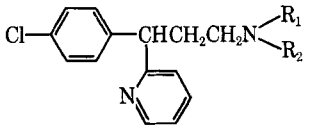
TABLE III—STRUCTURAL FORMULA OF CHLORPHENIRAMINE AND ITS METABOLITES



| Compd. | $R_1$ | $R_2$ |
|---|---|---|
| Chlorpheniramine | $CH_3$ | $CH_3$ |
| Metabolite *X* | $CH_3$ | H |
| Metabolite *Y* | H | H |

diurnal excretion pattern. The calculated *average* daily excretion rate was 0.502 mg., or 12.6% of the repeated 4-mg. chlorpheniramine dose. This compares favorably with the 12% that was estimated from the single-dose experiment.

On termination of the drug-intake regimen, the chlorpheniramine excretion rate decreased. From the total amount of free chlorpheniramine excreted after the last dose is taken on day 28.0, and using the average 0.502 mg. of chlorpheniramine excreted per 4-mg. dose, one can calculate the equivalent amount of chlorpheniramine maleate present in the subject immediately after the last dose. This calculation yields a figure of 9.3 mg. of chlorpheniramine maleate (equivalent to 2.4 tablets, each containing 4 mg. of chlorpheniramine maleate).

**Metabolites X and Y**—The *N*-dealkylation reaction, especially of monomethyl and dimethyl-substituted primary amines is a major pathway of drug metabolism (7). If these metabolites were present, the gas chromatographic method of Beckett and Wilkinson (2) should have revealed them, albeit with much higher retention times (6, 8) than the parent compounds. Unfortunately, in the single dose regimen, it is difficult to differentiate the desired metabolites from other constituents of urine. The metabolites, *X* and *Y*, which were found to be *N*-desmonomethylchlorpheniramine and *N*-desdimethylchlorpheniramine, respectively (Table III), built up, leveled off, and declined like chlorpheniramine but more slowly.

During the steady-state condition, an *average* of 0.510 mg. of *N*-desmonomethylchlorpheniramine and 0.216 mg. of *N*-desdimethylchlorpheniramine were excreted daily (13.2% and 5.8% of the repeated 4-mg. oral dose). Together with the free chlorpheniramine excreted daily, this accounts for 32% of the dose, leaving the fate of the remaining 68% still unaccounted for.

## SUMMARY AND CONCLUSIONS

A repetitive dosage regimen using a commercially available pharmaceutical dosage of chlorpheniramine has been successfully used to identify two metabolites of chlorpheniramine, *N*-desmonomethylchlorpheniramine and *N*-desdimethylchlorpheniramine, in the urine of a single human subject.

## ADDENDUM

Kamm and Van Loon (9) have recently reported the presence of the metabolites, *N*-desmonomethylchlorpheniramine and *N*-desdimethylchlorpheniramine, in the urine of the rat and the dog.

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*Keyphrases*

Chlorpheniramine—urinary excretion
Metabolites, chlorpheniramine—isolated, identified
GLC—analysis, urine
TLC—separation
Mass spectroscopy—identity

# A Micro-Extraction Technique with Compounds Isolated from Thin-Layer Chromatograms

By ALMA L. HAYDEN, WILSON L. BRANNON, and NEUMAN R. CRAIG

**A micro-apparatus is described for the extraction of drugs and related compounds from thin-layer or paper chromatograms by continuous elution with 1 ml. solvent. The sample, after extraction, is ground with KBr, mounted on a micro potassium halide plate, and placed in the center of a paper frame with a window area for infrared analysis. Spectra can be determined on 5- or 10-mcg. samples without beam condensers, ordinate expanders, or micro-dies.**

RECENT INVESTIGATIONS in this laboratory have involved structure characterization and identification of microgram quantities of drugs and related materials by infrared spectroscopy. Most often, these have been polar compounds which have been isolated from thin-layer and paper chromatograms where impurities from the matrix and eluting solvent may interfere. To minimize these effects while

