centage lipid in beef-soy blends in relation to amount added. One purpose of this study was to determine if presence of soy in ground beef would influence percentage lipids as determined by different methods. Generally it was not, in that no significant interaction was found between method of lipid analysis and percentage of soy.

Precision of Methods. Variances of values for the four methods of lipid analysis for raw and cooked ground beef and beef-soy blends are presented in Table IV. For raw meat, thermal extraction was more precise than was the methanol-chloroform-water or the ether extraction method, but it was not significantly more precise than the acid predigestion with ether extraction method. Perhaps the reason for low variance with the thermal extraction method is the insensitive reading scale (values were read only to the nearest quarter of a percent). When the thermal extraction method was eliminated from the comparisons, the other three methods used for raw meat had essentially the same precision. For cooked meat, values varied more for the acid predigestion with ether extraction and the chloroform-methanol-water extraction methods than for ether extraction.

CONCLUSIONS

Before selecting a lipid extraction method, three factors to consider are the values obtained, the variance of the values, and suitability of the method for laboratory conditions. Because it obtains values that vary little and that are only slightly lower than those of methanol-chloroform-water extraction, we recommend ether extraction. That method requires a longer extraction time, but less laboratory handling time, than do the other methods; it is suitable for a laboratory that does not require immediate results. Thermal extraction, which requires only 15 min per sample, is useful in quality control of beef-soy blend products.

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Received for review September 16, 1974. Accepted January 10, 1975. Contribution No. 311 from Kansas Agricultural Experiment Station.

Residue Determination of Thompson-Hayward 6040 in Bovine Manure by High Performance Liquid Chromatography

Residues of TH 6040, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea, were determined in bovine manure at levels between 2.0 and 0.5 ppm. Samples were cleaned up by liquid-liquid partition and elution through a Florisil column. Analysis was performed with reverse-phase high performance liquid chromatography.

One of the new "insect growth regulator" insecticides is Thompson-Hayward 6040, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea. TH 6040 inhibits the synthesis and deposition of cuticle during the molting process as insects mature through immature stages to become adults (Mulder and Gijswijt, 1973). When this compound is applied to larval growth media, it prevents emergence of adults of the house fly, *Musca domestica* L. (Wellinga et al., 1973), and the stable fly, *Stomoxys calcitrans* (L.) (Wright, 1975). In both cases the adult fly is the pest, especially in the case of the stable fly as both sexes are blood-sucking and feed upon livestock and man.

Since conditions in cattle feedlots are ideal for the production of stable flies (the large amounts of manure offer an excellent environment for larvae, and cattle are available for adult feeding), an insecticide such as TH 6040 that would kill the nonpestiferous immature stages in manure would be desirable (Wright, 1975). However, before a compound such as TH 6040 can be used as a control agent, its residual properties must be investigated. No suitable methods have been available for quantifying TH 6040 at low parts per million levels since TH 6040 is refractory to GLC analysis and methods employing hydrolysis followed by derivatization have not proved satisfactory (Oehler, 1973). We therefore attempted to develop a method for determining residues of TH 6040 using high performance liquid chromatography (HPLC) to quantify TH 6040 extracted from bovine manure. The results of these investigations are reported here.

MATERIALS AND METHODS

Fresh samples of bovine manure were frozen immediately after collection and stored at −18°. Fortified samples were prepared by adding the appropriate amount of a dichloromethane solution of TH 6040 to 20 g of manure to produce TH 6040 levels of 0.5, 1.0, and 2.0 ppm. The solvent was removed with a stream of dry nitrogen.

The samples were homogenized in 75 ml of acetonitrile for 2 min with a Polytron Model PT-10 homogenizer. Insolubles were removed by filtration through a 150-ml coarse fritted glass funnel. The homogenizer was rinsed three times with 50-ml aliquots of acetonitrile, and the rinses were filtered and pooled with the initial filtrate. After evaporation of the sample to dryness with a rotary evaporator at 50°, the residue was taken up in 50 ml of acetonitrile and partitioned twice against 200 ml of hex-

