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Antimicrobials: identification of 3,4,4′-trichlorocarbanilide and 4,4′-dichloro-3-(trifluoromethyl) carbanilide in deodorant bars

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Synopsis:

The ANTIMICROBIALS 3,4,4′-TRICHLOROCARBANILIDE and 4,4′-DICHLORO-3-(TRIFLUOROMETHYL) CARBANILIDE were IDENTIFIED in DEODORANT BARS by analysis of their degradation products. The antimicrobial(s) were first concentrated by solvent extraction of the deodorant bar. The extract was fused with phthalic anhydride to form mixed phthalimides, which were then hydrolyzed with hydrazine to yield a mixture of aromatic amines. Gas-liquid chromatographic analysis of the amine mixture was used to identify the antimicrobial(s) originally present in the deodorant bar. 3,4,4′-Trichlorocarbanilide when degraded yielded a mixture of 4-chloroaniline and 3,4-dichloroaniline. 4-Chloroaniline and 4-chloro-3-(trifluoromethyl) aniline were the products obtained from 4,4′-dichloro-3-(trifluoromethyl) carbanilide. The procedure was then evaluated as a method for the determination of the antimicrobials, using 3,5-dichloroaniline as the internal standard. Recoveries of added antimicrobials were 85 to 90 per cent of theoretical.

INTRODUCTION

Deodorant bars are composed of soaps and/or detergents and fragrance oils to which antimicrobial compounds have been added to retard the growth of skin bacteria that may give rise to body odor. For many years hexachlorophene was the principal active antimicrobial agent in deodorant bar formulations. A regulation restricting the use of hexachlorophene has resulted in the use of a number of substitute antimicrobials in these preparations. Two antimicrobial compounds that are frequently used in deodorant bars are 3,4,4′-trichlorocarbanilide (TCC) and 4,4′-dichloro-3-(trifluoromethyl) carbanilide (DCTFMC). Deodorant bars commonly contain mixtures of these two compounds, making their identification by chromatographic methods difficult. Wilson (1) was unable to separate TCC and DCTFMC by thin-layer chromatography. Sheppard and Wilson (2) reported that TCC and DCTFMC were eluted in the same fraction, using partition chromatography. The ultraviolet spectra of TCC and DCTFMC are nearly identical and the compounds, therefore, cannot be characterized

