Detection of Contamination of Pharmaceutical Preparations with Foreign Active Ingredients

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Abstract □ General procedural notes are submitted of methods of detecting contamination of pharmaceutical products with undeclared active ingredients. Several specific methods are also discussed in detail. The suspected contaminant is extracted with a suitable solvent and separated from other extracted ingredients, generally by column chromatography. A portion of the concentrated purified extract is examined by TLC for the presence of the suspected contaminant. A control sample, containing a small quantity of added contaminant and similarly extracted and purified, is spotted along the unspiked sample for comparison purposes.

Keyphrases □ Pharmaceutical preparation contamination—determination □ Contamination determination, pharmaceutical preparation—methodology □ Column chromatography—separation □ TLC—separation, identification

Finished pharmaceuticals are required by Good Manufacturing Practice Regulations under the Federal Food, Drug, and Cosmetic Act to possess the identity, strength, quality, and purity they purport to possess (Section 133.8). They should consequently be free of undeclared active ingredients. Section 133.8(*c*) in particular indicates some precautions to prevent mixups or contaminations. Contamination with potent drugs, even at very low levels, constitutes an obvious hazard. Contamination with low potency (or high dosage) drugs does not generally present a health hazard but is indicative of manufacturing conditions, which are potentially hazardous.

Food and Drug Administration laboratories occasionally test pharmaceuticals for foreign active contaminants, especially when inspection of a firm has indicated poor manufacturing controls. Rogavitz (1) developed a method for detecting contamination of drugs with corticosteroids. A portion of ground sample not exceeding 300 mg. is mixed with 2 g. of diatomaceous earth[1] and transferred to a glass column already containing superimposed layers of diatomaceous earth-K_3PO_4 solution and diatomaceous earth-$NaHCO_3$ solution. Chloroform elutes any corticosteroids present in the sample together with other neutral substances. Organic acidic compounds are trapped on the column. An aliquot of the concentrated eluate is spotted on a paper chromatogram. Development and detection of the corticosteroids are the same as previously indicated by Kunze and Markham (2).

Shevling (3) also employs paper chromatography for the detection of sulfonamides as contaminants of drug products. A 10-g. ground sample is shaken with $CHCl_3$ and then extracted with a total of 20 ml. 0.1 *N* HCl. The aqueous extracts are mixed with 15 g. of diatomaceous earth and transferred to a glass column, from which sulfonamides, if present, are eluted with ether. After development in $CHCl_3$–*tert*-butyl alcohol (50:1), the paper chromatogram is sprayed in succession with acidified $NaNO_2$ solution and *N*-(1-naphthyl)ethylenediamine dihydrochloride solution, in accordance with Bratton-Marshall reaction (4). TLC development was also tried but was found less sensitive by the same author (3).

Several other methods for the detection of foreign active ingredients as contaminants of drug products were developed in this laboratory. Generally the suspected contaminant (or contaminants), extracted from the sample with a suitable solvent, is separated as far as possible from other extracted ingredients by column chromatography. The purified extract is concentrated to a small volume and examined by TLC for presence of contaminants.

[1] Celite, Johns-Manville Products Corp., New York, N. Y.

EXPERIMENTAL

Apparatus—Standard equipment for TLC including 10 × 20 and 20 × 20-cm. glass plates; 100-μl. micropipet or microsyringe; Chromato-Vue (Black Light Eastern Corp., New York, N. Y.); chromatographic glass column (*a*), about 2.2-cm. inner diameter, 25 cm. long constricted end plugged with glass wool or cotton before use; chromatographic glass column (*b*), same as (*a*) but with a medium porosity fritted-glass disk fused above constricted end, and 2 and 5-cm. microcells and holders (Pyrocell Manufacturing Co., Westwood, N. J.).

Reagents and Solutions—Diatomaceous earth,[1] acid wash as described in USP XVII, p. 822; silicic acid 100 mesh (Mallinckrodt Chemical Works); Silica Gel G [Brinkmann Instruments, Westbury, N. Y. (or equivalent)]; phosphor for TLC (Research Specialties Co., Richmond, Calif.); iodoplatinate solution, dilute 3 ml. of 10% platinum chloride with 97 ml. of water and mix with 100 ml. of 6% aqueous potassium chloride; 2,7-dichlorofluorescein solution, 0.2% in methanol; silver acetate, 1% aqueous solution, warm on steam bath to dissolve; diphenylcarbazone solution, 0.1% in alcohol, freshly prepared; phosphoric acid, 40% solution in water; sulfuric acid, 10% solution (v/v); sodium bicarbonate, 8% solution (w/v), freshly prepared; tripotassium phosphate 20% solution (w/v), and chloramine-trichloroacetic acid: mix 20 ml. of freshly prepared 3% aqueous chloramine with 80 ml. of 25% alcoholic trichloroacetic acid.

