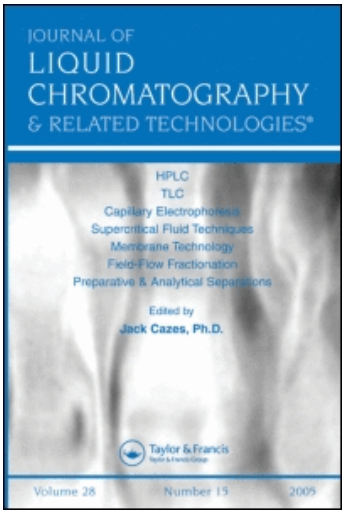

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CLEANUP OF SAMPLES FOR HIGH PERFORMANCE CHROMATOGRAPHY AND INSTRUMENTAL ANALYSIS

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ABSTRACT

Cleanup methods have been developed which allow utilization of modern chromatographic techniques and instrumental readouts. A cellulose cleanup for organophosphorus pesticides and carbamates has already been reported. A particulate carbon column has been described for Rumensin. These columns maybe combined to improve the cleanup for organophosphorus and carbamate pesticides. This same column cleanup maybe used for Ochratoxin. Powder charcoal maybe used for the mycotoxins aflatoxin, and zearalenone. It maybe used for T-2 toxin and trichothecenes with some loss (50-75% recovery).

The thin layer plates themselves provide a background reference for spectroscopy. This background maybe removed by "annealing" the plates at 400°C for 2 hours.

Sweepco distillation maybe used to cleanup biological samples so that carbamates and organophosphorus pesticides maybe analyzed by thin layer chromatography. Trichothecenes mycotoxins maybe cleanuped with about 40% loss.

Modern toxicology requires analysis, both quantitative and qualitative, at the submicro level of concentration in complex biological media.

The analysis of complex matrices for toxic compounds requires sample preparation before the system, which can be used for chemically clean samples such as analytical standards, may be used.

If quantitative Thin Layer Chromatography (TLC) and/or Instrumental Confirmation GC-Mass Spectroscopy, fluorescence spectroscopy are to be done routinely, the samples which are used must be sufficiently clean to (1) allow detection of substances without interference of the analyte matrix, and (2) to allow analysis without severely impairing continued use of the instruments and equipment.

This paper will discuss means of greatly improving the matrix "junk" to analyte ratio without causing destruction of the analyte.

The first method will deal with the use of carbon to remove pigments and absorbable substances, the second a volatalization with a Sweepco Distillation Unit and the third is a means to remove "background" materials from thin layer plates used for analytical separations.

Methods using a carbon cleanup by Storherr[1], Getz[2], et al, Stahr[3], et al; have been proposed to cleanup pesticide extracts (1 & 2) and (3) plant tissue extracts for the analysis of alkaloids, nitrates and sugars. These methods all were successfully applied to the appropriate need.

EXPERIMENTAL/REAGENTS & APPARATUS

Nanograde® hexane, petroleum ether, methanol, chloroform, and ethyl acetate were obtained from Mallinkrodt or the equivalent quality was made by distillation or absorption. Trichloromethyl silane (TCMS) was obtained from Applied Science Laboratories. A mixture of 80% petroleum ether, 20% TCMS was prepared for deactivation.

Thin layer chromatography plates (E. Merck) were obtained from Brinkman Instruments or equivalent pour plates were made with E. Merck Silica gel G. Whatman Whatman Silica Gel Multi K, and reverse phase TLC plates were also used.

A Kontes Sweepco distillation apparatus was used. It was modified as discussed below.

A Finnigan 4000 gas chromatograph mass spectrometer was used for mass spectra.

A self cleaning Admiral range was used to clean apparatus - glass and TLC plates.

Darco G-60 powdered carbon, Barneby Chaney #72462 carbon. Whatman CF-4 cellulose was vacuum drained, and stores in a dessicator until used.

Anhydrous, crystalline, Na_2So_4-was obtained from Mallinkrodt Chemical Co.

Carbons used in methods 1 & 2 are no longer available, and alternative carbons were discussed by Carson[4]. Obioha and Stahr[5] reported that the analysis of aflatoxin sample extracts could be greatly improved by carbon treatments, and the procedure was extended to other mycotoxins and pesticides by Stahr[6], as well as to Rumensin[7].

EXPERIMENTAL/METHODS

1.) Sample Cleanup

After preliminary preparation of the sample, extracting, defatting, and solvent partitioning, the sample may be treated with Darco G60 decolorizing carbon (0.100 g/100 ml of CHCl solvent). The carbon is filtered off and the sample concentrated for further instrumental analytical techniques.