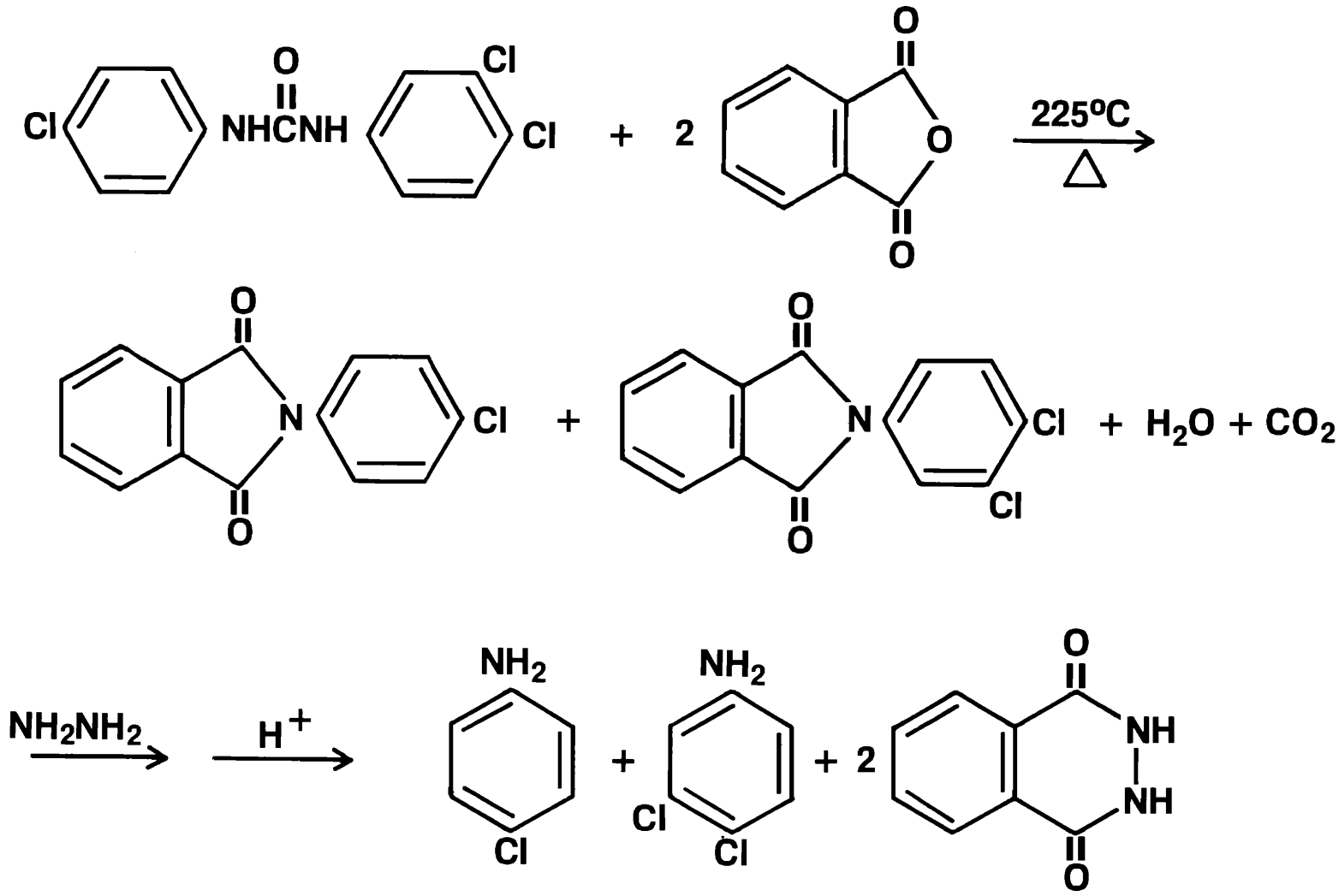

Figure 1. Degradation of TCC, using modified Gabriel synthesis

by this method. Investigations in this laboratory with high pressure liquid chromatography and gas-liquid chromatography (GLC) were likewise unsuccessful.

The primary purpose of this investigation was to develop a method for the identification of these compounds. The second part of this investigation involved evaluation of the qualitative method as a technique for the determination of TCC and DCTFMC.

Tingle and Brenton (3) reported that alkyl carbamides react with phthalic anhydride to give alkyl phthalimides in high yield. Manske (4) synthesized primary alkyl amines by hydrazine hydrolysis of alkyl phthalimides. These studies form the basis of the method reported here.

DCTFMC and/or TCC were isolated from the deodorant bars by solvent extraction. The residue remaining after evaporation of the solvent was reacted with phthalic anhydride (see Fig. 1). The reaction mass was hydrolyzed with hydrazine, and the resulting aromatic amines were analyzed by GLC. By comparison of peak retention times with those of standards, the degradation products of DCTFMC were identified as 4-chloroaniline and 4-chloro-3-(trifluoromethyl) aniline (5-amino-2-chlorobenzotrifluoride), the expected products. Similarly, TCC yielded 4-chloroaniline and 3,4-dichloroaniline. The procedure was then evaluated as a method for the quantitative estimation of DCTFMC and TCC. Known amounts of each were added to solutions of a commercial deodorant bar. Solutions were then carried through the procedure, and the resulting amines were determined by GLC, using 3,5-dichloroaniline as the internal standard. Initial recoveries of added TCC and DCTFMC varied from 25 to 29 per cent. After the method was modified to eliminate losses during evaporation of the ether extract, recoveries of 85 to 90 per cent were obtained.

METHOD

APPARATUS

A gas chromatograph (Model 810*) equipped with dual flame ionization detectors and operated under the following conditions was used: column 210° C, injection port 250° C, detector 260° C, carrier gas flow rate (helium), 80 ml/min.

