Acetonitrile–Diatomaceous Earth Column for Corticosteroids

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Abstract □ A column of acetonitrile on diatomaceous earth is used to hold the corticosteroid on the column during the wash of the column with *n*-heptane to remove decomposition products and interfering substances, after which the corticosteroid and acetonitrile are eluted from the column with chloroform. The recommended procedure significantly reduces or completely eliminates the interference of various substances and certain decomposition products in the blue tetrazolium, phenylhydrazine, isonicotinic acid hydrazide, and UV methods of determination. The column can be readily modified to include acidic, basic, or neutral aqueous-trap layers when necessary. There is no significant difference in precision between the proposed procedure and the normal precision of the determinative methods. Results on typical pharmaceutical preparations, some of which show evidence of extensive decomposition, are given.

Keyphrases □ Corticosteriod dosage forms—analysis □ Acetonitrile–diatomaceous earth column—corticosteroid separation □ Column chromatography—separation □ Blue tetrazolium, phenylhydrazine, isonicotinic acid hydrazide methods—corticosteroid analysis □ UV spectrophotometry-analysis

The determination of corticosteroids in many pharmaceutical preparations, other than those official in USP (1) and NF (2), can be difficult due to the interference of certain ingredients and because official methods do not always detect the presence of decomposition products of corticosteroids in the preparation. As a consequence, many "cleanup" procedures have been proposed to remove interferences and/or decomposition products before the actual final determinative step in the analysis of corticosteroids.

Levine (3) has reviewed the column partition chromatography of steroids and listed solka floc (4), silica (5, 6), siliconized diatomaceous earth (7), and diatomaceous earth (8) as the solid supports used. Jakovljevic *et al.* (9) used magnesium–silica gel[1] column chromatography with various solvent systems to remove the more polar interferences. Bracey *et al.* (10) utilized a column of methanol and water on acid-washed diatomaceous earth to remove interfering antibiotics from corticosteroid preparations.

This paper reports a new column partitioning chromatographic procedure which effectively traps the corticosteroid in an acetonitrile layer on a diatomaceous earth column while interferences are removed by washing with *n*-heptane. The acetonitrile and corticosteroid are then removed from the column with chloroform. The method may be modified readily for the removal of acidic, basic, and/or other water-soluble interferences.

EXPERIMENTAL

Apparatus—The following were used: Cary model 15 recording spectrophotometer; glass chromatographic columns, 2.2 × 25 cm. constricted at one end to 0.4 × 5 cm.; and aluminum tamping tool to fit chromatographic column.

Reagents—*Solvents*—All solvents were spectro, certified, analytical reagent, USP, or distilled-in-glass grade.

Acetonitrile, *n*-heptane, chloroform, methanol, and alcohol USP were used.

Acetonitrile–*n*-heptane (mutually saturated): mix 30 ml. of acetonitrile and 400 ml. of *n*-heptane in a separator. Agitate vigorously for at least 2 min. and separate when both layers are completely clear. These saturated solutions are to be used whenever *n*-heptane or acetonitrile is called for in these directions.

Chloroform (water saturated): add 50 ml. of water to 400 ml. of chloroform in a separator. Agitate vigorously for 2 min. and separate only when both layers are clear.

Diatomaceous earth,[2] acid washed, was used.

Standards—Hydrocortisone, hydrocortisone acetate, prednisolone, prednisolone acetate, prednisone (all USP reference standards); dexamethasone (NF reference standard); betamethasone (Schering Corp.); and flurandrenolone (Eli Lilly) were used.

All standard solutions were prepared as 1.00 mg./100 ml. in alcohol USP or as 100 mg./100 ml. in alcohol USP.

Reagents used in the determinative steps were prepared as specified in the method reference.

Proposed Column Procedure—*Preparation of Column*—Acetonitrile layer: insert a glass wool plug in the bottom of the chromatographic column. Thoroughly mix 4.0 g. of diatomaceous earth with 4.0 ml. of acetonitrile, transfer to the column, and pack firmly.

Aqueous trap layer: when it is necessary to remove water-soluble neutral impurities, an aqueous trap layer is used above the acetonitrile layer. Mix 2.0 g. of diatomaceous earth with 2.0 ml. of water, transfer to the column above the acetonitrile layer, and tamp firmly. If this type of trap is used, the chloroform must be saturated with water.