In all cases, the sample is ammended with the material of interest and this serves as a check on possible loss. It is often possible to add more carbon;

in fact, it may be necessary to sufficiently remove the colored "junk" which occurs with the sample.

The "Rumensin cleanup"[7], involved the use of a particulate carbon. This has the advantage of preventing the distribution of carbon beyond the point of use. The carbon column (as shown in Figure 1), 2 cm in diameter, is packed with a volume of 40 ml with Barneby Chaney particulate carbon and air bubbles are recovered with petroleum ether and a rubber suction bulb. One-half inch of sand is placed on the glass frit on the bottom of the column and one inch of sand is placed on top of the carbon to "anchor" it in a band. Ten grams of crystalline, anhydrous sodium sulfate is added to top the column. The extract is placed on top of the column in 1 ml of chloroform. One hundred milliliters of pet ether are added and allowed to elute through the column, then 100 ml of chloroform are added to the column and this eluent is saved and concentrated to near dryness. The volumne is adjusted to a measured volume and thin layer chromatography, GLC or other techniques are done.

Sweepco Cleanup-the Sweepco Unit is Shown in Figure 2.

A cleanup procedure first developed for cleaning up fats and pesticides may be extended to organophosphorus pesticides, carbamates and scirpene mycotoxins. The column was modified to contain a sand layer, glass bead layer (80% of tube), and a final sand layer, all supported by glass wool plugs (Figure 3).

The sample extract which is the usual equivalent of a PAM® pesticide preparation, may still have a matrix residue. A flow of 700 ml/minute of dry nitrogen is used with the distillation unit at 150°C temperature for organophosphorus and carbamate pesticides other than (DDVP) "Dichlorovos". Cleanup of Dichlorovos is done at 100°C with 500 ml/minute of nitrogen flow. Scir-