The GLC column was glass, 10 ft x 4 mm i.d., packed with Chromosorb W (60 to 80 mesh)† coated with 5 per cent KOH and 4 per cent PEG 20M.‡ The column packing was prepared as follows: dissolve 8 g of PEG 20 M and 10 g of KOH in 150 ml. of warm methanol. After the solution is complete, add methanol to make the volume 200 ml. Pour methanol solution into a 1 liter round-bottom flask followed by 25 g of 60 to 80 mesh Chromosorb W. Mix well and then apply vacuum for *ca.* 1 min to remove entrained air. Release the vacuum and swirl the flask vigorously; rapidly transfer the slurry to a 500 ml sintered glass (coarse) funnel set in a side-arm filter flask. Apply suction to the funnel for 2 to 3 min. Transfer the damp support to a large crystallizing dish or stainless steel pan and dry on the steam bath. Mix (do not stir) the support thoroughly every 5 min. by shaking the dish or pan. After the odor of the solvent has disappeared, pack the dried support into the GLC column. The packed column was conditioned for 24 h at 225° C with a carrier gas flow of 10 ml/min.

An oil bath (Model 11-48)‡ was used only for reaction of phthalic anhydride with preservative.

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†Applied Science Laboratories, Inc., State College, PA.

‡Fisher Scientific Co., Pittsburgh, PA.

REAGENTS

The following reagents were used: 3,4-dichloroaniline (98 per cent, No. D5560-1);* 3,5-dichloroaniline (98 per cent, No. D5579-2);* 4-chloroaniline (98 per cent, No. C2241-5);* 4-chloro-3-(trifluoromethyl) aniline (99 per cent, No. A-4565-3);* phthalic anhydride (ACS, No. 331);† and hydrazine hydrate (85 per cent, No. H-318).†

STANDARD SOLUTIONS

Prepare separate standard solutions of 3,4-dichloroaniline, 4-chloro-3-(trifluoromethyl) aniline, and 3,5-dichloroaniline by accurately weighing *ca.* 0.2 g of each compound into separate tared 50 ml beakers. Dissolve the compounds in benzene; quantitatively transfer each solution to a 100 ml volumetric flask and dilute to volume with benzene.

PREPARATION OF SAMPLE

Using a knife or spatula, reduce 2 to 3 g of deodorant bar to powder or fine shavings. Accurately weigh *ca.* 2 g of shavings into a tared 100 ml beaker. Add 50 ml of warm water and 1 pellet of KOH, and stir to dissolve. Transfer the solution to a 125 ml separatory funnel; rinse the beaker with 1 x 10 ml and 1 x 5 ml portions of ethanol, adding rinsings to the separatory funnel. Extract the solution with 3 x 20 ml of ethyl ether. If a stable emulsion forms during the second or third extraction, add a small amount of ethanol to break the emulsion. Combine the ether extracts and wash with 50 ml of 1 per cent NaCl solution, 50 ml of water containing 1 ml of HCl, and, finally, 50 ml of 2 per cent $NaHCO_3$ solution. Dry the ether extract over anhydrous Na_2SO_4 for 30 min, transfer to a 100 ml beaker, and evaporate on the steam bath to *ca.* 20 ml. Transfer the extract to a 50 ml centrifuge tube and carefully evaporate to dryness on a steam bath. Add *ca.* 50 mg of phthalic anhydride to the residue and heat the lower portion of the centrifuge tube in an oil bath at 225° C for 15 to 20 min. Cool, and add 2 ml of ethanol:dimethylformamide (1:1) to dissolve the reaction mass. Add two drops of hydrazine hydrate, warm for several minutes, and then add 15 ml of dilute HCl (1:10). Transfer the contents of the tube to a 125 ml separatory funnel, extract with 30 ml of ethyl ether, and discard the ether layer. Make the aqueous solution distinctly basic (litmus) by adding 10 per cent NaOH solution and then extract with 2 x 30 ml portions of ethyl ether. Combine ether extracts, wash with 30 ml of water, and dry 30 min over anhydrous Na_2SO_4. Transfer this extract to a 100 ml beaker; add 0.5 to 1 ml of xylene and evaporate to 15 to 20 ml. Transfer to a 50 ml centrifuge tube and evaporate carefully on the steam bath, leaving only xylene. Stopper the tube and reserve for analysis.