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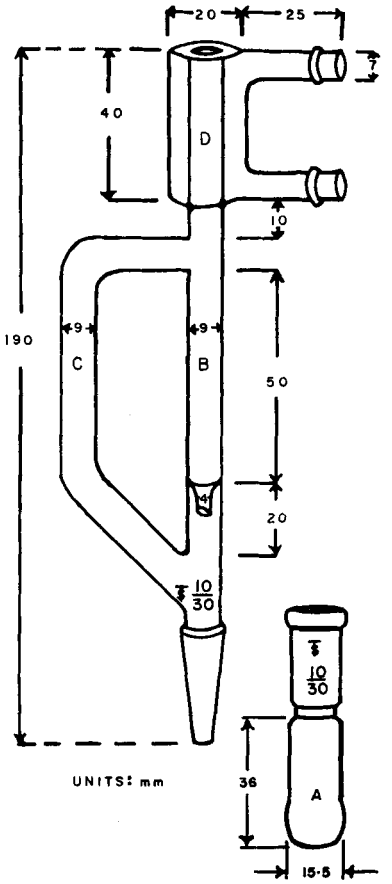


*Fig. 1—A continuous extractor with flask (A), column (B), delivery tube (C), and condenser (D).*

obtaining spectra on generally available equipment, the development of micro-sample handling techniques has been required.

The potassium halide disk technique can be easily modified, since the matrix is simply a means of mounting the sample. By localizing the compound in the center of the disk to approximate the final slit size of the beam, the required quantity of sample can be reduced significantly (1, 2). In this way, identifiable infrared spectra have been obtained in this laboratory without the aid of beam condensers or ordinate expanders on as little as 5 mcg. of a pure compound. As little as 10 mcg. of material isolated from a thin-layer chromatogram can be identified when this technique is used in combination with a continuous extractor (3). A further 5–10-fold reduction in sample size is possible if beam or signal intensifiers are used.

In previous reports of greater sample sensitivity (0.05–1.0 mcg.), other sampling and pelleting techniques were combined with beam condensers, ordinate expansion, and micro-dies (4–6). Bisset *et al.* (2) obtained similar results by using a reflecting microscope. The technique described here should be of particular interest when the above-mentioned accessories are not available.

The extraction apparatus shown in Fig. 1 operates efficiently with 1 ml. of solvent. The extractor consists of a boiling flask (A), a small column (B) with a side-arm delivery tube (C), and a condenser (D). The side-arm is wrapped with asbestos tape; the condenser is water or air cooled.

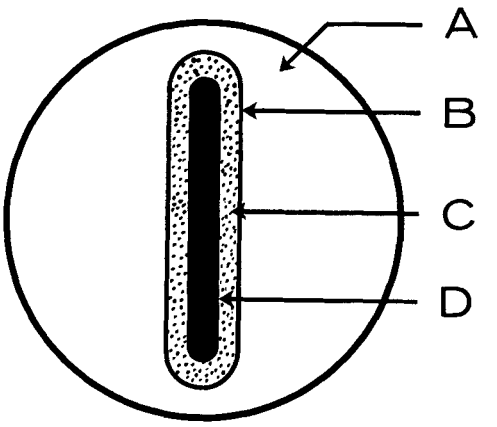


*Fig. 2—Micro-plate in paper frame: A, frame; B, window; C, KBr periphery; D, KBr sample mixture.*

In operation, the chromatographic zone or sample is placed in the column, which contains a pledget of glass wool. The zone may be prewashed with a solvent if desired. The apparatus is then assembled and about 1 ml. of the extracting solvent is poured through the column and collected in the flask. Single or mixed solvents may be used. The solvent is refluxed and the condensate continuously elutes the compound from the column. The cycle is continued until the extraction is completed. The extract or solution containing the compound is cooled and filtered through chloroform-washed fine filter paper or glass wool into a 50-mm. millite mortar. The filtrate is evaporated to dryness under nitrogen and the crystalline residue is dried in a vacuum over phosphorus pentoxide.