Aqueous basic trap layer: when it is necessary to remove water-soluble acidic interferences, a basic trap layer is used in the column above the acetonitrile layer. Mix 2.0 g. of diatomaceous earth with 2.0 ml. of either 8% (w/v) $NaHCO_3$ or 10% (w/v) Na_2CO_3, transfer to the column, and pack firmly above the acetonitrile layer. When this type of trap is used, the chloroform must be water saturated.

Aqueous acidic trap layer: when it is necessary to remove water-soluble basic interferences, both an aqueous layer and an acidic layer are used above the acetonitrile layer. Mix 1.0 g. of diatomaceous earth with 1.0 ml. of water and transfer to the column above the acetonitrile layer. Mix 2.0 g. of diatomaceous earth with 2.0 ml. of 1 *N* HCl, transfer to the column above the aqueous layer, and tamp firmly. Water-saturated chloroform is required in this case.

Sample layer: dissolve the sample residue, prepared as directed under *Sample Preparation*, in 1.0 ml. of acetonitrile and 2.0 ml. of *n*-heptane. Mix the solution thoroughly with 3.0 g. of diatomaceous earth, transfer to the column above the acetonitrile or other trap layer, and pack firmly. Dry wash the sample beaker with 1 g. of diatomaceous earth and with glass wool, both of which are transferred to the top of the column. Retain the sample beaker for washing with *n*-heptane and chloroform.

Elution of Column—Wash the sample beaker successively with six 25-ml. portions of *n*-heptane, which are transferred to the column. The liquid head should be maintained between 8 and 12 cm. above the column bed until the last wash, which is allowed to drain completely from the column. Wash the tip of the column with alcohol and discard the *n*-heptane and alcohol wash. Rinse the sample beaker with five 25-ml. portions of chloroform and pour each through the column, maintaining a liquid head of 8–12 cm. above the column bed until the last portion, which is allowed to drain completely from the column. Rinse the tip of the column with chloroform and add to the eluate. Evaporate the eluate carefully to dryness under air in the hood to ensure that the acetonitrile is evaporated completely and that fumes of acetonitrile are not generated in the open laboratory since acetonitrile vapors are toxic.

[1] Florisil, Floridin Co., Pittsburgh, PA 15222

[2] Celite 545, Johns-Manville Products Corp., New York, NY 10016

Table I—Precision of Column Procedure

Sample Number	Taken, mg.	Recovery[a] of Hydrocortisone Standards, mg. BT Method	PH Method	INH Method	UV Method
1	2.01	2.01	1.98	2.00	2.04
2	2.01	2.02	1.98	2.01	2.03
3	2.01	2.01	2.01	2.01	2.03
4	2.01	2.01	2.01	2.02	2.03
5	2.01	1.99	2.00	2.03	2.03
6	2.01	2.02	2.01	2.03	2.04
Av.	2.01	2.01	2.00	2.02	2.03
SD^b (%)		0.60	0.60	0.60	0.20

[a] Compared to aliquots of the same sample determined identically but without going through the column procedure. [b] Calculated from the range by the method of Dean and Dixon (14).

Preparation of Sample Solution for Determinative Procedures—Dissolve the residue from the chloroform eluate in alcohol USP and dilute accurately to a volume that will contain approximately 1 mg. of corticosteroid/100 ml. Use proper size aliquots of this solution for determination by one or more of the determinative procedures.

Determinative Procedures—Blue tetrazolium (BT) method: the procedure given in USP XVII (1) and NF XII (2) was followed, except that 10.0-ml. aliquots and 1.0 ml. each of the BT reagent and of the tetramethylammonium hydroxide reagent were used.

Phenylhydrazine (PH) method: the procedure of Silber and Porter (11) was followed without modification.

Isonicotinic acid hydrazide (INH) method: the procedure of Umberger (12) was used, except that the INH reagent was modified by using twice the recommended concentration of hydrochloric acid to increase the sensitivity of the reaction (13).

UV spectrophotometry: The alcohol USP solution was scanned directly in a 1-cm. cell.