TLC—Slurry 30 g. of Silica Gel G with 60 ml. of water and coat five 20 × 20-cm. or ten 10 × 20-cm. plates to a thickness of 0.25 mm. To prepare fluorescent plates add 100 mg. of phosphor to each 30 g. of silica gel. If 10 × 20-cm. plates are used, spot along one of the shorter sides. Keep size of spots small by drying drops with a gentle air current between applications. Develop until the solvent front is near the top of the plate. Detect developed spots under shortwave UV light or visually after spraying with a suitable solution.

General Procedural Notes—Weigh a portion of finely ground sample and transfer to a centrifuge tube or other container suitable for the extraction to be performed. Although larger samples can be occasionally taken, it is desirable that the sample weight does not exceed 5 g. Shake or macerate the sample with a solvent capable of extracting the suspected contaminant. Perform additional extractions or cleanups to separate, as far as possible, the contaminant from other extracted ingredients, both active and inactive. This can be often done by trapping and later eluting the contaminant from a diatomaceous earth column. Alternately diatomaceous earth

chromatography can be used to retain some sample ingredients while the contaminant is eluted. A silicic acid column, alone or in combination with a diatomaceous earth column, can also be used to retain a contaminant, which is then eluted with a methanolic chloroform solution. Concentrate the purified extract to a small volume, transfer to 5-ml. conical graduated tube, evaporate to 1.0 ml. or less with a current of air, and record volume. Spot 100 μl. on a prepared silica gel plate. Prepare a control as follows. To an equal amount of sample add in solution a small quantity of the suspected contaminant and continue as described above. The quantity of contaminant added to the control sample must be determined in each instance by the amount of sample taken for analysis, the desired lower limit of detection, and the percentage of the final solution spotted. The aliquot spotted should however contain at least 15–20 mcg. of the contaminant since at lower levels detection might be difficult, especially if the cleanup was not too extensive or too satisfactory. If the sample submitted for contamination analysis is small, the control portion can be taken from another sample of similar composition.

Spot the aliquot of the control sample alongside the aliquot of the unspiked sample. Also spot separately a quantity of contaminant equal to that contained in the spotted control solution. Develop, spray if necessary, and examine plate. Locate and mark the spots produced by the contaminant alone and in the chromatogram of the control solution. Compare these two with the chromatogram of the unspiked sample solution to determine whether the sample is contaminated with the suspected compound.

If the contaminant cannot be definitely located in the chromatogram of the control aliquot, because of the presence of coloring substances or active ingredients that could not be adequately removed, it might be desirable to repeat analysis increasing detection limit of contaminant or varying extraction and cleanup processes.

If, despite procedural changes introduced, the detection of the contaminant in the control chromatogram is still impossible or uncertain, the sample may not be suitable to be tested for the particular type of contamination. The unsuitability of a sample for a certain contamination analysis can be often determined in advance from a study of the extraction and TLC behavior of its active ingredients, and the contaminant of interest.

Specific Procedures for Different Contaminants—Suggested procedures are described for the individual contamination tests. To avoid continuous repetitions, those procedural steps that are clearly implied by the preceding section are omitted. For diatomaceous earth column chromatography use water-washed solvents. Columns are generally prepared by mixing 2 g. of diatomaceous earth with 2 ml. of the indicated solution, but minor deviations from these quantities can be made. Indications are given when fluorescent thin-layer plates must be used. R_f values are often related since, though not always reproducible, they give an indication of the order of succession of the spots in the chromatogram.

Contaminants—Barbiturates—To sample in 100-ml. round-bottom centrifuge tube add 15 ml. of 0.1 N H_2SO_4 and 55 ml. $CHCl_3$. Stopper, shake well 1 min., and centrifuge. Quickly immerse a 50-ml. pipet into the tube, blow out any aqueous solution from the pipet, and withdraw 50 ml. of the $CHCl_3$ layer. Repeat extraction with two additional 50-ml. portions of $CHCl_3$. Trap the barbiturates on a diatomaceous earth-tripotassium phosphate column, as indicated by Heuermann and Levine (5). After pre-elution is completed, elute barbiturates with 5 ml. of 10% CH_3COOH in $CHCl_3$ followed by 100 ml. of 1% CH_3COOH in $CHCl_3$. Develop chromatogram with $CHCl_3$–CH_3COOH (96:4).

