PP#2F1230

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PP #2F1230. Method tryout for DAC 3701 in meat and milk.

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The Diamond Shamrock Company's methods entitled: "Daconil, 4-hydroxy metabolite (DAC 3701) in meat" and "Daconil, 4-hydroxy metabolite (DAC 3701) in milk" were tried on beef kidney and milk fortified with 0.2 ppm and 0.4 ppm DAC 3701 respectively. DAC 3701 (2,5,6-trichloro-4-hydroxy isophthalonitrile) is a metabolite of Daconil (2,4,5,6-tetrachloroisophthalonitrile).

Our results indicate that the method is satisfactory for determining DAC 3701 in kidney and milk at these levels. Apparent DAC 3701 in the crop blanks was estimated at less than 0.01 ppm. Recoveries were 65-70% and 70-72% for kidney; 65-68% and 61-76% for milk at 0.2 and 0.4 ppm levels respectively. Crop blanks and recoveries were determined in duplicate. Petitioner's methods used Dohrmann microcoulometric titration cell (T-100) for the determinative step. We used an electron-capture detector which is more sensitive than T-100 cell. One ng DAC 3701 gave 50% FSD response and eluted in three minutes (i.e. 0.59 relative to Aldrin) under PAM I ECGLC operating conditions.

Details of the Method for Milk

a. Extraction of Milk Samples

Mix 400 gm milk, 20 ml of 10% potassium oxalate and 38 gm of anhydrous $MgSO_4$ with 150 ml of ethanol in a two-liter separatory funnel. Add 10 ml of conc. H_2SO_4 and 1200 ml ether to the sample. Shake five minutes with a gentle, rolling motion. Wash the ether with 800 ml of distilled water. Evaporate the solvent on a Rinco rotary evaporator.

We modified this extraction step as described below for the remainder of our study, and we recovered 60-68% of the added DAC 3701. Following this extraction step, there was much water present in the ether extract and method does not tell us what to do about this extracted water. We contacted the petitioner and he suggested extracting the acidified aqueous layer with isopropyl ether, then evaporate the isopropyl ether to dryness on the Rinco rotary evaporator. However we found this to be unnecessary when we extracted only 40 gm milk and used 1/10 amount of reagents used in the extraction. The sample had only small amounts of water and when it was evaporated to dryness without going through the partition as suggested by the petitioner, recovery was comparable to that of the first set of values.

b. Clean-up of Milk Extract

Transfer the sample with 60 ml hexane, then 30 ml acetonitrile to a 125 ml separatory funnel. Shake for three minutes. Collect the acetonitrile in a beaker and evaporate to dryness in a well-ventilated hood. Transfer the residue in the beaker to a 125 ml separatory funnel, using three 20 ml hexane and two 20 ml 5% NaOH rinses. Shake gently for three minutes, collect the aqueous phase in a 60 ml separatory funnel. Acidify with 5 ml of 18N sulfuric acid, then extract the sample with 15 ml of isopropyl ether. Remove the ether to a scrupulously clean flask and evaporate to dryness with a gentle flow of nitrogen. Add 1-2 ml of diazomethane solution to convert DAC 3701 to the methyl ether derivative. Dilute the sample to appropriate volume for the gas chromatograph determinative step.

c. Gas Chromatography

A Barber-Colman Model 8000 gas chromatograph equipped with an electron capture detector was used for the method trial. The method specified a Dohrmann Model G-100 chromatograph equipped with a Model T-100 microcoulometric titration cell. Petitioner used a 6' x 1/4" aluminum column, packed with 30/60 mesh acid-washed Chromosorb P containing 20% DC-200. We used a 10% DC 200 column and operated our instrument as prescribed in PAM 1.

d. Results

Milk (40 gm sample)

	DAC 3701 added (ppm)	DAC 3701 found (ppm)	DAC 3701 % recovered
1	---	<0.01	--
2	---	<0.01	--
3	0.2	0.13	65
4	0.2	0.14	70
5	0.4	0.244	61
6	0.4	0.304	76

Details of Method for Beef Kidney

(a) Extraction of Tissue Samples;

Extract 200 gm ground kidney with 800 ml chilled acetone in a Waring Blender. Filter through a Buchner funnel. Transfer the filter cake and paper to the blender and reblend for three minutes with a mixture consisting of 200 ml of acetone, 200 ml of dichloromethane. Filter and combine theffiltrates in a round bottom flask. Remove all the organic solvent on a Rinco evaporator.

(b) Clean-up of Tissue Extract;

Add 90 ml of saturated sodium chloride solution to the concentrated extract and transfer to a clean 500 ml separatory funnel. Rinse with 30 ml 18N sulfuric acid and 400 ml ethyl ether, and transfer both rinses to a separatory funnel. Mix well. Reextract the acid layer with 100 ml of diethyl ether, then discard the aqueous phase. Combine the ether extracts and wash with 30 ml of saturated salt solution. Concentrate to dryness on a Rinco evaporator.

(c) Hexane-Acetonitrile Partitioning;

Transfer the extract to a clean one-liter separatory funnel with successive rinses of 400 ml hexane, 60 ml acetonitrile, 400 ml hexane and 60 ml acetonitrile. Shake well. Collect acetonitrile layer in a 500 ml separatory funnel and reextract the hexane with 60 ml of acetonitrile. Combine the acetonitrile extracts and evaporate to dryness in a round bottom flask.

(d) Liquid Column Chromatography;

Pack a five inch 18 mm. I.D. chromatographic column with Florisil containing 2% water. Prewash the column with 50 ml dichloromethane. Transfer the extract to the column with a total rinse of 30 ml dichloromethane. Elute the column first with 50 ml of 5% acetone in dichloromethane, then elute the DAC 3701 from the column with 100 ml of 50% acetone-dichloromethane solution. Evaporate the eluate to dryness. Transfer the residue to a 60 ml separatory funnel using two 10 ml portions of isopropyl ether, 2 ml of 1:1 sulfuric acid and 10 ml water. Shake well, then discard the bottom layer; pour the isopropyl ether extract out of the top of the separatory funnel into a clean 25 ml Erlenmeyer flask with care so as not to transfer droplets of the acid into the flask. Evaporate the isopropyl ether extract to dryness. Add 20 ml of diazomethane to convert DAC 3701 to its methyl ether derivative for the GLC determinative step.

(e) Gas Chromatography: As with milk (above)

(f) Results

We followed the steps with no major changes and encountered no difficulties with tissue samples. We pre-tested our Florisil column with 40γ DAC 3701 and recovered 86%. Our recovery data are not corrected by this column efficiency.

Beef Kidney

	DAC 3701 added (ppm)	DAC 3701 found (ppm)	DAC 3701 % recovered
1	---	<0.01	--
2	---	<0.01	--
3	0.2	0.14	70
4	0.4	0.13	65
5	0.4	0.28	70
6	0.4	0.288	72

Comments

1. Both methods are suitable to detect DAC 3701 in meat (beef kidney) and milk at the 0.2 and 0.4 ppm levels.

2. We only need inject 2-4 mg sample into our ECGLC system. It is reasonable to assume that the method is sensitive enough to detect lower levels of DAC 3701, if necessary.

3. We would suggest extracting only 40gm of milk sample instead of 400 gm. This smaller sample size is still adequate and enables the analyst to perform the analysis more easily.

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