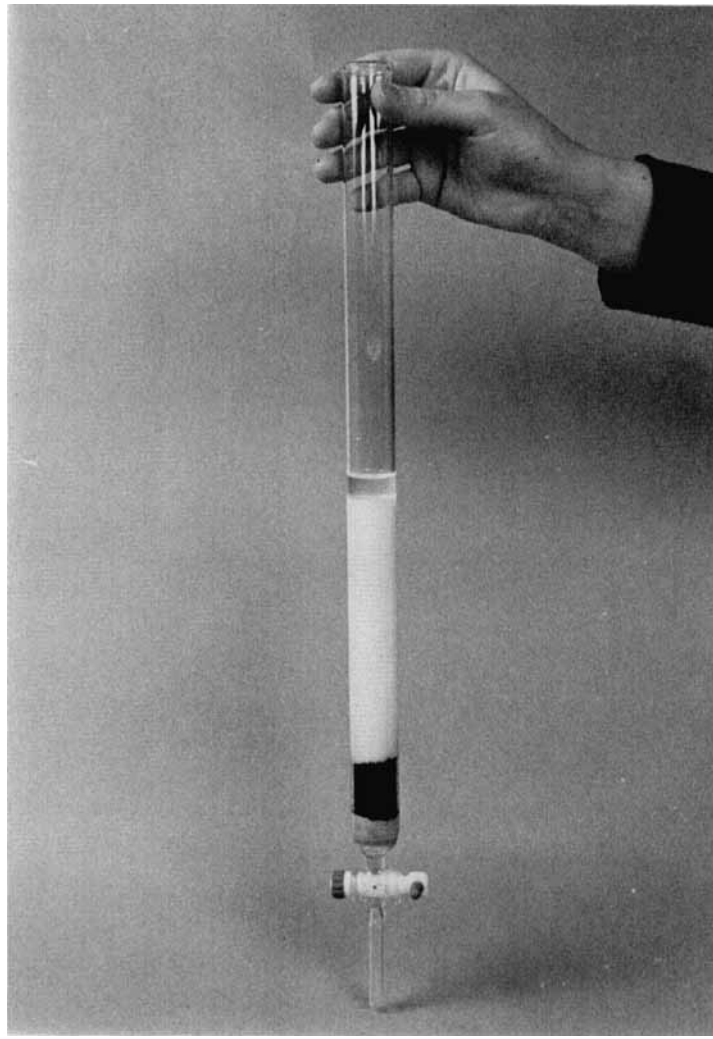

FIGURE ONE

Carbon Cellulose Column

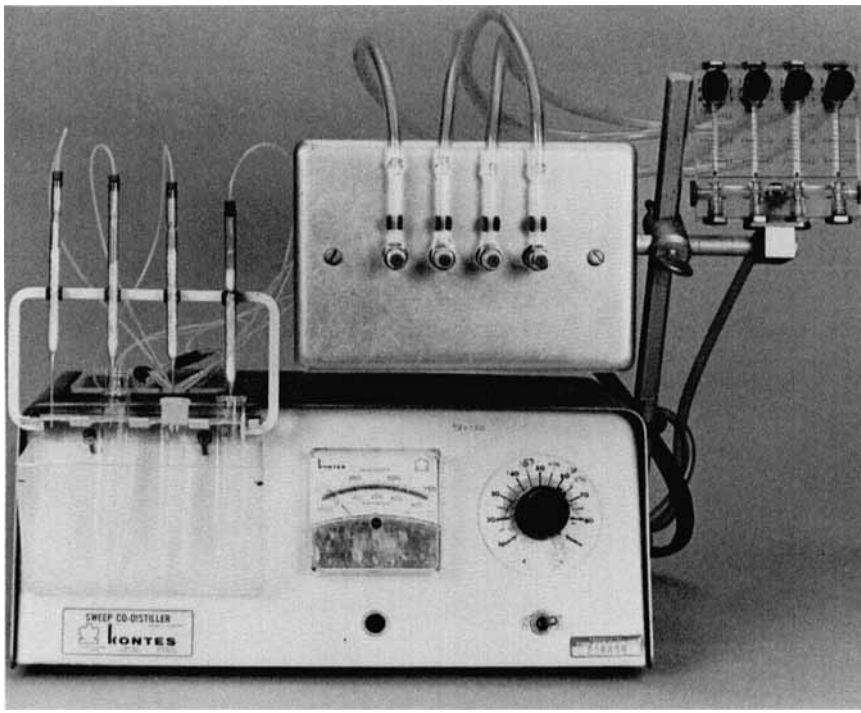

FIGURE TWO

Sweepco Distillation Unit

pene mycotoxins are analyzed like organophosphorus pesticides. A temperature of 210°C-220°C is used for T-2 toxin.

The extract is injected on the column in 1 ml or less volume and allowed to elute into the Teflon® loop condenser and collection tube, maintaining a fluid seal in the Teflon® tube. "Rinses" of 1 ml (total 10 ml) portions of petroleum ether are added at intervals to elute the sample and maintain a fluid seal for fifteen minutes.

FIGURE THREE

Sweepco Distillation Column

Between samples, the glass column is annealed to reduce any "memory" and silanized with 2 injections of 1 ml trichloromethylsilane at operating temperature and condition with 2-2 ml injections of pet ether, before the next sample is injected.

Figure 4 shows the recoveries with silanization of the Sweepco distillation unit. Figure 5 shows the typical concentration curve obtained by analyzing Furadan^{R} directly on TLC after the Sweepco^{R} cleanup.

2.) Thin Layer Plate Cleaning

Samples which have been separated in bands on thin layer plates may still be too contaminated to run by the

Carbofuran Recovery

from Sweep Co-Distillation

Oven Temp	Column Condition	Sample Matrix	Recovery Percent
160°	unsil	--	40 → 80
160°	sil	--	95 → 100
140°	unsil	--	80 → 85
140°	sil	--	95 → 100
140°	sil	Feed ext.	∿90
120°	sil	--	85 → 95

FIGURE FOUR

CarboFuran Recovery
from Sweepco Distillation

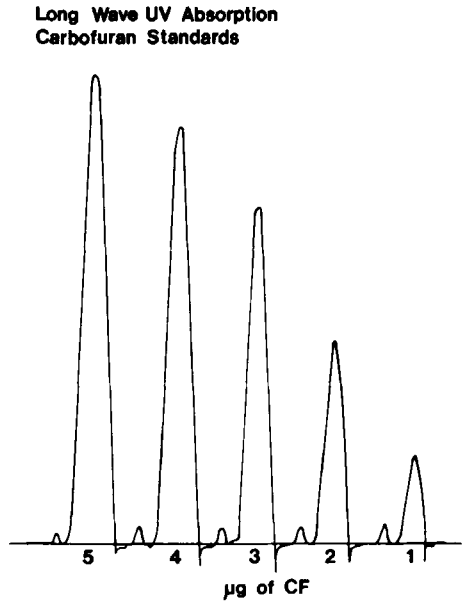

FIGURE FIVE

Standard Curve
CarboFuran (Kontes Densitometer)

solid inlet system of a mass spectrometer. A typical mass spectrometer blank from a thin layer plate extraction is shown (Figure 6). A UV spectral blank in shown (Figure 7).