Detect barbiturates by spraying plate with silver acetate followed by diphenylcarbazone (6, 7). Barbiturates can also be detected visually or under UV light after spraying with 2,7-dichlorofluorescein solution, but this detection is less specific.

Contaminant—Diethylstilbestrol—Extract sample as under *Barbiturates* but do not subject to column chromatographic cleanup. Develop chromatogram with $CHCl_3$–ether (95:5). Examine developed plates visually and circle yellow areas, if any. Irradiate plate under short-wave UV light for 10 min. After irradiation, the diethylstilbestrol forms a yellow spot. Be sure, however, that the diethylstilbestrol is not in an area that was colored yellow before irradiation.

Contaminant—Reserpine—Extract sample as under *Barbiturates*. If desired, 0.1 N NaOH can be substituted for 0.1 N H_2SO_4. A $NaHCO_3$ solution wash of the chloroform extract might also be helpful (8).

Pack a chromatographic column (*b*) with silicic acid to a height of 3 cm. Pour extracts into column, elute, add 100 ml. $CHCl_3$, and discard all eluates. Change receiver and elute with 50 ml. of 20% CH_3OH in $CHCl_3$. Use fluorescent TLC plates. Develop chromatogram with $CHCl_3$–CH_3OH (98:2). Detect reserpine under short-wave UV light.

Contaminants—Cardiac Glycosides—To sample in centrifuge tube, add about 15 ml. $NaHCO_3$ solution and extract with $CHCl_3$ as described under *Barbiturates*. Cleanup in a silicic acid column as under *Reserpine*. Develop chromatogram with $CHCl_3$–CH_3OH (90:10). Spray develop plate evenly with chloramine-trichloroacetic acid solution, then heat in oven at 100° for 5 min. (9).

Contaminants—Quinine or Tropane Alkaloids, Pyrilamine or Chlorpheniramine—Extract sample as under *Barbiturate* substituting 0.1 N NaOH for the acid solution. Pass the extracts through a diatomaceous earth-0.2 N H_2SO_4 column, as used by Levine and Roe (10) for the determination of atropine. After the extracts have eluted, add 100 ml. $CHCl_3$ and discard all eluates. Change receiver and elute with 1 ml. of ammonium hydroxide followed by 100 ml. of $CHCl_3$.

Use fluorescent plates; develop chromatogram with $CHCl_3$-diethylamine (96:4). Quinine and quinidine can be detected under UV light even at the 5–10-mcg. level. Atropine and scopolamine are not visible under UV light, except in relatively large amounts (100 mcg. or more). All four compounds produce colored spots if plate is sprayed with iodoplatinate solution (11). They also produce yellow spots, detectable visually or better under UV light, if plate is sprayed with 2,7-dichlorfluoroescein. Note: if sample contains amphetamine, methamphetamine, ephedrine, phenylpropanolamine, or their salts, these compounds will be present in the spotted solutions. They also react with iodoplatinate and 2,7-dichlorfluoroescein solution. With the developing solvent of $CHCl_3$-diethylamine (96:4), they are all carried near the solvent front, where they form a wide band. They do not consequently interfere with the detection of quinine or tropane alkaloids.

If a sample containing quinidine and no phenylethylamines is tested for atropine contamination (or vice versa) use the following developing solvent: $CHCl_3$–CH_3OH–CH_3COOH (90:10:4). For detection of pyrilamine and chlorpheniramine, develop chromatogram with $CHCl_3$-diethylamine (98:2). These compounds can be located under short-wave UV light. They also react with iodoplatinate and 2,7-dichlorfluoroescein solution. Difficulties may arise if sample contains alkaloids or phenylethylamines.