If the sample is to be prepared for quantitative analysis, all of the above transfers should be quantitative by rinsing the prior container with several small portions of the appropriate solvent.

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†Eastman Organic Chemicals, Rochester, NY.

IDENTIFICATION

Using an initial range and attenuation settings of 10^2 x 8 as a starting point, inject 2 to 3 μl of a solution containing 2 to 3 μg of 4-chloroaniline/μl benzene onto the GLC column. If the initial chromatogram is unsatisfactory, adjust the injection volume and/or recorder attenuation to bring the peak on scale. If the 4-chloroaniline peak does not elute in 4–5 min, adjust the column temperature accordingly. Record the retention time of 4-chloroaniline. Similarly determine the retention times of 3,4-dichloroaniline and 4-chloro-3-(trifluoromethyl) aniline. The retention times for 4-chloroaniline, 4-chloro-3-(trifluoromethyl) aniline and 3,4-dichloroaniline relative to 3,5-dichloroaniline are 0.30, 0.48, and 1.13, respectively.

Inject 3 to 4 μl of the sample solution onto the GLC column. Record the retention values for the eluted peaks and compare with those obtained for the standards. Identify TCC by peaks corresponding in retention time to 4-chloroaniline and 3,4-dichloroaniline, and DCTFMC by peaks corresponding to 4-chloroaniline and 4-chloro-3-(trifluoromethyl) aniline.

DETERMINATION

3,4,4'-Trichlorocarbanilide (TCC): Pipet 1.0 ml of the 3,5-dichloroaniline (internal standard) solution into the sample that was previously determined to contain 4-chloroaniline and 3,4-dichloroaniline. Inject *ca.* 5 μl of this solution onto the GLC column at range and attenuation settings to keep the 3,5-dichloroaniline and 3,4-dichloroaniline peaks on scale. Measure the peak heights and, assuming a linear relationship between the concentration and peak height, estimate to the nearest 1.0 ml how much additional 3,5-dichloroaniline is needed to obtain approximately equal ($\pm$10 per cent) peak heights. Pipet the calculated amount of 3,5-dichloroaniline solution into the sample solution. Again inject the sample solution to determine if the peak heights of 3,4-dichloroaniline and 3,5-dichloroaniline are approximately equal. Adjust the volume injected and/or the attenuation to keep the peaks within 50 to 90 per cent full-scale recorder deflection. Prepare a standard solution by mixing 10.0 ml each of the 3,5-dichloroaniline and 3,4-dichloroaniline standard solutions. Using the same range and attenuation settings used for the sample, inject 3 to 4 μl of the standard solution onto the GLC column. From the chromatogram, determine the volume of either 3,4-dichloroaniline or 3,5-dichloroaniline that must be added to the standard solution to obtain peaks of approximately the same ratio as the peaks in the sample. Also determine the injection volume of standard needed to give peaks that are about the same height as those in the sample. Using these conditions, alternately inject the standard and sample solutions, making a minimum of two injections of each.

4,4'-Dichloro-3-(trifluoromethyl) carbanilide: Use the above procedure, substituting 4-cholor-3-(trifluoromethyl) aniline for 3,4-dichloroaniline. Prepare the initial standard solution by mixing 5.0 ml of 4-chloro-3-(trifluoromethyl) aniline standard solution with 10.0 ml of 3,5-dichloroaniline standard solution.

Mixtures of TCC and DCTFMC: Measure the smallest GLC peak first. Add the necessary additional internal standard to the sample and measure the second GLC peak.

Calculations: Calculate the weight of amine being determined in the sample as follows:

$$\text{Weight (mg) unknown} = (Ru/Rs) \times Ks \times (ISu/ISs)$$

where Ru is the ratio of the peak height of the unknown in the sample to that of the internal standard added to the sample; Rs is the ratio of the peak height of the known standard to that of the internal standard in the standard solution; Ks is the weight (mg) of the known standard in the standard solution; ISu is the weight (mg) of the internal standard in the sample; and ISs is the weight (mg) of the internal standard added to the standard solution.