Alternatively, the plate may be developed to remove soluble materials (3X in clean solvent, methanol was used). This is a long preparation procedure. Another means is to merely immerse the plate repeatedly in solvent. This has the obvious advantage of speed. It suffers from its inability to simulate actual sample conditions and a blank residue may still remain for sample eluents.

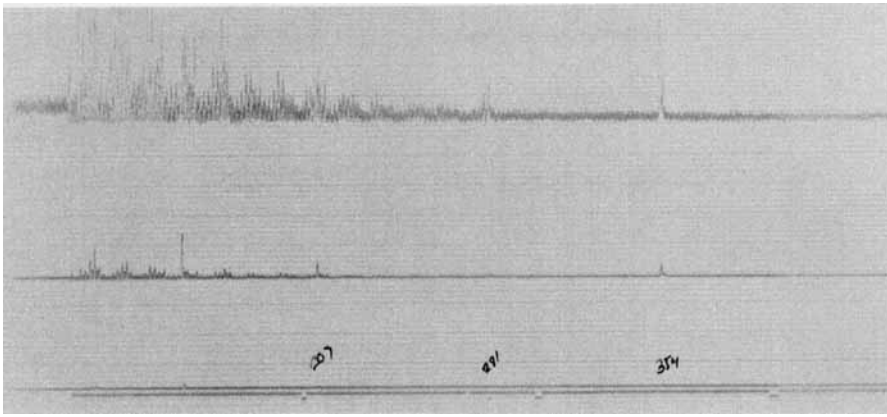

FIGURE SIX

Thin Layer Chromatographic
Mass Spectrum of (TLC) Blank

The preferred method is to anneal the plates in a self cleaning oven for three hours. This completely removes the background. If the solvent is weak enough so that sample loss or extremely long developments are required, the plate may be "deactivated" by methanol immersion. Air dried to remove excess methanol and then activated to the appropriate level of activity. "Conditioning" of the plate to a desirable level of activity may also be done.

RESULTS AND DISCUSSION

By combining cellulose as described by Stahr[3], et al., with the carbon very clean, extracts of rumen contents and chlorophyll bearing foods and feeds can be obtained. Rumensin, organophosphorous pesticides and carbamate pesticides elute in the chloroform fraction.

Figure 8 shows thin layer chromatographs before and after cleanup. Figures 6 and 7 show backgrounds from the solid probe and UV spectrometer from thin layer chromatography bands eluted with methanol. Finally, we should say that all compounds cannot be removed from TLC plates with methanol, although it is our solvent of choice for most compounds not extremely polar so that spectroscopic measurement may be made directly.

Polar compounds like Dicumarol may be removed from TLC plates by spraying the band with dilute HCl and elution into ethyl acetate or 4 to 1 benzene methanol. It is quite insoluble in methanol. Better yet, 0.2N HCl acid may be used to quantitatively remove Dicumarol from TLC plates.

We conclude that with proper cleanup preparation, even very difficult samples may be analyzed by analytical chromatography using direct analysis or removing bands for spectroscopic measurements to complete the analysis. Gas chromatographic column life is greatly extended and the potential harm to GC/MS are relieved. The quality of separations are greatly enhanced and also the amount of sample that can be spotted may be increased making the sensitivity of the analysis much greater if cleanup procedures are used.

Recoveries from the carbon column is shown in Tables I and II T-2 toxin, Diacetoxyscirpenol and Vomitoxin have been recovered by the Sweepco process.

Roridan A must be converted to the acetate to achieve a recovery by the Sweepco. 80-90% of most pesticides can be recovered by Sweepco Distillation. 50-60% of Scirpene mycotoxins are recovered by the Sweepco process. A Furadan and T-2 toxin sample before and after cleanup are shown in Figure 9 and 10.

