

# MULTICOMPONENT SPECTROPHOTOMETRIC ASSAY OF SULPHACETAMIDE AND PHOTODEGRADATION PRODUCTS

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## ABSTRACT

A multicomponent, spectrophotometric method has been developed for the simultaneous determination of sulphacetamide, sulphanilamide and major degradation products in photolysed solutions. It is based on a three component (Sulphacetamide, sulphanilamide and azobenzene-4, 4'-disulphonamide) assay at pH 4.0 using 258, 268 and 320nm as analytical wavelengths. The blue oxidation product of sulphanilamide is determined independently at 550 nm. The reproducibility of the method judged by the analysis of synthetic mixtures, is within  $\pm 5\%$ . The method is stability indicating for the assay of sulphacetamide in degraded eye-drop preparations and could also be conveniently used for photodegradation studies.

## Introduction

Colorimetric methods in conjunction with chromatography are generally employed for the assay of sulphonamide mixtures. The most commonly used method is Bratton-Marshall method (1) which is based on diazotization of the sulphonamide with sodium nitrite, coupling the diazo compound with N-(1-naphthyl)-ethylenediamine, and measuring the absorbance at 545nm. Several workers have applied direct spectrophotometry to the assay of sulphonamide mixtures (2-5) and azo and azoxy derivatives of sulphanilamide (6) or used colorimetry after chromatographic separation of sulphanilamide (7) and sulphacetamide (8) in degraded solutions.

In the present work an attempt has been made to develop a multicomponent spectrophotometric method for the assay of sulphacetamide and its major photodegradation products present in photolysed solutions. The method is stability - indicating for the assay of sulphacetamide and could be conveniently used for the determination of the drug in eye-drop preparations.

## Materials and Method

Sulphacetamide (Sigma) and sulphanilamide (BDH) were recrystallised from water-ethanol mixture and water respectively. Azobenzene-4, 4'-disulphonamide was synthesised by the method previously reported (9). The blue oxidation product of sulphanilamide ( $C_{30}H_{25}O_2N_5$ ) was prepared by the method of Barkan and Goldsmith (10). All reagents and solvents were of analytical grade unless otherwise stated.

### *Photolysis of Sulphacetamide*

A  $10^{-3}$ M solution of sulphacetamide (or sulphanilamide) at appropriate pH was placed in a 250ml stoppered silica flask and deoxygenated for 30 minutes by bubbling oxygen free nitrogen. The solution was irradiated with a Philips 30w mercury discharge UV tube (emission energy at 254nm=8874%, this wavelength almost corresponds to the absorption maxima of sulphonamides). A magnetic stirrer was used to stir the solution during irradiation. The flask was immersed in a water-bath maintained at  $25 \pm 1^{\circ}\text{C}$ . Assay Procedure:

A 1ml aliquot of the photolysed solution was transferred, at suitable intervals, into a 20ml volumetric flask and made up to volume with citrate buffer of pH 4.0. The contents were thoroughly mixed and the absorbance was measured at selected wave-lengths on Shimadzu-240 spectrophotometer. The absorbance of blue oxidation product of sulphanilamide was determined independently at 550nm. The assay was carried out in a dark room under dim light.

Aqueous solutions of sulphacetamide (So), on photolysis, yield a number of products some of which have been identified (11). A scheme has been developed to assay sulphacetamide and its major photodegradation products, viz. Sulphanilamide(s), azobenzene-4, 4'-disulphonamide (a) and the blue product(b). It is based on the assumption that the total number of moles involved in the reactions are to be accounted for as a criterion for the reproducibility of the assay method. One mole of sulphacetamide on photolysis produces one mole of sulphanilamide. Since two molecules of sulphanilamide are involved in the formation of azobenzene-4,4'-disulphonamide, its mole equivalent in terms of sulphanilamide would be twice (2a). As five molecules of sulphanilamide are assumed to participate in the formation of blue product ( $\text{C}_{30}\text{H}_{25}\text{O}_2\text{N}_5$ ), its mole equivalent in terms of sulphanilamide would be five times (5b). Thus the total mole equivalent in terms of sulphacetamide in a particular reaction would be  $S_o + S + 2a + 5b$ . Similarly with photolysis of sulphanilamide, which produces the same compounds, the total mole equivalent would be  $(5 + 2a5b)$ .

Absorbance measurements are made at two or three suitably selected wavelengths and the concentration of various components in photolysed solutions are evaluated by solving the simultaneous equations directly or by using matrix methods (12).