Sample Preparation—*Lotions, Creams, and Ointments*—The sample size should be sufficient to contain approximately 5 mg. of the corticosteroid. For preparations declared on a weight–volume basis, transfer the selected volume to a beaker, using a pipet calibrated "to contain," and wash the contents of the pipet into the beaker with warm alcohol USP. For preparations declared on a weight–weight basis, weigh an accurate sample as rapidly as possible into a beaker. In either case, add warm alcohol USP up to a total volume of 30 ml. and heat on the steam bath with periodic agitation to incipient boiling. Cool in an ice bath until the residue solidifies and then decant the liquid into a 100-ml. volumetric flask. Repeat the extraction with three 20-ml. portions of warm alcohol, decanting each into the flask after cooling. Adjust to room temperature and dilute to volume with alcohol USP. Filter if necessary. Carefully evaporate a 20.00-ml. aliquot to dryness on the steam bath and continue as directed under "Sample Layer."

Drops, Injectables, and Suspensions—Accurately measure a volume of the sample containing approximately 1 mg. of corticosteroid into a beaker. Carefully evaporate to dryness under air on a steam bath and continue as directed under "Sample Layer."

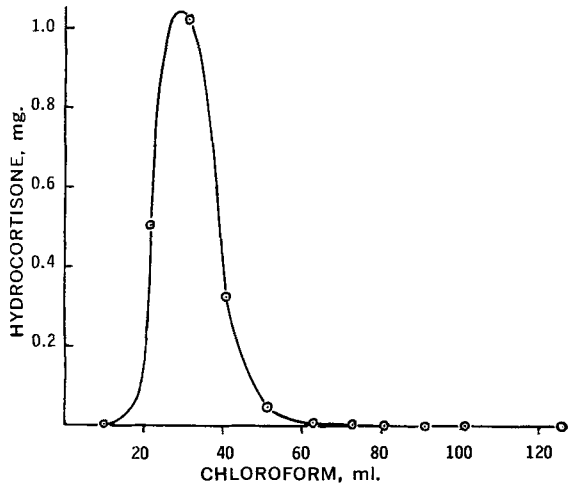

Figure 1—*Elution curve for removal of corticosteroid and acetonitrile from column with chloroform.*

Table II—Precision of Determinative Methods

Sample[a] No.	Absorbance BT Method	PH Method	INH Method	UV Method
1	0.548	0.387	0.340	0.451
2	0.551	0.394	0.338	0.451
3	0.551	0.387	0.340	0.450
4	0.551	0.391	0.340	0.450
5	0.550	0.386	0.347	0.447
6	0.551	0.386	0.339	0.451
Av.	0.550	0.388	0.341	0.450
SD^b	0.0012	0.0032	0.0036	0.0016
SD (%)	0.22	0.82	1.05	0.35

[a] Samples of hydrocortisone standard (1.00 mg.) were dissolved in chloroform, evaporated to dryness, dissolved, and diluted to volume in alcohol USP; aliquots were determined by each method. [b] Calculated from the range by method of Dean and Dixon (14).

Tablets—Weigh 20 tablets to obtain the average tablet weight. Grind the 20 tablets to pass a 60-mesh screen, mix thoroughly, and accurately weigh a sample containing approximately 1 mg. of corticosteroid into a beaker. Cover the sample with 1.0 ml. of methanol, swirl periodically during a 10-min. period, and add 1.0 ml. of water. Mix the solution thoroughly with 3.0 g. of diatomaceous earth; continue as directed under "Sample Layer" beginning with "...transfer to the column above the acetonitrile...." Use water-saturated chloroform in the elution step.

Buffered tablets that contain alumina or magnesium carbonate will not disperse properly under the conditions listed and must be dissolved by the following procedure. Prepare the column with the acetonitrile layer and an aqueous trap layer as directed previously. Add 1.0 ml. of dilute HCl to the accurately weighed sample and allow to stand for 5–10 min. to ensure complete solution of the oxide and/or carbonate. Add 2.0 ml. of water and 3.0 g. of diatomaceous earth and mix thoroughly. Transfer to the column; continue as directed under the "Sample Layer" beginning with "Dry wash the beaker...." Use chloroform saturated with water in the elution step.

