nanograms of Daconil 2787 and DAC 4824 was linear in the tested range of 0.20 to 1.0 ng.

Methylation of DAC 3701

The petitioner had supplied us with small quantities of pure standards labeled (a) DAC 3701 - the metabolite of Daconil 2787; and (b) DAC 4824 which is the methyl ester of DAC 3701.

A standard solution containing 50γ/ml of DAC 3701 in ethyl ether was prepared. One ml aliquots containing 50γ were pipetted into volumetric flasks, the solvent blown off gently and several attempts were made to methylate the residue with 2 ml of diazomethane prepared as outlined in the method on page "iii". The ethyl ether was blown off with gentle stream of dry air and the final volume made to 50 ml with benzene. 5λ aliquots were injected into the GLC system and compared with an equivalent quantity of DAC 4824. (Based on molecular weights). However, we did not observe a peak at the retention time of DAC 4824 with the methylated compound. We then tried a new batch of diazomethane, added a drop of HCl and ethanol as catalysts, but did not succeed in our attempts. The melting point of the standard labelled DAC 3701 provided by the petitioner was determined and found to be 240°C. Inasmuch as the petitioner informed us that the melting point for DAC 3701 should have been 262°C he supplied us with a new standard of DAC 3701. The melting point and infra-red curves, were obtained for the new standard of DAC 3701 and matched and specifications given to us by the petitioner. It was clear that the original standard of DAC 3701 supplied to us by the petitioner on September 14, 1970 was incorrect.

5.0 mgm of the new standard of DAC 3701 was dissolved in 100 mls of 1:1 mixture of acetone and methylene chloride. 1 ml aliquots equivalent to 50 γ were methylated with 2 ml of diazomethane, the methyl ether was blown off and the residue dissolved in 50 mls of benzene. The methylated product was compared to a known standard of DAC 4824 and we noted that the methylation was quantitative with this new standard of DAC 3701 (molecular weight of DAC 3701 - 247.5, molecular weight of DAC 4824 - 261.5). In this way, we ascertained that the new standard was the correct one.

Testing of Florisil

490 g of Florisil, PR grade 60/80 mesh (LA #88) as supplied by the Floridin Company was mixed with 10 ml of distilled water as directed. After equilibration overnight, the elution pattern of both standards through de-activated Florisil was checked. We noted that it was most important that the standards be dissolved in 10 ml of ethyl ether, mixed with 3 g Florisil and then the ether blown off. The column should be prepared in the manner indicated under the heading "Column Chromatographic Cleanup" on page v of the method. Under no circumstances should DAC 3701 be transferred over the layer of sodium sulfate, as this seems to have an adverse affect on the recovery of DAC 3701. The 5% and 50% acetone in methylene chloride eluates were collected, concentrated and injected into the GLC system (DAC 3701 fraction was methylated prior to injection). The recovery was quantitative in both the fractions.

Reagent Blanks

All the reagents and solvents (all B/and J-distilled-in-glass except isopropyl ether) used in the extraction and cleanup procedures for peanuts were first taken through the entire procedure. No interfering peaks were found in both the fractions at the elution time of DAC 2787 and DAC 4824.

Analysis of Peanuts

A large batch of peanuts (unroasted nutmeats) were ground in a Waring Blendor, 100 g samples were fortified with 0.3 and 0.6 ppm of each compound in a mixture, then extracted with 600 mls of acetonitrile. The macerate was transferred to a two-quart jar with 300 mls of hexane and skaken for 2 hours as outlined in the modification submitted by the petitioner on September 14, 1970. The filtration was very slow and most of the hexane was lost in this step. The filtrate was transferred to a separatory funnel, along with hexane washings as directed and shaken for 2 minutes. An emulsion was formed which was very slow to break.

At this stage we modified the petitioner's extraction procedure as under:

(a) 50 g of nutmeats were extracted twice with 150 ml portions of acetonitrile in a Waring Blendor and the supernatant liquid

was filtered through a Buchner funnel after each extraction.