### *Selection of Analytical Wavelengths*

The absorption spectra of sulphacetamide and sulphanilamide are markedly altered with changes in the pH region below 5.0. This enabled us to make a distinction between the absorption maxima of these compounds. Sulphacetamide and sulphanilamide exhibit absorption maxima at 268nm and 258nm respectively (pH 4.0) which may be advantageously utilised for a two component assay. At pH 4.0 the absorption maximum of azobenzene-4, 4'-disulphonamide lies at 320nm.

The blue product exhibits a maximum at 550nm at which there is no interference from any other photoproduct (Fig. 1, 2-). Choice of pH: In view to the susceptibility of

sulphonamides to pH changes in acidic solutions, pH 4.0 was chosen as the optimum pH for assay on the basis of maximum distinction achieved among the absorption maxima of these compounds. This necessitated the final adjustment of pH to 4. using citrate buffer, of Beer's Law:

The Validity of Beer's law for each compound, singly and in mixture, has been established by preparing synthetic mixtures of varied composition. The reproducibility of multicomponent assay method of is of the order =5 % (Table 1, 2).

#### *Determination of molar extinction coefficients*

The values of molar extinction coefficients calculated by the method of least squares for sulphacetamide, sulphanilamide and azobenzene-4, 4'-disulphonamide are reported in Table 3. The molar extinction coefficient of the blue product at 550nm has been calculated as 40800 (pH 4.) on the basis of its molecular weight.

### **Results and Discussion**

A set of typical results for the analysis of 3-4 products in photolysed solutions of sulphacetamide and sulphanilamide is reported in Table 4 and 5. The assay method, when applied to the photolysis of sulphacetamide, give uniformly increasing values of sulphanilamide, azobenzene-4,4'-disulphonamide and the blue product. The assay data on examination for conformity with the proposed analytical scheme showed an almost constant molar balance with time.

The values of the molar balance are in good agreement with the initial concentration of sulphacetamide. Similar assay results were obtained for sulphanilamide and degradation products (Table 5). The overall loss in total molar concentrations of sulphacetamide and sulphanilamide reactions at 50% photolysis is to the extent of  $0.3 \times 10^{-4} \text{M}$  (3%) and  $0.6 \times 10^{-4} \text{M}$  (6 %) respectively which suggests that certain photoproducts could not be accounted for, in addition to minor quantities of azoxybenzene-4, 4'-disulphonamide. However, a gradual increase in its concentration, as the reaction proceeds, would affect the absorbance at 320nm. one of the wavelengths chosen for the multicomponent assay. Since azoxybenzene-4, 4'-disulphonamide has a higher molar extinction coefficient at this wavelength (30900) than that of the azobenzene -4, 4'-disulphonamide (13200), its presence would then to increase slightly the calculated values of molar concentration of the latter. Azoxybenzene, 4, 4'-disulphonamide is formed on the photolysis of sulphacetamide solution by the oxidation of azobenzene-4, 4'-disulphonamide (6). Similarly any interference from the blue product, assayed independently at 550nm in the region of the analytical wavelengths, would lead to a little apparent increase of the molar concentrations of other compounds. Obviously the discrepancy in assay results leading to an overall decrease in the total molar balance appears to be due to the presence of minor substances such as sulphanilic acid (11), p-hydroxylaminobenzenesulphonamide (13) and unknown products which could not be assayed.