A pressed paper or cardboard frame[1] (12.7 mm. in diameter, 0.6 mm. in thickness) containing a 2 × 11 mm. window is placed on the anvil of a 0.5-in. potassium bromide die. The dry residue is ground by hand with 2 mg. of potassium halide, and the mixture is aligned vertically (1 × 10 mm.) in the center[2] of the window area of the paper frame. Approximately 25 mg. of potassium halide powder is used to rinse the mortar and then to fill in the remaining window area. The die is assembled and evacuated, and the micro-plate is pressed in the usual manner. After pressing, the micro-plate with paper frame (Fig. 2) is mounted in the micro-sample position of a double-beam spectrophotometer. Perkin-Elmer models 21 and 237 were used in this laboratory to record the spectra.

Figure 3 shows the spectra of 1-methylhydantoin: A is 10 mcg. isolated from a thin-layer chromatogram, B is 10 mcg. crystallized directly from chloroform. Both spectra were obtained on micro-plates. For comparison, a standard 0.5% disk (C) is included. Figure 4 shows a spectrum, obtained as described above, of 5 mcg. of 1-methylhydantoin recrystallized from chloroform. In the reference beam, an attenuator was used for the 10-mcg.

[1] Supplied as 13-mm. frames from Limit Research Corp., Darien, Connecticut.

[2] For use with a beam condenser, the mixture is pressed in a 1 × 1 mm. central area of the window.

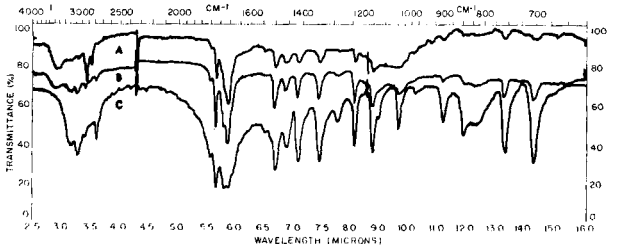


*Fig. 3—Spectra of 1-methylhydantoin: 10 mcg. isolated from TLC (A), 10 mcg. crystallized from $CHCl_3$ (B), and a standard 0.5% disk (C).*

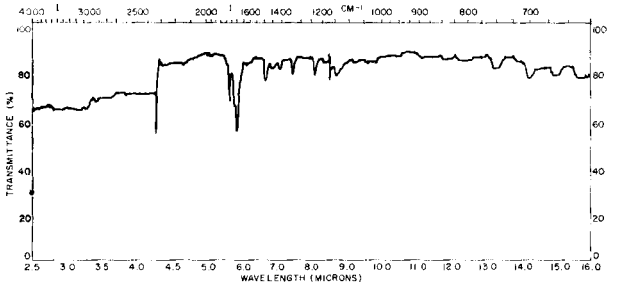


*Fig. 4—Spectrum of 5 mcg. 1-methylhydantoin crystallized from $CHCl_3$.*

spectra; a potassium bromide reference disk or plate was used with the 0.5% and 5-mcg. spectra. The spectra are essentially the same except for additional absorption in the C-H and 9 $\mu$ spectral regions of the sample from the thin-layer chromatogram. These spectra were recorded on a Perkin-Elmer model 237 grating spectrophotometer. Comparable, though less intense, spectra were examined in the micro-sample position of a NaCl prism spectrophotometer.

It must be emphasized that spectral identification of mcg. quantities of pure compounds depends on sample handling techniques as well as on crystal form and absorption characteristics of the molecule. In addition, the identification of materials isolated in microgram quantities from thin-layer and paper chromatograms requires adequate extraction with the inclusion of minimal solvent and substrate residues. By using the continuous extractor, microgram quantities of polar compounds can be isolated from thin-layer chromatograms with 1 ml. of solvent. However, the effects of solvent and substrate residues must be further reduced by washing glassware, thin-layer plates, and paper with the extracting solvent before use. In this application, the extraction was performed with freshly distilled, dried, and ethanol-stabilized chloroform. The extractions and disk preparations should be made on the same day. In this way, reproducible spectra of suitable intensity are obtained. Effects of excipient residues on the spectra can be further reduced by using a reference micro-plate prepared under the same conditions as the sample.

This technique has been used also in the identification of barbiturates, steroids, and other compounds isolated from thin-layer and paper chromatograms.

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**Keyphrases**

Micro—extraction from chromatograms
Diagram—extraction apparatus
Extraction technique—microgram quantities
IR spectrophotometry—identity