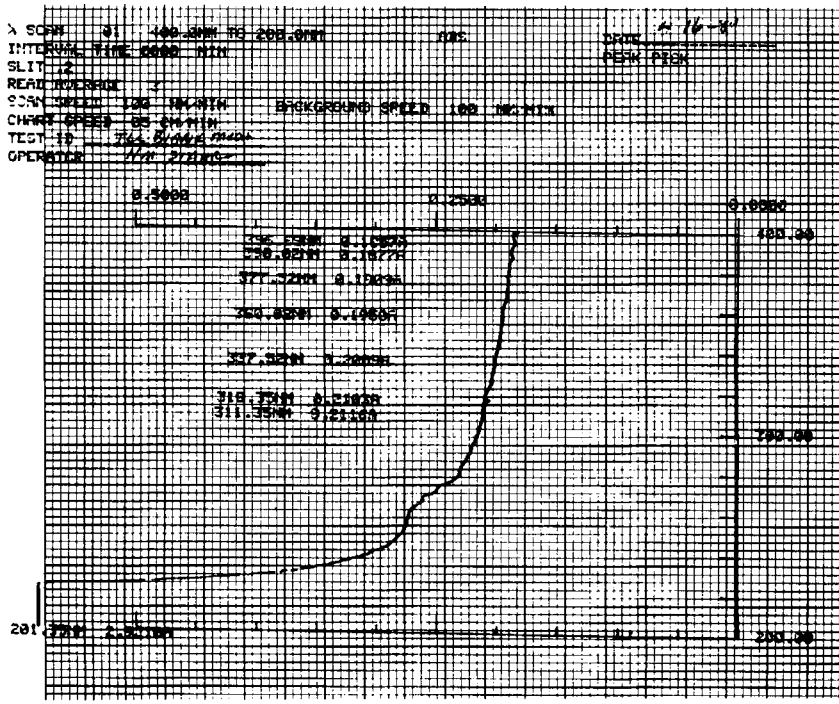

FIGURE SEVEN A
Ultraviolet Spectrum Of TLC
Blank Before Annealing Plate

TABLE I

Recovery of Organophosphorus
Pesticides from Cellulose Column

Pesticide	Cellulose Column	Without Cellulose column
Thimet	80%	100%
Methyl Paration	82%	86%
Malathion	87%	95%
Parathion	90%	96%

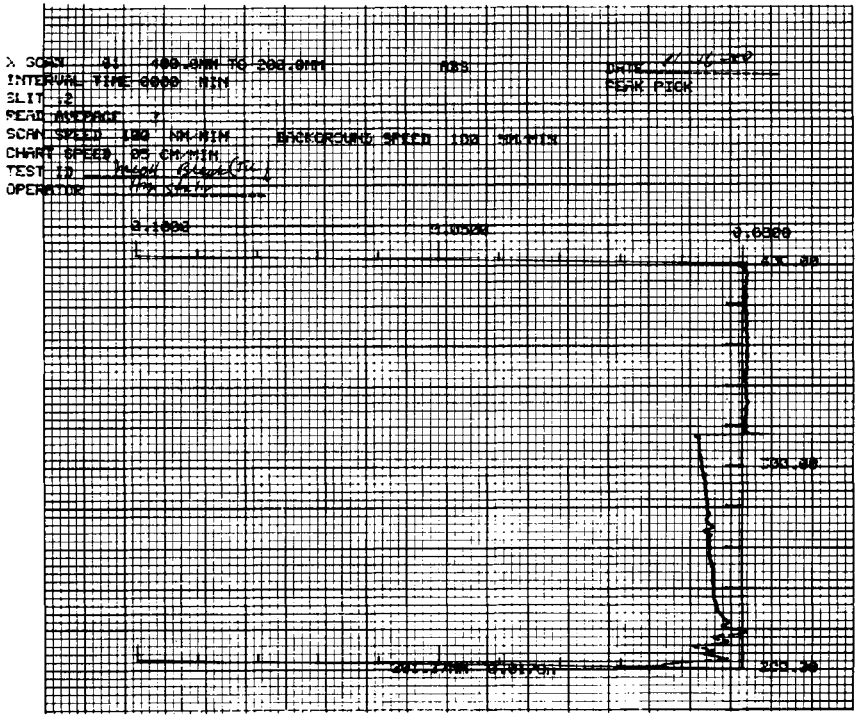

FIGURE SEVEN B
Ultraviolet Spectrum of TLC
Blank After Annealing Plate

TABLE II

Carbon-cellulose cleanup

Compound	% Recovery of Added Pesticide in a Sample Matrix	
Ciodrin®	50%	± 10%
Thimet®	89%	± 16%
Parathion® (ethyl/methyl)	81%	± 10%