Capsules—Weigh the net contents of 20 capsules to determine the average capsule content. Mix thoroughly and accurately weigh a sample containing approximately 1 mg. of the corticosteroid into a beaker. Proceed as directed under *Tablets* beginning with "Cover the sample with 1.0 ml. of methanol...."

METHOD DEVELOPMENT

Elution Curve—The column was prepared as directed under *Column Preparation*, including a sample of approximately 2 mg. of reference standard hydrocortisone. To ensure constant flow conditions, a 12-cm. liquid head was maintained in the column during elution by use of a separator. The 150-ml. *n*-heptane wash was evaporated to dryness, dissolved in alcohol USP, and scanned by UV. The spectrum showed no evidence of the presence of hydrocortisone. The chloroform eluate was caught in ten 10-ml. fractions and two 25-ml. fractions. Each fraction was taken to dryness,

Table III—Capacity of Column

Hydrocortisone Taken, mg.	Hydrocortisone Recovered, mg.	Recovery, %
1.00	1.02	102
2.00	2.02	101
4.00	4.00	100
6.00	5.92	99
8.00	7.89	99
10.0	9.84	98
15.0	15.1	101
20.0	19.8	99
	Av.	99.9%
	SD^a	1.4%

[a] Calculated from the range by method of Dean and Dixon (14).

Table IV—Applicability of Column Procedure to Various Corticosteroids

Corticosteroid	Taken, mg.	Recovered, mg.	Recovered, %
Betamethasone	2.120	2.155	101.7
Cortisone acetate	2.036	2.054	100.9
Deoxycorticosterone acetate	2.015	2.026	100.5
Dexamethasone	2.080	2.104	101.2
Flurandrenolone	2.003	1.994	99.6
Hydrocortisone acetate	2.000	1.952	97.6
Prednisolone	2.000	2.000	100.0
Prednisolone acetate	2.180	2.176	99.8
Prednisone	2.080	2.105	101.5
		Av.	100.3
		SD[a]	1.39%

[a] Calculated from the range by the method of Dean and Dixon (14).

Table V—Removal of Interferences in Products by Proposed Procedures

Method of Analysis	Declared Value, %[a] BT	PH	INH	UV
Hydrocortisone Cream, 0.125%[b]				
Direct	101.4[c]	95.0	113.8[c]	ND[d]
Recommended procedure	99.2[c]	94.6	96.9	ND[d]
Buffered Prednisolone Tablets, 5 mg.				
Direct	65.8[c]	44.8	84.6	ND[d]
Recommended procedure	50.5[c]	45.3	64.8	65.1

[a] Average of duplicates. [b] Interferences present according to manufacturer's declaration were lanolin, stearic acid, and parabens. [c] Variation of absorbance with time indicates unidentified interference is present. [d] ND—not determined by this method.

dissolved in alcohol USP, and determined by UV spectrophotometry. The elution curve, shown in Fig. 1, indicates that the corticosteroid was completely eluted in the first 100 ml. of eluate. Since over 99.5% was eluted in the first 80 ml. of eluate, the use of 125 ml. of chloroform in the recommended procedure includes a definite safety factor.

Replication Studies—Six 2.00-ml. aliquots of a standard hydrocortisone solution containing 1.00 mg./ml. were determined by the suggested column procedure. Aliquots of the final alcohol solution were analyzed by all four determinative steps and compared to values obtained from aliquots of the same standard solution determined directly without being put through the column. The values are shown in Table I. The standard deviation, as calculated from the range by the method of Dean and Dixon (14), varies from 0.20% for the UV method to 0.60% for the PH method, with an average of 0.50% for all four determinative procedures. This is approximately the same as the average standard deviation for six replicate 1.00-mg. samples of standard hydrocortisone which were dissolved in 100 ml. of chloroform, evaporated carefully to dryness, made to volume with alcohol USP, and determined by all four determinative procedures. The results, shown in Table II, have an average standard deviation of 0.61%.

Column Capacity Study—Samples of from 1.00 to 20.0 mg. of hydrocortisone were placed on and eluted from the column by the suggested procedure. The eluates were evaporated to dryness, dissolved in alcohol, and analyzed by UV spectrophotometry. The results were compared to identical samples which were not placed on and eluted from the column and are reported as percent recovery in Table III. Samples containing at least 20 mg. of corticosteroid can be determined safely by the suggested procedure.