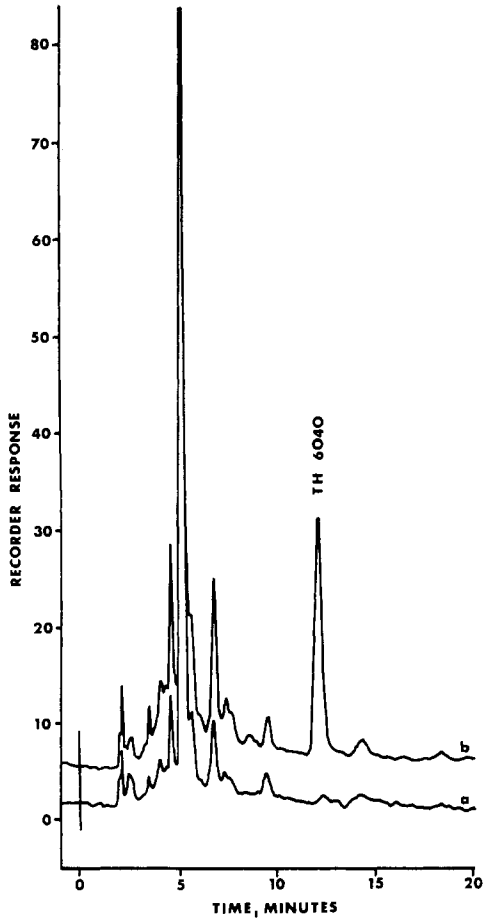

Figure 1. High-performance liquid chromatograms of (a) an extract of bovine manure and (b) an extract of manure containing 0.5 ppm of TH 6040. The LC conditions are given in the text.

ane. The hexane was subsequently back-extracted with two 50-ml aliquots of acetonitrile, and the combined acetonitrile fractions were evaporated to dryness.

Florisil columns were prepared by adding (dry), in order, 5 g of Na_2SO_4, 20 g of Florisil, and 5 g of Na_2SO_4 to a 2.0 × 30 cm glass column equipped with a fritted glass disk. The sample was transferred to the column with a total of 50 ml of a solution of dichloromethane–hexane (1:1). The TH 6040 was eluted from the column with 150 ml of dichloromethane, which was evaporated (rotary evaporator, 30°). The residue was dissolved in a known volume of acetonitrile, 1 ml or more, and held for subsequent analysis.

Samples were analyzed on a Waters ALC-100 liquid chromatograph equipped with a Model 6000 high pressure pump and a UV_{254} detector. A 6 mm × 30 cm μ-Bondapak C-18 "reverse phase" column was utilized with a solvent system composed of acetonitrile–water (57:43). The flow rate was 1.5 ml/min, and the range setting on the detector was 0.08 absorbance unit full scale (AUFS). Twenty-microliter aliquots of prepared manure samples and standard acetonitrile solutions of TH 6040 were injected. Quantification of TH 6040 in the manure samples was accomplished by comparison of peak heights with those for known amounts of TH 6040.

RESULTS AND DISCUSSION

The HPLC analytical system that we employed is based on reverse-phase partition chromatography instead of the more common normal-phase adsorption chromatography utilizing silicic acid. Reverse-phase packings are compatible with an extremely wide range of solvents including such protonating solvents as water. Activated silicic acid adsorbents cannot be utilized with protonating solvents because exposure to these solvents results in a loss of adsorption that decreases separation efficiency. Also, polar compounds are often adsorbed strongly to silicic acid, and polar solvents are required to elute these compounds. However, with reverse-phase systems, the compounds with high polarity are eluted first, and less polar compounds are retained longer.

Since our extraction system removed polar compounds along with TH 6040, the reverse-phase system seemed to be the logical choice. This indeed was the case. Virtually all the 254-nm absorbing compounds extracted from manure by our system eluted before TH 6040 (Figure 1), which appeared 12 min after injection. A small peak present in all manure extracts eluted 20 sec after TH 6040 (Figure 1), but interference of this material with the quantitation of TH 6040 was small because it never amounted to more than 1.5% deflection of full scale.

Two additional small peaks were present at 14 and 18 min, respectively. Following these peaks the base line stabilized completely, and injections could be made at 25-min intervals. Even when the samples were concentrated to 1 ml, the extraneous compounds did not interfere with quantitation of TH 6040 (Figure 1).

Samples of manure containing 0.5, 1.0, and 2.0 ppm of TH 6040 were extracted, purified, and analyzed by HPLC (each treatment was replicated twice). The percentage recovery averaged 97% (range 92–106%). Based upon the recorder response (25% full-scale deflection for 200 ng) and the minimal background interference, TH 6040 residues of somewhat less than 0.5 ppm could be quantified in this system; 0.25 ppm (100 ng) would produce a 13% full-scale deflection (the detector response is linear).

Although high-performance liquid chromatography is not nearly as sensitive as some GLC analyses, it can provide valuable data on residues of compounds unsuitable for GLC analysis. However, detection systems that are sensitive and reasonably specific must be chosen (uv or visible absorption, fluorescence), and suitable extraction and clean-up procedures must be developed so that injections representing relatively large amounts of the initial sample can be analyzed.

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Received for review August 23, 1974. Accepted January 31, 1975. Mention of a pesticide or a commercial or proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the USDA.