(b) The residue was further extracted with 150 mls of hexane and filtered through the Buchner funnel. The filter cake was broken a few times with a spatula to expedite filtration.

(c) The blendor was washed with 2 x 50 ml portions of hexane which also were filtered through the Buchner funnel.

(d) The combined filtrates were transferred to a separatory funnel and the filter flask was rinsed with small portions of hexane and combined with the filtrate in the separatory funnel.

(e) The separatory funnel was shaken for 2 minutes and the layers were allowed to separate. The acetonitrile layer was drained into a round bottom flask and concentrated to an oily residue on a "Calab" rotary evaporator at 50°C, using house vacuum.

(f) The oily=residue was dissolved in 10 mls of ethyl ether, 3 g of deactivated Florisil was added and mixed thoroughly while evaporating the ether to complete dryness under a stream of air.

(g) The rest of the clean-up procedure is the same as described under "Column Chromatographic Cleanup" on page v of the method.

We noted that most of the oil came along with the eluate of 5% acetone.in methylene chloride. The quantity of oil was between 0.2 and 0.25 ml in the 5% acetone in methylene chloride fraction and ca. 0.025 to 0.05 ml in the 50% acetone in methylene chloride fraction (the fraction containing the metabolite DAC 3701). On extraction with isopropyl ether the oil and the pigments were taken up in the isopropyl ether layer. No difficulty was experienced in the methylation step.

Both the fractions were diluted in benzene as tabulated below and 5λ aliquots injected into the GLC system equipped with electron capture detector. All the injections of samples and standards were kept constant at 5λ.

Sample No.	Fortification Level	Volume of Benzene used for dilution	Quantity of crop injected in 5λ aliquots
1 and 2	none	50 ml	5 mg
3 and 4	0.3 ppm	150 ml	1.665 mg
5 and 6	0.6 ppm	300 ml	0.833 mg

The residue in ppm and percent recoveries from the fortified crops samples were calculated on the basis of peak heights found in the sample as compared to the peak heights of standards analysed concurrently. Both the check samples did not show peaks at the retention times of Daconil 2787 or DAC 4824 (methylated DAC 3701).

The recoveries are as under:

Fortification Level ppm (of each compound)	Daconil 2787 found (ppm)	Daconil 2787 %Recovery	DAC 3701 found (ppm)	DAC 3701 % Recovery
1. zero	<0.02*	--	<0.02*	--
2. zero	<0.02*	--	<0.02*	--
3. 0.30	0.26	87	0.27	90
4. 0.30	0.28	98	0.30	100
5- 0.60	0.52	87	0.66	110
6. 0.60	0.50	83	0.57	95

NOTE: *The sensitivity of the method is estimated at <0.02 ppm based on response of less than 5% FSD.

Broccoli

A bunch of broccoli was chopped in a Hobart Chopper and 100 g samples were fortified at 2.5 and 5.0 ppm with each compound (in a mixture) and extracted with 250 mls of acetone plus 5 ml of dilute sulfuric acid (1:1). We followed the extraction and cleanup procedure submitted by the petitioner under the heading "Extraction of Crops" and "Column Chromatographic Cleanup" with the following minor changes:

(1) We used a "Calab" rotary evaporator fitted to a dry ice trap and attached to a house vacuum with a water bath temperature at 40 to 45°C to remove acetone. Under these conditions, acetone did not boil. When water vapor was seen in the trap, the flasks were disconnected and the samples carried through the cleanup procedure.

No emulsion difficulty was experienced during clean-up. The samples were passed through the chromatographic columns as directed and 5% and 50% acetone in methylene chloride fractions were collected. Both the fractions had a considerable amount of color which persisted when diluted with benzene as tabulated below and injected into the GLC system equipped with an electron capture detector. All the injections of samples and standards were kept constant at 5λ.

