## Spectrophotometric Determination of Three Sulfa Drugs in Combination

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The principle of simultaneous equations has been applied to a tertiary mixture containing three sulfa drugs. A rapid procedure for the spectrophotometric deter-mination, without prior separation, of sulfacetamide, N<sup>1</sup>-benzoylsulfanilamide, and sulfathiazole in combination has been developed.

THERE the individual amounts of three sulfa drugs appearing in combination with each other in a product are to be estimated, the official procedures (1, 2) utilize paper strip chromatography to first separate each drug and then colorimetric procedures to estimate the individual amounts of the components. The official procedures are quite time-consuming and not so accurate. There is a need, therefore, for a method which will permit the quick and accurate estimation of sulfacetamide, N1-benzoylsulfanilamide, and sulfathiazole in combination with each other in a water-dispersible cream base.

Marzys (3) has developed a procedure for the individual estimation of the components of sulfathiazole, sulfamerazine, and sulfadiazine mixtures utilizing a combination of colorimetric and spectrophotometric procedures. Since no specific color reaction was known for any of the three sulfas studied, their ultraviolet absorption spectra in 0.1 Nhydrochloric acid were used to determine whether or not the amounts of the individual sulfas could be estimated from the absorption at three wavelengths and solving simultaneous equations with three unknowns (4).

#### EXPERIMENTAL

Apparatus: Beckman spectrophotometer model DU 4700 with 1-cm. matched quartz cells was used. Cell correction was applied where necessary. Unless otherwise mentioned the measurements were done against 0.1 N hydrochloric acid as blank.

Reagents: Hydrochloric acid, A.R. grade, solvent ether, reagent grade, sulfacetamide U.S.P. XV (5), sulfathiozole N.F. X (6), N<sup>1</sup>-benzoyl sulfanilamide, supplied by Bengal Immunity Co. Ltd., conforming to the specifications of Basu & Sikdar (7).

The absorption spectra for the three sulfas were determined at 220, 235, and 280 m $\mu$  (see Fig. 1). They conformed to Beer's law between 5 and 25 mcg./ml.; the absorption being additive. The absorptivities of the three compounds, determined using 1-cm. quartz cells and 0.2-mm. slit width, are given in Table I.

By solving the following three simultaneous equations from the absorbances at the indicated wave. lengths, one obtains the concentrations of the three ingredients:

$$460 \ a + 465 \ b + 345 \ c = 1000 \times A_{220}$$
  
$$56.5 \ a + 584 \ b + 106.5 \ c = 1000 \times A_{235}$$
  
$$167.5 \ a + 189 \ b + 489 \ c = 1000 \times A_{280}$$

where a = concentration of sulfacetamide,  $b = N^{1}$ benzoylsulfanilamide, and c = sulfathiazole, expressed as mg. per 100 ml. of solution.

Analysis of Known Synthetic Mixtures.---Known mixtures were prepared by taking suitable aliquots of solutions of individual components. The acidity in the final solution was maintained at 0.1N. The absorbances were measured at 220, 235, and 280 mµ and the content of each component calculated using simultaneous equations. The results are given in Table II.

Analysis of the Cream Containing Sulfacetamide, N1-Benzoylsulfanilamide, and Sulfathiazole.--About 1 Gm. of the cream (containing about 100 mg. of total sulfonamides) was mixed with a little solvent ether to get a good dispersion and transferred to a 250-ml. separator with the aid of about 50 ml. of

TABLE I.—Absorptivity Values (E 1%, 1 cm.)

Wavelength, mµ	220	235	280
Sulfacetamide	460	56.5	167.5
N <sup>1</sup> Benzoylsulfanilamide	465	584	189
Sulfathiazole	345	106.5	489

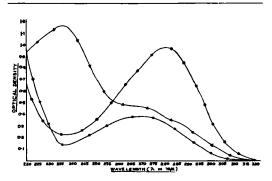


Fig. 1.—Absorption spectra determined for the three sulfa drugs studied. Sulfacetamide—••-•, N'-benzoylsulfanilamide  $- \blacktriangle - \bigstar -$ , sulfathiazole -**■**-, (conen., 20 mcg./ml.)