Contaminants—Phenylethylamines—To sample in 100-ml. centrifuge tube, add 10 ml. 0.1 N H_2SO_4 and swirl well; then add 55 ml. $CHCl_3$ and 5 ml. 1 N NaOH. Extract and cleanup as in preceding section. If the sample to be tested contains alkaloids, use a distillation and extraction procedure similar to that used by McCullough (12) for the determination of amphetamine. Prepare a distillation apparatus consisting of a 500-ml. Kjeldahl flask, a bulb-type distilling head, a connector, and a water condenser. Keep end of condenser immersed in a beaker containing 25-ml. 1 N H_2SO_4. Macerate sample in a beaker with two successive 25-ml. portions 1 N H_2SO_4 and filter into Kjeldahl flask. Wash beaker with a total of 175 ml. of water in several portions and filter into flask. Quickly add 25 ml. 20% NaOH solution, connect to rest of distilling apparatus, heat with burner, and distill 150 ml. Control heating continuously to prevent foaming or back suction. Transfer distillate to 250-ml. separatory funnel, washing beaker with small portions of water. Add to separator 50 ml. $CHCl_3$ and 25 ml. 20% NaOH solution. Shake well 1 min. and filter $CHCl_3$ layer. Extract with two more 50-ml. portions of $CHCl_3$, filter, and combine with previous extract. Develop chromatogram with $CHCl_3$–CH_3OH–CH_3COOH (90:10:4).

Spray develop plate with iodoplatinate or 2,7-dichlorofluorescein solution. Note: if sample contains quinine, quinidine, or their salts, small quantities of these compounds or decomposition products of them are distilled. They do not interfere with detection of the phenylethylamines, since they are carried near solvent front. Pyrilamine and chlorpheniramine (or decomposition products of them) distill in greater amounts; presence of these compounds in a sample may make difficult or impossible the phenylethylamine contamination test.

Contaminants—Steroids—Extract sample as under *Barbiturates* substituting water for 0.1 N H_2SO_4 solution. Prepare a column suitable to retain the declared active ingredients of the sample. For example; a diatomaceous earth-tripotassium phosphate column can be used to retain weakly acidic compounds such as barbiturates (5),

Table I—TLC Data of Some of the Drugs Tested for Contamination

Drug	R_f	Developing Solvent
Phenobarbital	0.44	Chloroform-Acetic Acid (96:4)
Butabarbital	0.64	Chloroform-Acetic Acid (96:4)
Amobarbital	0.72	Chloroform-Acetic Acid (96:4)
Digoxin	0.39	$CHCl_3$-CH_3OH (90:10)
Gitoxin	0.46	$CHCl_3$-CH_3OH (90:10)
Digitoxin	0.63	$CHCl_3$-CH_3OH (90:10)
Quinine	0.11	$CHCl_3$-diethylamine (96:4)
Quinidine	0.23	$CHCl_3$-diethylamine (96:4)
Atropine	0.26	$CHCl_3$-diethylamine (96:4)
Scopolamine	0.50	$CHCl_3$-diethylamine (96:4)
Amphetamine, methamphetamine, ephedrine, phenylpropanolamine	0.71	$CHCl_3$-diethylamine (96:4)
Atropine	0.32	$CHCl_3$-CH_3OH-CH_3COOH (90:10:4)
Ephedrine	0.36	$CHCl_3$-CH_3OH-CH_3COOH (90:10:4)
Amphetamine	0.48	$CHCl_3$-CH_3OH-CH_3COOH (90:10:4)
Phenylpropanolamine	0.54	$CHCl_3$-CH_3OH-CH_3COOH (90:10:4)
Methamphetamine	0.60	$CHCl_3$-CH_3OH-CH_3COOH (90:10:4)
Quinine-quinidine	0.96	$CHCl_3$-CH_3OH-CH_3COOH (90:10:4)
Scopolamine	0.99	$CHCl_3$-CH_3OH-CH_3COOH (90:10:4)
Testosterone	0.14	$CHCl_3$-CH_3OH (99:1)
Methyltestosterone	0.17	$CHCl_3$-CH_3OH (99:1)
Ethinyl estradiol	0.20	$CHCl_3$-CH_3OH (99:1)
Desoxycorticosterone	0.32	$CHCl_3$-CH_3OH (99:1)
Estrone	0.36	$CHCl_3$-CH_3OH (99:1)
Progesterone	0.41	$CHCl_3$-CH_3OH (99:1)
Prednisolone	0.13	$CHCl_3$-CH_3OH (99:5)
Hydrocortisone	0.17	$CHCl_3$-CH_3OH (99:5)
Prednisone	0.27	$CHCl_3$-CH_3OH (99:5)
Cortisone	0.30	$CHCl_3$-CH_3OH (99:5)
Prednisolone acetate	0.50	$CHCl_3$-CH_3OH (99:5)
Hydrocortisone acetate	0.56	$CHCl_3$-CH_3OH (99:5)
Cortisone acetate	0.62	$CHCl_3$-CH_3OH (99:5)
Methapyrilene	0.55	$CHCl_3$-CH_3OH (80:20)
Acetaminophen	0.75	$CHCl_3$-CH_3OH (80:20)

a diatomaceous earth-pH 10.1 solution column to retain acetaminophen (13), a diatomaceous earth-H_2SO_4 column to retain alkaloids and phenylethylamines (10), and a diatomaceous earth-$NaHCO_3$ solution column to retain aspirin (14). A multilayer column can also be used.