Calculate weight of TCC or DCTFMC by using the following conversions:

$$\text{Weight (mg) TCC} = W\text{-}3,4 \times 1.95$$
$$\text{Weight (mg) DCTFMC} = W\text{-}4 \times 1.79$$

where W-3,4 is the weight (mg) found for 3,4-dichloroaniline and W-4 is the weight (mg) found for 4-chloro-3-(trifluoromethyl) aniline.

RESULTS AND DISCUSSION

Before the proposed method was applied to the analysis of commercial deodorant bars, it was necessary to determine conditions suitable for the stepwise degradation of the antimicrobial compounds. TCC and DCTFMC reacted smoothly with phthalic anhydride at 225° C; evolution of carbon dioxide and water ceased after several minutes. The reaction mass, however, was difficult to dissolve in ethanol, the usual solvent. A mixture of dimethylformamide and ethanol was suitable. Hydrolysis of the phthalimides with hydrazine hydrate and hydrochloric acid proceeded rapidly under mild temperature conditions. Aromatic amines were isolated from the reaction mixture by the usual methods.

One of our concerns was the possible interference by amines formed by the degradation of other antimicrobials used in deodorant bars. If, for example, tribromosalicylanilide reacted analogously, *p*-bromoaniline would be the expected product. This compound has approximately the same GLC retention time as 4-chloro-3-(trifluoromethyl) aniline and is, therefore, a serious interference. Tribromosalicylanilide was carried through the degradation procedure. GLC analysis of the reaction products demonstrated the absence of *p*-bromoaniline.

The GLC columns used for the analysis of primary amines are usually packed with a nonsilanized support containing several per cent of potassium hydroxide to reduce adsorption. Polyester or other liquid phases containing functional groups that react with strong bases must not be used. We found that a 10 ft glass column containing potassium hydroxide-treated Chromosorb W coated with PEG 20M gave satisfactory separation. 3,5-Dichloroaniline was selected as the internal standard because of its chemical similarity and the nearly equal specific detector response to those compounds being determined. The extraction procedure used in this study was designed to separate the neutral ether-soluble fraction from the acidic and basic water-soluble compounds present in the deodorant bar. Solvent extraction of solutions containing surfactants nearly always results in the formation of emulsions. This problem can be ef-