Corticosteroid Applicability Studies—Since all of the method-development work utilized hydrocortisone, other corticosteroid standards were analyzed by the proposed column procedure utilizing the INH procedure and compared to identical standards which were not put on and eluted from the column. The results are shown in Table IV. The recoveries ranged from 97.6 to 101.7%, with an average recovery of 100.3% and a standard deviation calculated from the range by the method of Dean and Dixon (14) of 1.39%.

Interference Studies—The efficiency of the suggested procedure for the complete removal or reduction of interferences in the various determinative methods was investigated in sample preparations and by the addition of the interfering substances to hydrocortisone standards. In each case, the measurement was made on aliquots of the alcohol extract of the sample or of the extract of the standard plus the interfering substance. One aliquot was measured directly and the second was put through the column separation step before measurement. In the investigation involving the standard plus added interference, a standard without the interfering substance was also determined directly.

The results for two typical samples are shown in Table V. The values for the hydrocortisone cream show interference in bot the BT and INH procedures, but none in the PH method when run directly. The interference in both BT and INH measurements was detected using the variation of absorbance with time method (15). The column separation procedure reduces the amount of interference in the BT method and completely removes the substances that interfere in the INH measurement.

Results for the buffered prednisolone tablets show the versatility of the suggested procedure in the detection and estimation of decomposition products in corticosteroid preparations. When run directly, interference was indicated only in the BT method, and this interference was not completely removed by the column procedure. Since the INH procedure depends upon conjugation in Ring A of the corticosteroid, and the BT and PH reagents react with the side chain at C_{17} (15), the difference between the values by INH and PH when run directly indicates that the C_{17} side chain in approx-

Table VI—Removal of Certain Interferences by Recommended Procedure

Interfering Substance	Interfering Substance, mg./mg. Hydrocortisone	Absorbance Method of Measurement: Blue Tetrazolium — Standard Only, Direct	Blue Tetrazolium — Standard + Interference, Direct	Blue Tetrazolium — Standard + Interference, Procedure	Phenylhydrazine — Standard Only, Direct	Phenylhydrazine — Standard + Interference, Direct	Phenylhydrazine — Standard + Interference, Procedure	Isonicotinic Acid Hydrazide — Standard Only, Direct	Isonicotinic Acid Hydrazide — Standard + Interference, Direct	Isonicotinic Acid Hydrazide — Standard + Interference, Procedure
Lanolin	600/1	0.580	0.839	0.600	0.540	T[a]	0.538	0.334	0.764	0.348
Polysorbate 60	25/1	0.580	0.611	0.573	0.540	T[a]	0.521	0.334	0.332	0.338
Sodium lauryl sulfate	20/1	0.580	0.574	0.573	0.540	T[a]	0.537	0.334	0.332	0.328
Sorbitan monooleate	250/1	0.580	0.742	0.637	0.540	T[a]	0.552	0.334	0.468	0.362
Sorbitan monostearate	250/1	0.580	0.674	0.607	0.540	T[a]	0.539	0.334	0.366	0.335
Sulfide[b]	0.133/1	0.580	0.703	0.580	0.540	0.540	0.540	0.334	0.336	0.333
Sulfur	0.05/1	0.580	0.732	0.580	0.540	0.540	0.538	0.334	0.334	0.332

[a] T means solution becomes turbid so that absorbance cannot be determined. [b] Acetonitrile column modified with aqueous trap layer.