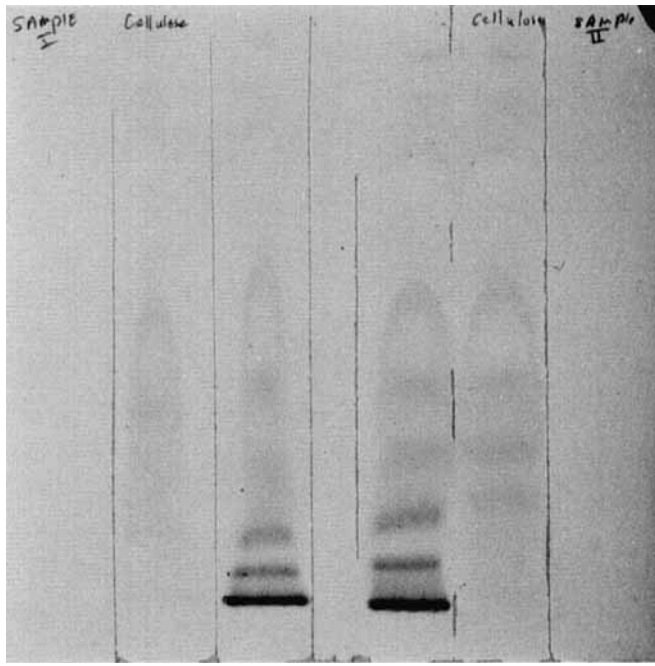

FIGURE EIGHT

Thin Layer Chromatogram (TLC) before and after Cleanup of Pesticide Extract

Particulate carbon cellulose cleanup for organophosphorus and carbamate pesticides are usually 75-85%.

CONCLUSION

Cleanup of samples before sophisticated analysis is done will increase sensitivity, selectivity, and prolong instrument life. It is possible to cleanup samples so that TLC quantitative analysis maybe done directly on a TLC plate. Cleanup of TLC plates reduces background interferences so that more definitive ultraviolet and mass spectrum maybe produced.

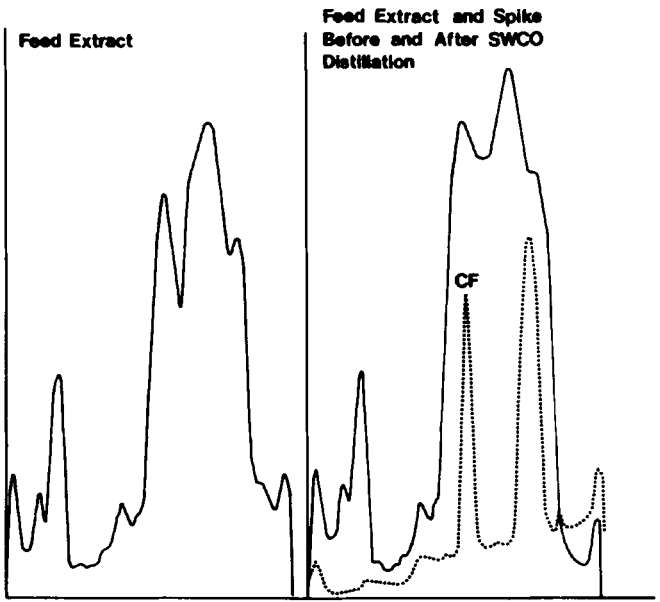

FIGURE NINE

TLC Densitometer Scan of
Furadan Sample
Before and After Cleanup

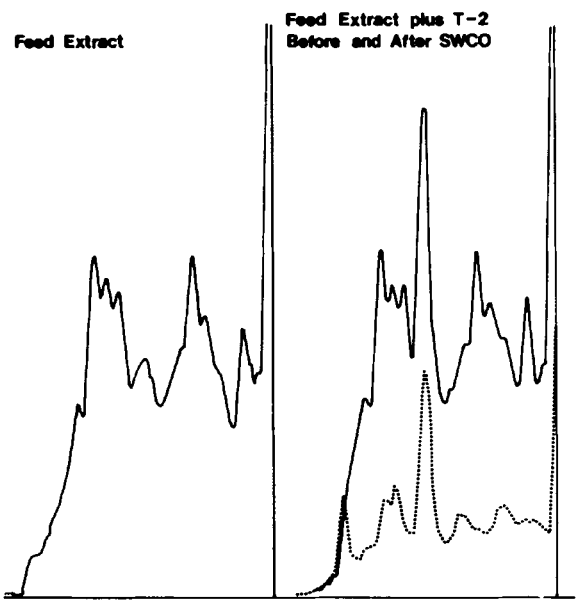

FIGURE TEN

TLC densitometer scan of T-2
toxin sample
before and after cleanup

ACKNOWLEDGMENTS

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