A Column Chromatographic Method for the Determination of Sulfanilamide in Pharmaceutical Preparations Containing Sulfacetamide or Its Sodium Salt

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Abstract □ A simple and rapid method, using column-adsorption chromatography on alumina and UV spectrophotometry, has been developed for the separation and determination of sulfanilamide in ophthalmic solutions and tablets containing sulfacetamide or its sodium salt.

Keyphrases □ Sulfacetamide formulation—sulfanilamide determination □ Sulfanilamide—sulfacetamide degradation product □ Column chromatography—separation □ UV spectrophotometry—identity

The presence of undeclared sulfanilamide in preparations containing sulfacetamide has been reported (1–4). Hayden (1) found 17% sulfanilamide in a commercial sample of sulfacetamide powder, using paper chromatography and IR spectroscopy. Klein and Kho (2) reported undeclared sulfanilamide in suspensions of mixed sulfas containing sulfacetamide, using TLC. Previous work has shown that sulfacetamide preparations form sulfanilamide as a degradation product when exposed to light, extremes of temperature, or prolonged storage (3, 4). Gruber and Klein (4) used TLC on silica gel to separate the degradation product from the parent compound; the amount of sulfanilamide present was determined colorimetrically with the Bratton-Marshall reagent. Ophthalmic solutions contained contamination of 13% sulfanilamide, but tablets showed no detectable degradation over a period of 4 months.

Alexander and Stanley (5) used alumina for qualitative thin-layer chromatographic determination of sulfa drugs in feeds. The separation of sulfanilamide from sulfacetamide or its sodium salt was much greater in this system using alumina than in the silica gel system used by Gruber and Klein (4). (In both systems sulfanilamide has the greater R_f.) This large separation was the basis for adapting the qualitative thin-layer chromatographic procedure to a quantitative column chromatographic determination for sulfanilamide.

Sulfanilamide was easily separated and collected from a neutral alumina column, using the thin-layer developing solvent system of Alexander and Stanley (5). The sulfanilamide in the column eluate was quantified by UV absorption. The residue from the column eluate was also identified qualitatively as sulfanilamide by IR spectroscopy.

EXPERIMENTAL

Apparatus and Reagents—The following apparatus and reagents were used: 1-cm. (i.d.) glass columns fitted with Teflon stopcocks and sintered-glass frits (Kontes); aluminum oxide, neutral, suitable for chromatographic adsorption (Merck)[1]; spectrophotometric grade methanol and chloroform (Burdick and Jackson); USP sulfanilamide reference standard solution, 5 mcg./ml. in chloroform–methanol (70:30); a suitable recording UV spectrophotometer.

Preparation of Chromatographic Column—Heat the aluminum oxide for 30 min. at 100° before use. With the stopcock open, transfer 5.0 g. of aluminum oxide to the column in a slurry with chloroform–methanol (70:30). The solvent should drain quickly as the adsorbent settles. Wash the column with 20–25 ml. of the solvent mixture, and cover the top of the column with a pledget of glass wool. Drain the solvent to a level of 1 cm. above the glass wool.

If a flow rate of 1–2 drops per 10 sec. is encountered due to an excess of fine particles in the alumina, the column can be packed by the following procedure: place 10–15 g. of the alumina into a 250-ml. separator. Add 50 ml. of the solvent mixture and shake. The fine particles will stay suspended in the solvent. Open the stopcock of the column and add the alumina slurry from the separator to the predetermined height for 5 g. (about 7 cm.). Maintain a level of solvent above the alumina and wash with 20–25 ml. of the solvent mixture as in the previous column-packing procedure.

Sample Preparation—Transfer an accurately measured volume of ophthalmic solution or an accurately weighed portion of ground solid sample equivalent to about 200 mg. of sulfacetamide to a 100-ml. volumetric flask. Add 30 ml. of methanol and mix thoroughly. Dilute to volume with chloroform and mix again.

Procedure and Analysis—Pipet a 5-ml. aliquot of the sample solution onto the column; collect the eluate in a 50-ml. volumetric flask. After all the sample aliquot has entered the column, elute to volume with chloroform–methanol (70:30). Maintain a liquid level about 5 cm. above the alumina so that the time for collecting the 50 ml. is about 30 min. Obtain the absorbance of the sample at

Table I—Results of Determinations of Recoveries of Sulfanilamide

Sample	Sulfanilamide Found, %	Sulfanilamide, mcg. Added per Column Aliquot	Sulfanilamide, mcg. Recovered	Recovery, %
Standard 1[a] (sulfacetamide)	0.04	211	209	99.0
	0.02		210	99.5
	0.03		210	99.5
	0.05		212	100.4
	0.05		212	100.4
Standard 2[a] (sodium sulfacetamide)	0.54			
	0.52			
	0.52			
	0.52			
	0.53			
Standard 3 (recrystallized form of standard 2)	0.30	422	421	99.8
	0.28		420	99.6
	0.27		420	99.6
	0.29		418	99.1
	0.28		422	100.0
10% Ophthalmic solution of sodium sulfacetamide	2.31	211	208	98.6
	2.33		209	99.1
	2.32		210	99.5
	2.29		215	101.8
	2.29		213	100.9
30% Ophthalmic solution of sodium sulfacetamide	2.35	169	168	99.4
	2.36		168	99.4
	2.33		169	100.0
	2.33		168	99.4
	2.34		167	98.8
500-mg. Sulfacetamide tablets	0.08	252	253	100.4
	0.04		256	101.6
	0.05		252	100.0
	0.05		254	100.8
	0.05		253	100.4

[a] K & K Laboratories, Plainview, N. Y.