Sample No.	Fortification level m	Volume of Benzene used for dilution	Qty. of Crop Injected in 5λ Aliquots.
1 and 2	none	100 ml	5 mgm
3 and 4	2.5 ppm	1250 ml	0.4 mgm
5 and 6	5.0 ppm	2500 ml	0.2 mgm

The residue in ppm and percent recovery from fortified crop samples were calculated on the basis of peak heights found for the sample as compared to peak heights for standards analyzed concurrently. Both the check samples did not show peaks at the retention time of Daconil 2787 or DAC 4824 (methylated DAC 3701).

The recoveries are as under:

Broccoli

Fortification Level (ppm of each compound)	Daconil 2787 found (ppm)	Daconil 2787 % Recovery	DAC 3701 Found(ppm)	DAC 3701 %Recovery
1. zero	<0.02*	---	<0.02*	---
2. zero	<0.02*	---	<0.02*	---
3. 2.5	2.2	88	2.0	80
4. 2.5	2.5	100	2.5	100
5. 5.0	5.1	102	4.6	92
6. 5.0	4.7	94	4.0	80

*The sensitivity of the method is estimated at <0.02 ppm based on response of less than 5% FSD.

Comments

1. We think that the modified extraction and cleanup procedure submitted by the petitioner on September 14, for Daconil 2787 and DAC 3701 for peanuts is different than what was actually used by the petitioner for gathering data. No chromatograms and raw data were submitted by the petitioner to support his results. If the residue method is run on peanuts as described in the petitioner's method, then:
(a) the filtration of acetonitrile plus hexane extract is very slow and most of the hexane is lost during filtration and the emulsion takes a long time to break.
(b) ca. 0.4 to 0.5 ml of oil are left in the final sample to be injected into the GLC system for quantitation of Daconil 2787. The volume of residual oil is greater than the final volume of the sample recommended by the petitioner. We do not think it possible to detect residues in samples cleaned up in this manner using the older Dohrmann Gas Chromatograph (Model G-100) equipped with the microcoulometric titration cell (T-100).

2. The petitioner's method for extraction of peanuts was modified by us to simplify the filtration and acetonitrile vs. hexane partitioning steps. The quantity of oil in the final sample varies from 0.2 to 0.25 ml (for a 50 g peanut sample). The latest model of the Dohrmann microcoulometric system is ten times more sensitive than the older model; thus the final sample can be dissolved in a larger quantity of benzene. Therefore, a smaller quantity of accompanying crop (or oil) will be injected into the GLC system. We have found that the quantity of oil in the final sample can be further reduced to ca. 0.02 ml by including a backwash step with hexane during the hexane vs. acetonitrile partitioning step. If this step is to be included, then the method should be verified further by running more recoveries with samples of peanuts.

3. Due to the very high sensitivity to Daconil 2787 and DAC 4824 (methyl ester of DAC 3701), of the electron capture detector, the final peanut and broccoli samples can be diluted to very large volume, thus reducing the quantity of crop extract to be injected into the GLC system. We are of the opinion that GLC system equipped with the electron capture detector should be the method of choice over the Dohrmann microcoulometric system for these two compounds. The only advantage of the Dohrmann microcoulometric system is that

it is specific for chlorinated pesticides. The electron capture detector is not so specific.

4. The elution pattern of Daconil 2787 and DAC 3701 through the Florisil column and methylation of DAC 3701 should be checked.

5. The 10% DC-200 on Chromosorb W/HP/80/100 mesh column packing commonly available in all Food and Drug laboratories can be used instead of the two column packings recommended by the petitioner.

Gobind P. Makhijani
Analytical Methods Section
Chemistry Branch
Pesticides Tolerances Division
Approved: Bart J. Puma
cc:
Tox. Br.
Chem. Br.
RO-130
MRO File
Mr. Puma
Mr. Beusch
PP#1F1024

Gobind P. Makijani:mae
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RD/init:BJPuma