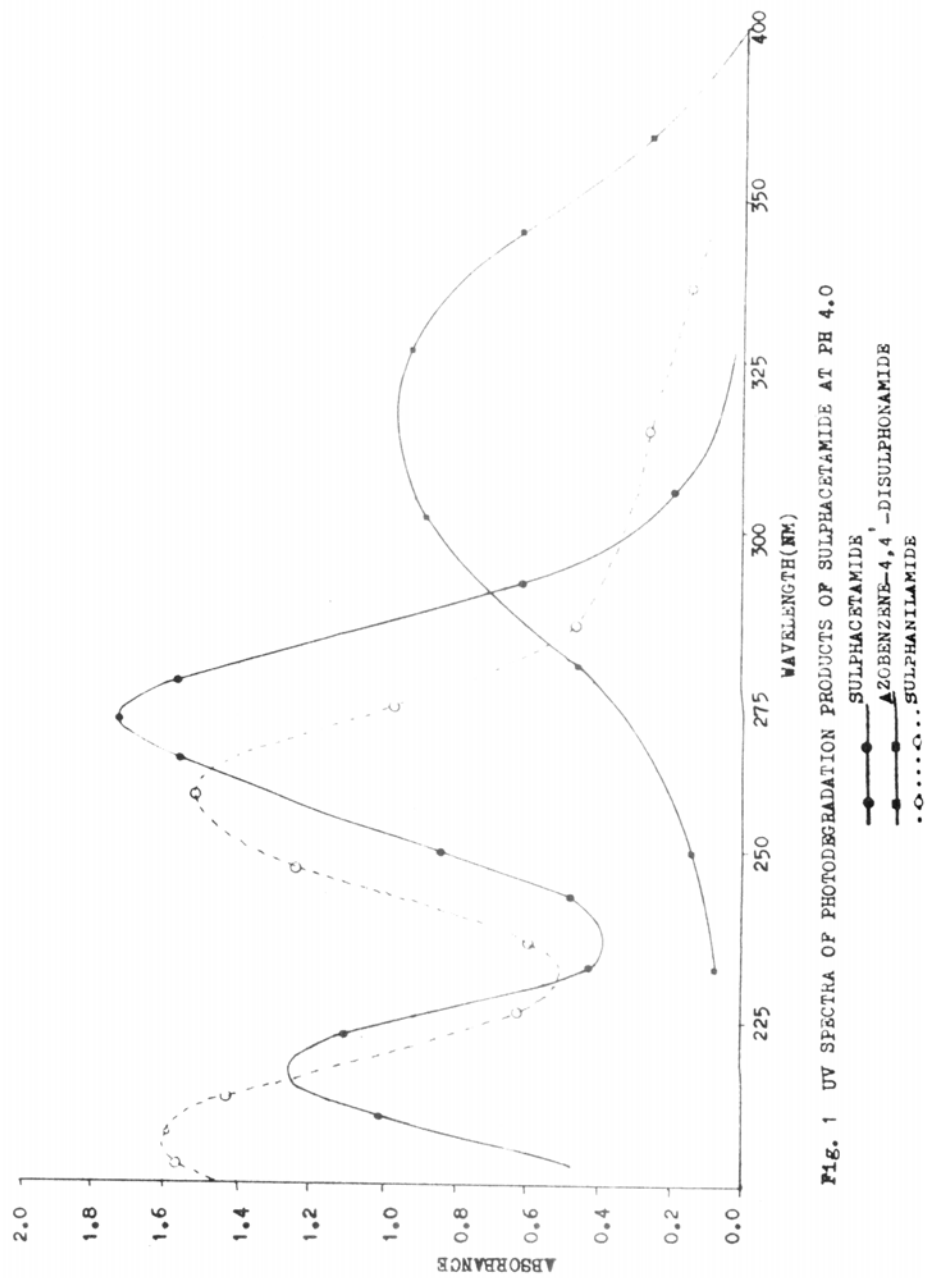


Fig. 1 UV SPECTRA OF PHOTODEGRADATION PRODUCTS OF SULPHACETAMIDE AT PH 4.0

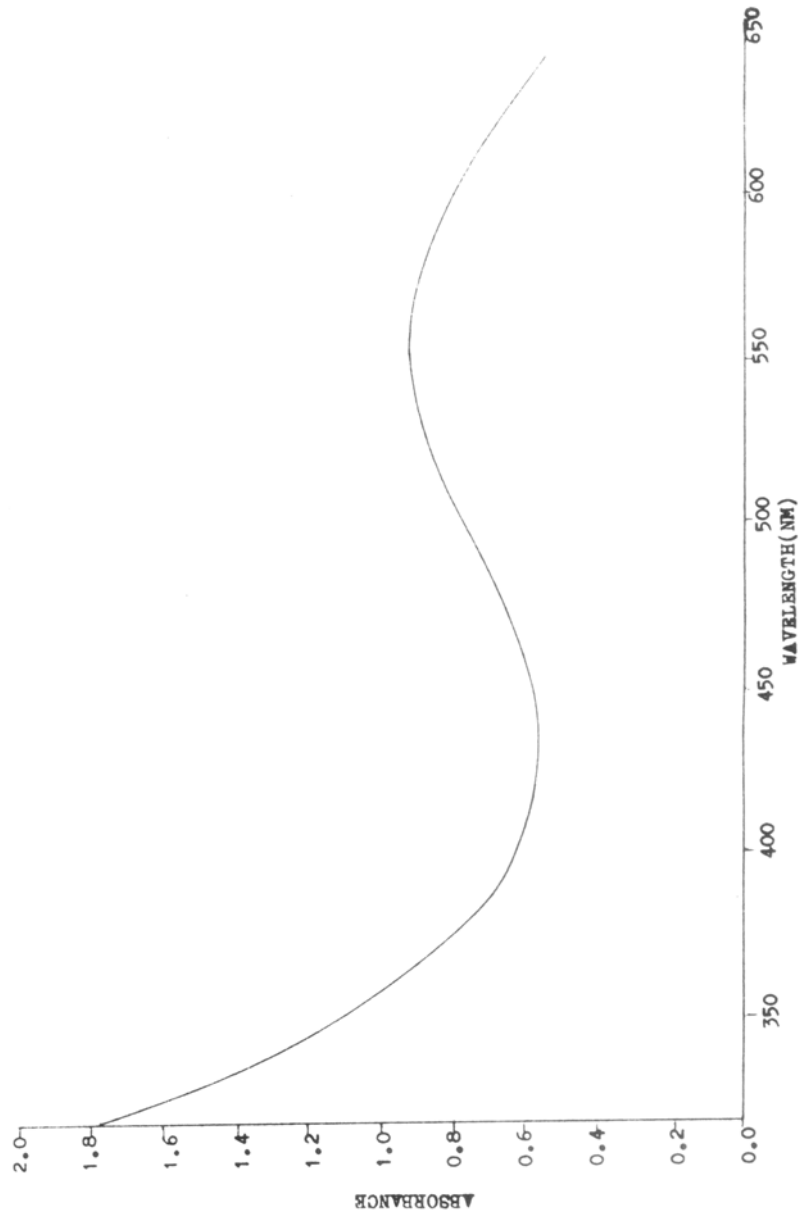


FIG.2 VISIBLE SPECTRUM OF BLUE OXIDATION PRODUCT AT PH 4.0

Table-2: Analysis of synthetic mixtures of sulphacetamide, sulphanylilamide and Azobenzene -4,4'-disulphonamide \*

Mixture No.	moles/1x10 <sup>4</sup>			disulphnoamide, mole/1x10 <sup>4</sup>		
	Added	Found	%Error	Added	Found	%Error
1.	8.50	8.32	-2.11	1.00	1.05	+5.00
2.	8.00	8.10	+1.25	1.50	1.50	0.00
3.	6.50	6.78	+4.30	2.50	2.50	0.00
4.	5.00	5.25	+5.00	2.00	2.05	+2.50
5.	4.00	4.08	+2.00	2.50	2.50	0.00
6.	3.50	3.55	+1.42	2.75	2.80	+1.82
7.	2.75	2.84	+3.27	5.50	5.42	-4.45
8.	2.25	2.36	+4.88	5.50	5.41	-1.64
9.	2.00	1.91	+4.50	2.50	2.63	+5.20
10.	1.25	1.30	+4.00	1.75	1.79	+2.28
11.	1.00	1.02	+2.00	1.00	0.95	-5.00
12.	0.50	0.48	-4.00	1.00	0.96	-4.00
				0.50	0.52	+4.00
				0.50	0.48	-4.00
				1.00	0.95	-5.00
				3.00	3.11	+3.67
				3.50	3.40	-2.86
				3.75	3.87	+3.20
				1.75	1.79	+2.28
				2.25	2.32	+3.11
				5.50	5.38	-2.18