[1] Merck Reagent 71707 and Fisher Cat. No. A-950 were both found suitable.

262 mμ against the elution solvent blank. Compare the absorbance of the sample to that of the sulfanilamide reference standard solution.

RESULTS AND DISCUSSION

Proprietary standards of sulfacetamide and sodium sulfacetamide were analyzed by the column chromatographic procedure. A portion of the sodium sulfacetamide was recrystallized and also analyzed. All three standards were eluted with an additional 30 ml. of elution solvent after the first 50 ml. was collected. The 50-ml. fraction exhibited absorbance maxima at 262 mμ; the subsequent eluates did not show UV absorbance. Therefore, the sulfanilamide was completely eluted and no additional sulfanilamide or any sulfacetamide was present in subsequent 30-ml. eluates. However, each batch of alumina should be tested to ensure that the desired separation occurs in the first 50 ml. of eluting solvent.

Sulfanilamide was added to the free sulfacetamide and to the recrystallized sodium salt, and recoveries were obtained. Data from analyses using these standards are reported in Table I.

Two buffered ophthalmic solutions of 10 and 30% sodium sulfacetamide and a tablet form of sulfacetamide, 500-mg. label declaration, were assayed for their sulfanilamide content. Known amounts of sulfanilamide were added and percent recoveries were determined. Data from these analyses showed that the ophthalmic solutions contained a larger percentage of sulfanilamide than the tablets. This agrees with the quantitative findings of Gruber and Klein (4).

The USP XVII (6) or NF XII (7) procedure failed to detect any degradation of sulfacetamide preparations since it is a general titrimetric procedure for sulfonamides.

The residue left after evaporation of the column eluate was confirmed as sulfanilamide by IR spectroscopy, employing micro-KBr disk techniques.

All standards and samples used in this study were analyzed by the screening procedure of Gruber and Klein (4) on silica gel. Amounts were spotted to yield about 0.1 mcg. of sulfanilamide as determined by the column chromatographic procedure. Sulfanilamide was detected in all cases except in the sulfacetamide standard and the tablet form of sulfacetamide. These also gave the lowest results by the column chromatographic procedure.

The method described in this paper can also be used to determine purity of reference standards of the free sulfa or its sodium salt to meet compendial requirements. Levels of sulfanilamide below 1% can be quantitated.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 29, 1969, from *Food and Drug Administration, Buffalo, NY 14202*

Accepted for publication September 17, 1969.

Selective Determination of Isoproterenol and Isoproterenol Sulfonic Acid in Pharmaceutical Dosage Forms

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Abstract □ Two simple, precise, and specific methods for the determination of isoproterenol in decomposed formulations are described. DEHP shakeout procedure, which depends upon ion-pair formation with the unchanged drug at a suitable pH, enables the quantitative determination of isoproterenol from the DEHP–ether phase and of its sulfonic acid from the aqueous buffer phase. Isoproterenol sulfonic acid can then be determined selectively by treating the aqueous buffer phase with Doty's reagents. The sodium metaperiodate method has been developed as an alternate checking procedure to validate the results obtained by the DEHP method and involves the quantitative formation of an aryl aldehyde of the unchanged drug. The merits of both the procedures over the conventional UV and visible spectrophotometric procedures are shown by their application to the analysis of simulated decomposed formulation, aged simulated inhalations and injections, and commercial formulations. A thin-layer chromatographic procedure for the separation and detection of isoproterenol and its sulfonic acid and other artifacts is described.

Keyphrases □ Isoproterenol dosage forms—analysis □ Isoproterenol sulfonic acid formed in products—analysis □ Degradation products presence—isoproterenol determination □ Di-(2-ethylhexyl)phosphoric acid extraction method—isoproterenol determination □ Metaperiodate sodium method—isoproterenol determination □ UV spectrophotometry—analysis □ TLC—analysis

The selective determination of isoproterenol (3,4-dihydroxy-α-[(isopropylamino)methyl]-benzyl alcohol hydrochloride), in the presence of isoproterenol sulfonic acid and other decomposition products or vice versa, presents an unusually difficult analytical problem. Recently it became evident that the composition of pharmaceutical dosage forms, especially inhalations and injections containing isoproterenol hydrochloride, can change with aging. The change is attributed to the interaction of isoproterenol with the bisulfite antioxidant, by which the alcoholic hydroxyl group of the drug is replaced by a sulfonic acid group (1) as shown in