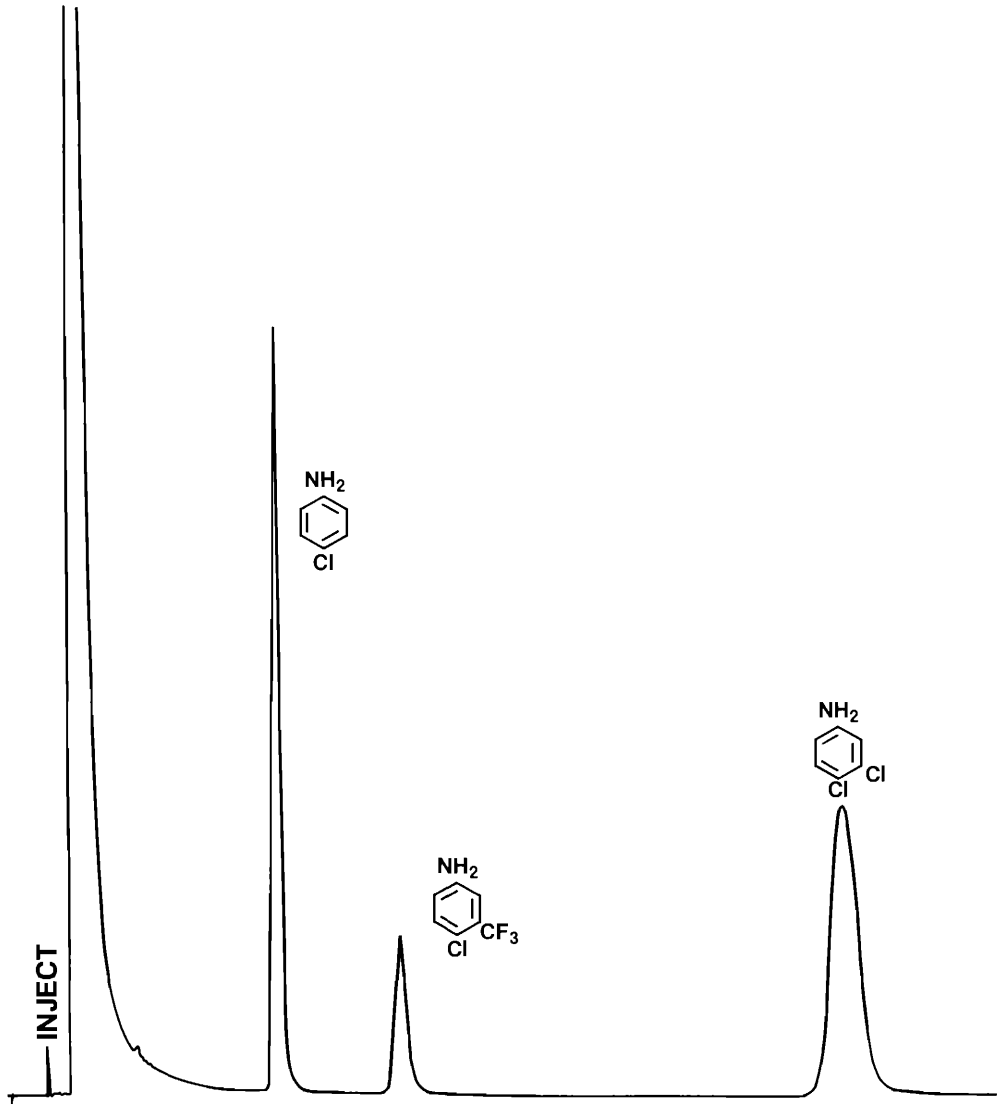

Figure 2. Gas chromatogram of degradation products of antimicrobials extracted from commercial deodorant bar: in order of elution, 4-chloroaniline, 4-chloro-3-(trifluoromethyl) aniline, and 3,4-dichloroaniline

fectively eliminated by adding ethanol. Our investigations indicate that an ethanol concentration of 20 to 25 per cent by volume is optimal. The ether-soluble fraction is usually small, generally 20 to 30 mg/g of product. Since occasionally the neutral fraction may be somewhat larger, we recommend that correspondingly larger amounts of phthalic anhydride be used for the degradation step. Likewise, the amount of hydrazine hydrate used should be increased accordingly.

Several commercial products known to contain TCC and/or DCTFMC were analyzed

Table I.
Recoveries of 3,4,4′-Trichlorocarbanilide (TCC) and 4,4′-Dichloro-3-(trifluoromethyl) Carbanilide (DCTFMC) Added to a Commercial Deodorant Bar

Preservative	Added (mg)	Recovery (mg)	Recovery Per Cent
TCC	10	9.0	90
TCC	10	8.6	86
DCTFMC	10	8.9	89
DCTFMC	10	8.5	85

by the proposed procedure. In all cases, clean chromatograms containing only those peaks corresponding to the expected products were obtained (see Fig. 2).

To evaluate the qualitative method as a procedure for the determination of TCC and DCTFMC, known amounts of each antimicrobial were added separately to solutions of a commercial deodorant bar and the solutions were analyzed by the described procedure. The isolated amines were determined by GLC, using 3,5-dichloroaniline as the internal standard. Initial recoveries were 25 to 29 per cent of theoretical, much lower than expected. A step-by-step evaluation of the procedure indicated substantial losses of the aromatic amines when the ether extract was evaporated to dryness. The procedure was modified by adding 0.5 to 1.0 ml of xylene to the extract to keep the sample from evaporating to dryness. This modification resulted in recoveries of up to 90 per cent (Table I). Other minor modifications in the procedure were not effective in increasing recoveries. Because we did not consider recoveries of 90 per cent sufficiently accurate for a quantitative method, further studies were not done. Therefore, it is recommended that the procedure be used only to identify the antimicrobials present.

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