				7.00	6.81	-2.71
				8.00	8.22	+2.75
				8.50	8.75	+2.94

\*Absorbance measurements at 268nm, 258nm and 320nm.

Table-2: Analysis of synthetic mixtures of sulphacetamide, sulphanilamide and Azobenzene -4,4'-disulphonamide \*

Mixture No.	sulphacetamide moles/1x10 <sup>4</sup>			sulphanilamide moles/1x10 <sup>4</sup>			Azobenzene -4,4'-disulphonamide moles/1x10 <sup>4</sup>		
	Added	Found	%Error	Added	Found	%Error	Added	Found	%Error
1.	8.50	8.32	-2.11	1.00	1.05	+5.00	0.50	0.52	+4.00
2.	8.00	8.10	+1.25	1.50	1.50	0.00	0.50	0.48	-4.00
3.	6.50	6.78	+4.30	2.50	2.50	0.00	1.00	0.95	-5.00
4.	5.00	5.25	+5.00	2.00	2.05	+2.50	3.00	3.11	+3.67
5.	4.00	4.08	+2.00	2.50	2.50	0.00	3.50	3.40	-2.86
6.	3.50	3.55	+1.42	2.75	2.80	+1.82	3.75	3.87	+3.20
7.	2.75	2.84	+3.27	5.50	5.42	-4.45	1.75	1.79	+2.28
8.	2.25	2.36	+4.88	5.50	5.41	-1.64	2.25	2.32	+3.11
9.	2.00	1.91	+4.50	2.50	2.63	+5.20	5.50	5.38	-2.18
10.	1.25	1.30	+4.00	1.75	1.79	+2.28	7.00	6.81	-2.71
11.	1.00	1.02	+2.00	1.00	0.95	-5.00	8.00	8.22	+2.75
12.	0.50	0.48	-4.00	1.00	0.96	-4.00	8.50	8.75	+2.94