Below the first column and receiving its eluates, place a chromatographic column (*b*), packed to a height of 3 cm. with silicic acid. Elute extracts through both columns, then add 100 ml. $CHCl_3$. After all $CHCl_3$ has eluted, remove top column, change receiver, add to column (*b*) 50 ml. of 20% methanol in $CHCl_3$, and elute. Develop chromatogram with $CHCl_3$-CH_3OH (99:1 or 99:5) depending on which steroid is to be detected. See Table I in this respect. Spray develop plate with 40% H_3PO_4, then heat in oven at 120° for about 30 min. (15). Brown, red, or dark spots form, according to compound. The method cannot be used when the sample's ingredients cannot be trapped on a column.

Contaminant—Methapyrilene or its Salts—Shake sample in 100-ml. volumetric flask with 40 ml. $CHCl_3$, fill to mark with $CHCl_3$, and mix. Prepare a diatomaceous earth-H_2SO_4 solution column. Pipet 5 ml. of solution to beaker, mix with 20 ml. of ether, and transfer to column. After first portion has eluted, add five successive 5-ml. portions of ether and discard eluates. Change receiver and elute with 1 ml. of ammonium hydroxide followed by 250 ml. $CHCl_3$. Use fluorescent plates. Develop with $CHCl_3$–CH_3OH (80:20). Examine developed plate under short-wave UV light. Note: if sample contains acetaminophen, traces of this compound will be present in the spotted solution, but will not interfere with detection of methapyrilene.

Contaminant—Acetaminophen—Shake sample in 100-ml. volumetric flask with 4 ml. of methanol, add $CHCl_3$ to volume, and mix. Prepare a column to retain declared active ingredients of sample, if possible. Pipet 5 ml. of solution to a beaker, mix with 20 ml. of ether, and transfer to column. After first portion has eluted, add five successive 5-ml. portions of ether.

Develop and examine plates as under *Methapyrilene*. Note: neutral compounds are similarly eluted. If sample contains methapyrilene and a diatomaceous earth-H_2SO_4 solution column is used to trap it, traces of this compound will be eluted but will not interfere with identification of acetaminophen.

Contaminant—Meprobamate—Macerate sample with three 50-ml. portions of $CHCl_3$ and filter. Develop chromatogram with $CHCl_3$–CH_3OH (90:10). Detect meprobamate visually or under UV light after spraying plate with 2,7-dichlorfluoroescein. Generally this method is not applicable if sample contains compounds soluble in $CHCl_3$.

RESULTS AND DISCUSSION

The column and thin-layer chromatographic conditions indicated in the specific methods are intended to serve as examples and can be modified to meet situations different from the ones considered. For additional column chromatography and TLC information consult the review article of Levine (16) and that of Comer and Comer (17).

The interpretation of a chromatogram must be done very carefully especially when sample compounds, that could not be completely removed during the cleanup process, have a TLC behavior such that they could be mistaken for the contaminant of interest. Generally when a positive contamination test is obtained for a sample, it is desirable to perform additional analyses. If the contaminant has sufficient UV absorbance and the detection is done in a nondestructive manner, the spot can be scraped from the plate and extracted with a suitable solvent. The centrifuged extract, concentrated to a small volume (1:3 ml.), can be read on a recording spectrophotometer in 2- or 5-cm. microcells. Micro IR identification can also be attempted, if the laboratory has the necessary equipment. If UV or IR identification cannot be obtained, additional TLC tests could be performed changing the conditions and using the spray solution most specific for the given contaminant.

If doubts remain, despite additional analysis, it is generally not advisable to report the suspected contamination.