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No. Sulfacetamid			N <sup>1</sup> -Benzoyl sulfanilamide		Sulfathiazole		Total	
	Found	Added	Found	Added	Found	Added	Found	Added
1	0.810	0.800	0.589	0.600	0.637	0.600	2.036	2.000
$^{2}$	0.404	0.400	1.005	1.000	0.630	0.600	2.039	2.000
3	0.366	0.400	0.812	0.800	0.803	0.800	1.981	2.000
4	1.227	1.200	0.409	0.400	0.368	0.400	2.004	2.000

TABLE II.—RESULTS WITH KNOWN MIXTURES, MG./100 ML.

#### TABLE III.-ASSAY RESULTS OF OINTMENT

No.	Sulfa- cetamide, %	N <sup>1</sup> -Benzoyl- sulfanil- amide, %	Sulfathia- zole, %	Total, %
1	2.765	3.749	$\begin{array}{c} 2.363 \\ 3.343 \\ 3.406 \\ 3.371 \\ 3.420 \end{array}$	9.877
2	2.947	3.744		10.034
3	2.799	3.840		10.045
Average	2.837	3.777		9.985
Claim	2.860	3.700		9.980

solvent ether. The suspension was extracted with four 50-ml. portions of 1 N hydrochloric acid. The combined acid extracts were washed in a 500-ml. separator with 25 ml. of solvent ether and filtered through a Whatman No. 41 filter paper into a 500ml. volumetric flask. The filter was washed and the volume made up with 1 N hydrochloric acid. A 10-ml. aliquot was pipetted into a 100-ml. volumetric flask and the volume made up with distilled water. A 200-ml. portion of 1 N acid was similarly treated to prepare the blank.

The absorbances of the sample solution at 220, 235, and 280 mµ, measured against the blank, were used in the calculations as above to obtain the results in Table III.

#### CONCLUSION

A spectrophotometric procedure has been developed for rapid and fairly accurate determination of three individual sulfa drugs in combination with each other without prior separation. Though the combination is not the one very frequently used, the procedure could, perhaps, be extended to other combinations as well.

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# Synthesis of Methylglyoxal-bis-guanylhydrazone-C<sup>14</sup>

### By VINCENT T. OLIVERIO and CHARLENE DENHAM

### Methylglyoxal-bis-guanylhydrazone (methyl GAG), an antitumor agent in current animal and clinical trials, has been synthesized with isotopic carbon ( $C^{14}$ ) in excellent yield and radiochemical purity for pharmacological studies.

**VUMEROUS** reports have recently appeared relat-ing the inhibitory effects of guanylhydrazones, particularly methylglyoxal - bis - guanylhydrazone (methyl GAG), on various animal and human neoplasms (1-7). To facilitate studies on the metabolic fate and mechanism of action of these agents in man and animals, we have synthesized radioactive methyl GAG-C14 (I) in excellent yield and radiochemical purity.

Methyl GAG may be prepared by the reduction of nitroguanidine with zine dust in acetic acid to aminoguanidine (8), followed by condensation with pyruvaldehyde (9). The aminoguanidine may also be prepared, alternatively, as originally described for pilot plant production (8), by methylation of

Received April 27, 1962, from the Clinical Pharmacology and Experimental Therapeutics Section, Medicine Branch, National Cancer Institute, Bethesda 14, Md. Accepted for publication May 25, 1962. Guanidine-C<sup>14</sup>, 1,1'-(methylethanedilidenedinitrilo)dihy-drochloride monohydrate, is the full chemical name. The abbreviated name, methyl GAG-C<sup>14</sup>, is employed throughout the text the text

The thiourea-C<sup>14</sup> was obtained from New England Nuclear

Corp., Boston, Mass. Pyruvaldehyde (43%) was obtained through the courtesy of Union Carbide Chemicals Co., New York, N. Y.

thiourea with dimethyl sulfate followed by hydrazinolysis of the S-methylisothiuronium sulfate.

For the preparation of methyl GAG-C14 on the milligram scale, the latter procedure was chosen because: (a) appreciable quantitites of zinc salts were coprecipitated with addition of sodium bicarbonate after reduction of nitroguanidine, even in the presence of ammonium chloride, resulting in an impure product; (b) the specific activity (mc./mmole) of the commercially available C14-guanidine nitrate was only one-fourth as high as the less expensive  $C^{14}$ -thiourea; and finally; (c) the overall reaction yield (30%) of the first procedure was appreciably lower than the yield (70%) of the alternate