\*Absorbance measurements at 268nm, 258nm and 320nm.

Table-3: Molar extinction coefficients ( $M^{-1} \text{ cm}^{-1} \times 10^{-3}$ ) of sulphacetamide and photodegradation products \*at pH 4.0

Compound	258nm	268nm	320nm
Sulphacetamide	13.53	17.13	0.10
Sulphanilamide	15.25	11.63	0.016
Azobenzene-4,4'-disulphonamide	3.13	3.95	13.15

\*Each Value is an average of 3-5 determinations.

Table-4: Photolysis of  $10^{-3}$  M sulphacetamide solution at pH 9.0 (Borate buffer) Concentrations of sulphacetamide and degradation products.

Time	Sulphacetamide	Sulphanilamide	Azobenzene-4,	4'-disulphonamide	Blue product	Total
Mins.	S	S	a	2a **	b	5b**
	moles/ $1 \times 10^4$	moles/ $1 \times 10^4$	moles/ $1 \times 10^4$	moles/ $1 \times 10^4$	moles/ $1 \times 10^4$	moles/ $1 \times 10^4$
0.	10.000	0.000	0.000	0.000	0.000	0.000
5.	9.533	-0.107	0.163	0.326	0.012	0.060.
10.	8.890	-0.080	0.394	0.788	0.017	0.085
15.	8.454	-0.060	0.579	1.158	0.019	0.095
20.	8.054	-0.165	0.826	1.652	0.029	0.145
25.	7.606	-0.118	0.981	1.962	0.034	0.170
30.	7.196	-0.107	1.096	2.192	0.044	0.220
35.	6.804	0.017	1.274	2.548	0.048	0.240
40.	6.301	0.199	1.434	2.868	0.053	0.265
45	6.180	0.199	1.449	2.899	0.063	0.315
50.	5.888	0.291	1.544	3.088	0.073	0.365
55.	5.598	0.378	1.648	3.298	0.083	0.415
60.	5.346	0.372	1.753	3.506	0.093	0.465
70.	4.842	0.559	1.895	3.790	0.102	0.510

\*( $s_0s + 2a + 5b$ ) \*\*mole equivalent in terms of sulphanilamide

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 Table-5: Photolysis of  $10^{-3}$  M sulphaniilamide solution at pH 9.0 (Borate buffer)  
 Concentrations of sulphaniilamide and degradation products.

Time mins.	Sulphaniilamide moles/ $1 \times 10^4$	Azobenzene-4, 4' moles/ $1 \times 10^4$	4'-disulphonamide moles/ $1 \times 10^4$	Blue product moles/ $1 \times 10^4$	Total* moles/ $1 \times 10^4$
	s	a	2a**	b	5b**
0.	10.000	0.000	0.000	0.000	10.000
2.	9.580	0.112	0.224	0.009	9.849
4.	8.786	0.309	0.618	0.017	9.489
6.	8.294	0.473	0.946	0.029	9.385
8.	7.708	0.774	1.548	0.041	9.461
10.	7.366	0.906	1.812	0.044	9.398
12.	6.996	1.046	2.092	0.049	9.333
14.	6.663	1.186	2.372	0.046	9.265
16.	6.358	1.279	2.558	0.061	9.221
18.	6.145	1.395	2.790	0.073	9.300
20.	5.908	1.557	3.114	0.073	9.387
24.	5.354	1.766	3.532	0.076	9.266
28.	5.073	1.951	3.902	0.078	9.365

\* (s + 2a + 5b)      \*\* mole equivalent in terms of sulphaniilamide

The proposed assay method is stability-indicating and has been successfully applied to the photodegradation studies of sulphacetamide and sulphanilamide. It is specific for the determination of sulphacetamide in eye-drop preparations which tend to deteriorate in the presence of oxygen and light.

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