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Chromatographic Analysis of Chlorpheniramine, Pyrilamine, and Methapyrilene in Combination

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Abstract ☐ A partition chromatographic method is presented for the separation and quantitation of chlorpheniramine, methapyrilene, and pyrilamine in combination. A solution of *p*-toluenesulfonic acid and potassium chloride on a column of diatomaceous earth is used to effect the separation. The isolated chlorpheniramine is determined by UV spectrophotometry. The methapyrilene and pyrilamine are separated and determined by GLC.

Keyphrases ☐ Chlorpheniramine, pyrilamine, methapyrilene combination—analysis ☐ Column chromatography—separation ☐ UV spectrophotometry—analysis ☐ GLC—analysis

Pharmaceutical preparations containing chlorpheniramine, methapyrilene, and pyrilamine in combination have been on the market for a number of years. However, the literature does not cite a method for the analysis of this combination. GLC has been used successfully to separate and analyze a large number of antihistamines (1–4). However, with these chromatographic procedures the separation of methapyrilene and chlorpheniramine is not complete enough to permit their analysis.

The separation and analysis of pharmaceutical amines based on ion-pair partition chromatography has been reported by Levine *et al.* (5–7). They have shown that *p*-toluenesulfonic acid (tosic acid) is effective in extracting a wide variety of these amines.

In the present study, a tosic acid-potassium chloride partition column is used to separate chlorpheniramine from methapyrilene and pyrilamine. Chlorpheniramine is determined by UV spectrophotometry; methapyrilene and pyrilamine are determined by GLC.

EXPERIMENTAL

GLC Column Preparation—The packing support was prepared and conditioned in the following manner (4): 200 mg. of cyclohexanedimethanol succinate[1] and 2.0 g. of methylphenyl silicone[2] were weighed into a 600-ml. beaker and dissolved in 350 ml. of benzene–toluene (1:2) by heating on the steam bath with mixing. To this solution, 20 g. of 80–100-mesh diatomaceous earth[3] was added. The solvent was removed by evaporation on a steam bath with frequent stirring. The packing material was slowly added to a 1.8-m. (6-ft.) × 4-mm. i.d. glass coiled column, using vacuum and tapping. The column was conditioned overnight at 250° with a 50 ml./min. nitrogen flow.

Apparatus—A gas chromatograph[4] equipped with a flame ionization detector and a 1-mv. recorder was used. The operating conditions were: column temperature, 250°; inlet temperature, 280°; detector temperature, 250°; voltage, 250; carrier gas, nitrogen at 100 ml./min.; sensitivity, 1×10^{-9} amp. full scale.

A recording UV spectrophotometer was used with 1-cm. silica cells.

Reagents—*Tosic Acid-KCl Solution*—Dissolve 10 g. of *p*-toluenesulfonic acid and 11.2 g. of KCl in 35 ml. of distilled water with heating. Cool, dilute to 50 ml., and mix.

Diatomaceous Earth[5]—Acid wash as outlined by USP XVII (8).

Ether and Chloroform—Reagent Grade—Wash twice with equal volumes of water.

Standard Solutions—Pyrilamine maleate NF reference standard and methapyrilene HCl,[6] 1 mg./ml. in methanol; chlorpheniramine maleate USP reference standard, 0.02 mg./ml. in 0.1 N H_2SO_4.

Column Preparation—*Column I*—Mix 2.0 g. of diatomaceous earth with 1.0 ml. of 1 N NaOH, transfer to a chromatographic column[7] containing a pad of glass wool, and tamp. Weigh a portion of finely ground sample equivalent to 5 mg. of pyrilamine maleate into a 100-ml. beaker. Add 2.0 ml. of 1 N NaOH and 3.0 g. of diatomaceous earth. Mix until uniform; quantitatively transfer to column and tamp. Cover with a pad of glass wool.

Column II—Mix 3.0 g. of diatomaceous earth and 2.0 ml. of 1 M $NaHCO_3$, transfer to a second column containing a pad of glass

[1] HI-EFF-8BP, Applied Science Laboratories, Inc., State College, PA 16801
[2] SE-52, Analabs, Inc., Hamden, Conn.
[3] Gas Chrom Q, Applied Science Laboratories, Inc., State College, PA 16801
[4] Packard model 7621S, Packard Instrument Co., Downers Grove, IL 60515
[5] Celite 545, Johns-Manville Corp., New York, N. Y.
[6] K & K Laboratories, Plainview, N. Y.
[7] Cat. No. 420300, 250 mm. × 22 mm. i.d., Kontes Glass Co., Vineland, N. J.