Table VII—Typical Samples Analyzed by the Proposed Procedure

Product	Corticosteroid	Interfering Substances Present[b]	Declared Value, %[a] Method of Analysis BT	PH	INH
A. Samples Showing Little or No Decomposition					
Cream 0.25%	Hydrocortisone	a, c	103.6[c]	105.6[c]	101.8[c]
Lotion 0.125%	Hydrocortisone	c, e	100.0[c]	99.2[c]	99.7[c]
Lotion 0.25%	Hydrocortisone	c	110.8	108.4	109.6
Lotion 0.50%	Hydrocortisone	a, c, g	99.2[d]	94.4	96.9
Lotion 1%	Hydrocortisone	a	107.2[c]	107.5[c]	107.4[c]
Drops 25 mg./ml.	Hydrocortisone acetate	—	78.4	80.6	79.0
Drops 0.2%	Prednisolone	—	89.0	ND[e]	89.5
Suspension 0.1%	Dexamethasone	—	106.3	106.8	107.0
Suspension 0.25%	Prednisolone acetate	c	111.8[c,d]	99.8[c]	102.1[c]
Tablets 0.75 mg	Prednisone	d	90.1[c,f]	ND[e]	88.0[c,f]
B. Samples Showing Decomposition					
Cream 0.125%	Hydrocortisone	a, c, g	48.4[d]	30.2	71.1
Cream 0.125%	Hydrocortisone	a, c, g	82.2[d]	45.0	60.0
Cream 0.50%	Hydrocortisone	a, c, g	111.7[d]	102.7	113.2
Lotion 0.125%	Hydrocortisone	a, c, g	24.3[d]	2.5	43.8
Lotion 0.25%	Hydrocortisone	c, e, f	97.6[c,d]	90.6[c]	100.3[c]
Lotion 0.50%	Hydrocortisone	a, c, g	95.3[d]	90.7	98.7
Ointment 0.50%	Hydrocortisone acetate	h	104.2[d]	93.4	96.9
Suspension 0.1%	Dexamethasone	—	98.5[c]	ND[e]	103.7[c]

[a] Average of duplicates. [b] Interfering substances present according to manufacturer's declared content: a, lanolin; b, magnesium stearate; c, parabens; d, salicylamide; e, sodium lauryl sulfate; f, sorbitan monostearate; g, stearic acid; and h, selenium sulfide. [c] Single determination. [d] Variation of absorbance with time indicates presence of unidentified interference. [e] ND, not determined by this method. [f] Salicylamide was removed by extraction of $CHCl_3$ eluate with 0.25 *N* NaOH before evaporation to dryness.

imately 40% of the declared amount of the corticosteroid had been oxidized to neutral or acidic products. The column procedure for buffered tablets of this type requires an aqueous trap layer to remove HCl from the chloroform, and this aqueous layer also removes any acidic decomposition products. The difference between the INH values before and after being put through the column indicates that approximately half of the decomposition products were acidic. The difference between the INH and PH results after being put through the column indicates that the other half of the decomposition products were neutral. These results also indicate that only 45% of the declared amount was actually present in the sample at the time of the analysis.

The results of the investigation using standards with added interfering substances are summarized in Table VI. The substances added have been reported to interfere in at least one of the determinative methods (15). The proposed procedure either eliminates the interference completely or greatly reduces it at the level shown. The first column of figures under each method is the absorbance for the standard alone run directly, the second column is the standard plus interference run directly, and the third column is the absorbance of standard plus interference after being separated by the proposed procedure. The differences in the values in the three columns indicate the extent of interference and the efficiency of removal.

RESULTS AND DISCUSSION

The results on typical undecomposed and partially decomposed corticosteroid preparations are summarized in Table VII. In each case in which there is significant disagreement between the BT and PH values, a study of the variation of absorbance with time indicates that some unidentified interfering substance is present. It is also apparent that such discrepancies are found more often in samples that have undergone decomposition, as indicated by significant differences between values obtained by the INH procedure and values obtained by the PH or BT method.

Diethyl ether was used instead of chloroform during part of this investigation for the removal of the corticosteroid from the column. It was found, however, that some bottles of ether contain ether peroxides, which cause decomposition of the corticosteroid during the evaporation step and also interfere with all four determinative methods. When ether is used, the column must be kept completely filled during the elution step to ensure that the corticosteroid is completely eluted from the column. Since chloroform has none of these disadvantages, it is the solvent of choice for the elution step. Isooctane (2,2,4-trimethylpentane) was substituted for the *n*-heptane in several analyses but did not improve the results obtained.

The few minor limitations of the proposed procedure include the care that must be used in the evaporation of acetonitrile, because it is toxic and because any acetonitrile left unevaporated will cause gross interference in the BT procedure. Also, if the liquid head in the column is maintained above 12 cm. during the *n*-heptane wash, some acetonitrile will be stripped from the column. This could lead to some of the corticosteroid also being removed during the wash step.

Some substances, which interfere in one or more of the determinative steps, are sufficiently soluble in both water and chloroform to be removed from an aqueous trap layer in the elution step. If this occurs, the eluate is evaporated, redissolved in acetonitrile–*n*-heptane, and transferred to a new column without an aqueous trap layer. One example of an interfering substance of this type is a decomposition product of corticosteroids thought to be a glyoxal.

Preliminary work with the column indicates that it can be used to improve the analysis of other types of steroids and may be used to separate with ease certain important steroids occurring in pharmaceutical preparations. Investigative work is continuing on the use of the column for such separations.

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Quantitative Determination of Butaperazine by TLC

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Abstract □ A method for the separation and determination of butaperazine in the presence of its degradation products is described. A sample is streaked onto a thin layer of silica gel G under a stream of nitrogen. The chromatogram is developed with isopropyl alcohol–ammonia (1 *N*) (4:1). The separated butaperazine is removed from the silica gel by elution with methanol and is determined quantitatively by UV spectroscopy. Details of the elution technique are described. Using the proposed method, quantitative recoveries are obtained from tablets and syrups.

Keyphrases □ Butaperazine in dosage forms—analysis □ TLC—separation □ UV spectrophotometry—analysis

The use of psychotropic drugs for the treatment of patients with emotional or mental disorders has led to widespread use of phenothiazine derivatives. One such derivative is butaperazine,[1] 2-(*n*-butyryl)-10-[3-(4-methyl-1-piperazinyl)-propyl]-phenothiazine.

The literature provides ample indication that several types of decomposition take place in these compounds. One type involves oxidation at the sulfur atom, leading to sulfoxide and eventually to sulfone (1). The quinonoid-type oxidation products of phenothiazine have been described (2–4). Huang and Sands (5, 6) studied the effect of UV irradiation on chlorpromazine solution under aerobic and anaerobic conditions. They found that under the former condition, oxidation prevails and the sulfoxide and *N*-oxide are formed; however, under the latter condition, the polymerization processes predominate.

The degree of deterioration and the type and amount of decomposition product pose difficult problems for the analyst. Consequently, for the purpose of establishing stability, it is necessary to devise a relatively simple but versatile separation, one that would be applicable to the quantitative determination of the phenothiazine derivative in experimental formulations.

Recently, Blazek (7) reviewed the procedures available for quantitative determination of phenothiazine derivatives. Included among the methods for these compounds are colorimetric (8), titrimetric (9), UV absorption spectrophotometric, and chromatographic procedures. One might choose any one of these except for the following considerations. The first two procedures are not selective for the undegraded compound. The UV method (10, 11) is an accurate and convenient means of assaying formulations containing phenothiazine derivatives, but it is unsuitable in badly degraded formulations because of the presence of other UV absorbing species. A paper chromatography technique (12, 13) was not selected because of degradation and tailing occurring during analysis (14, 15). Gas chromatographic procedures have been used for phenothiazines, but the present authors observed that butaperazine, because of its high boiling point and low thermal stability, was too low in volatility to be eluted quantitatively without extensive thermal decomposition from the several columns that were tried. Thus, they eliminated GLC from further consideration.

Since its introduction by Stahl (16), TLC has assumed a position of analytical importance for both the separation and analysis of complex inorganic, organic, and biological mixtures. Several papers have been published which describe quantitative thin-layer techniques (16–19). These methods can be classified as direct or indirect.

In the direct method, the developed chromatogram is quantitatively evaluated by measuring spot size or area or by densitometry. Thus, measurement is accomplished without removing the sought-for substance from the support. An indirect method implies removal of the separated substance from the plate. This may be followed by elution of the sample from the adsorbent and analysis of the eluant, usually by spectrophotometry or colorimetry. Indirect methods have the advantage in that spectra of the samples are readily obtained as part of the analysis. These may provide important additional information concerning identity and purity (20). Spencer and Beggs (21) have pointed out certain precautions which must be taken if an indirect method is to give precise, accurate results.

The authors report an application of the indirect method, utilizing TLC for the physical separation of butaperazine from its degradation products and, subsequently, quantitative determination of butaperazine using UV spectroscopy. Possible sources of error in the method are examined. The initial steps in the oxidative decomposition of butaperazine are shown.

[1] Marketed as Repoise by A. H. Robins Co., Richmond, VA 23220