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Short Communication

Determination of mixtures of sulphonamides by titration with chloramine-T

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Introduction

Single organic compounds can be determined by a wide variety of methods but problems may arise in the analysis of individual substances in mixtures or in the presence of interfering substances [1]. Procedures are described in this paper for the analysis of mixtures of sulphonamides in drug formulations without any preseparation of their constituents.

Because of the extensive use of sulphonamides in the treatment of bacterial infections, their determination in formulations, either alone or in mixtures with other sulpha drugs, has received considerable attention [2–7]. The official sulphonamides are all derivatives of sulphanilamide and are analysed by diazotization of the aromatic amino group [8, 9]. However, sulphafurazole has been reported to consume more than one equivalent of nitrite [9, 10]. Drug excipients that are acidic interfere with the alkalimetric titration of sulphonamides [11–15], e.g. with diiodohydroxyquinoline. Titration with bromine has been recommended [16–19] but this technique is not widely used mainly because of the varying stoichiometry of the reaction [20–24]. In theory, four equivalents of bromine are consumed in the substitution at both positions *ortho* to the aromatic amino group. However, it has been found that certain substituents attached to the sulphonamido side-chain also take up two equivalents of bromine. The *ortho*-dibromination is prevented by acetylation of the aromatic amino group as a result of its steric and electronic deactivation factors but bromine consumption by the side-chain substituent remains unaffected. Similar deactivation effects have also been observed when the amino group is diazotized. These two prereactions permit the analysis of acylated and non-acylated sulphonamides and other sulpha drugs when present in the same sample.

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Experimental

Reagent and materials

Chloramine-T solution (0.02 M) was prepared by dissolving 5.62 g of sodium *N*-chloro-4-toluenesulphonamide trihydrate in, and diluting to, 1 l, with water and standardizing the solution iodometrically [25].

Individual sulphonamides were standardized by the methods of diazotization [9] or nonaqueous titration [14]. All drugs used were fresh and purchased from local suppliers. Methyl red indicator solution was 0.02% in ethanol.

Procedures

Assay of individual sulphonamides in tablets. An accurately weighed amount of powdered tablets containing 0.05–0.2 mmole of the sulphonamide was treated with 10 ml of anhydrous acetic acid and 2 ml of acetic anhydride and warmed in a boiling water bath for 10 min. The cooled solution was mixed with 0.5 g of potassium bromide, 10 ml of 5% sulphuric acid, 25 ml of methanol and two or three drops of methyl red indicator, then titrated with 0.02 M chloramine-T until the red colour of the indicator was sharply bleached. The titration could also be followed potentiometrically using a calomel and platinum electrode pair. The quantity of sulphonamide in a tablet of average weight was calculated using the following equation:

$$\text{sulphonamide (mg)/tablet} = (\text{mol wt})\left(\frac{VMA}{w}\right), \tag{1}$$

where V is the volume of chloramine-T (molarity M) required in the titration, w is the weight (mg) of sample taken for analysis and A is the average weight (mg) of a tablet.

Assay of mixtures of sulphonamides. A known number of tablets were weighed and ground to a fine powder. Accurately weighed portions containing about 0.2 mmole of total sulphonamide were used for each determination as follows. (The sample and compound numbers refer to pharmaceutical preparations and their constituent sulphonamides respectively as cited in Tables 3 and 4.)

(i) Sample Nos I–III, VI–VIII and X. Two determinations were necessary using two separate portions of the powdered tablet.

Portion 1 was mixed with 10 ml of 5% sulphuric acid, 0.5 g of potassium bromide, 25 ml of methanol and two or three drops of methyl red, then titrated with 0.02 M chloramine-T until the red colour of the indicator had almost faded when two drops more of the indicator were introduced and the titration was continued to the sharp decolorization of the red colour. An indicator blank on the same number of drops of methyl red was determined and subtracted from the sample titre.

Portion 2 was treated with 10 ml of anhydrous acetic acid, 2 ml of acetic anhydride and warmed in a boiling water bath for 10 min. After the solution had cooled, 0.5 g of potassium bromide, 10 ml of 5% sulphuric acid, 25 ml of methanol and two or three drops of methyl red were added and the solution was titrated with 0.02 M chloramine-T.

$$\underset{\text{(mg/tablet)}}{\text{Compound Nos 1, 13, 15 and 17}} = \left(\frac{\text{mol wt}}{2}\right) M\left(\frac{V_1A}{w_1} - \frac{V_2A}{w_2}\right) \tag{2}$$

$$\underset{\text{(mg/tablet)}}{\text{Compound Nos 2, 14, 16 and 18}} = (\text{mol wt})\, M \left(\frac{1.5\, V_2 A}{w_2} - \frac{0.5\, V_1 A}{w_1}\right) \quad (3)$$

$$\underset{\text{(mg/tablet)}}{\text{Compound Nos 3, 5 and 21}} = (\text{mol wt}) \left(\frac{M V_2 A}{w_2}\right) \quad (4)$$

$$\underset{\text{(mg/tablet)}}{\text{Compound Nos 4, 6 and 22}} = \left(\frac{\text{mol wt}}{2}\right) M \left(\frac{V_1 A}{w_1} - \frac{3 V_2 A}{w_2}\right) \quad (5)$$

where V_1 and V_2 are the volumes of chloramine-T (molarity M) required in the titration of Portion 1 (w_1 mg) and Portion 2 (w_2 mg), respectively of the powdered tablet, and A is the average weight (mg) of a tablet.

(ii) Sample Nos IV and V. To analyse these mixtures, three determinations were needed using three separate portions of the powdered tablet.

Portion 1 was combined with 10 ml of 5% sulphuric acid, 0.5 g of potassium bromide, 25 ml of methanol and two or three drops of methyl red then titrated with 0.02 M chloramine-T to the sharp decolorization of the indicator.

Portion 2 was treated with 10 ml of anhydrous acetic acid and 2 ml of acetic anhydride, then warmed. The cooled solution was mixed with 0.5 g of potassium bromide, 10 ml of 5% sulphuric acid, 25 ml of methanol and two or three drops of methyl red, then titrated with 0.02 M chloramine-T.

Portion 3 was shaken with 10 ml of 5% sulphuric acid to dissolve the sulphonamide then treated dropwise with 2 ml of 1% sodium nitrite and allowed to stand for 2 min. A 1 g portion of urea was then added to decompose excess nitrous acid, the solution was shaken well and allowed to stand for 2 min. A 0.5 g portion of potassium bromide, 5 ml of 5% sulphuric acid, 25 ml of methanol and two or three drops of methyl red were added and the mixture was titrated with 0.02 M chloramine-T.

$$\underset{\text{(mg/tablet)}}{\text{Compound Nos 8 and 1}} = (\text{mol wt})\, M \left(\frac{V_3 A}{w_3}\right) \quad (6)$$

$$\underset{\text{(mg/tablet)}}{\text{Compound Nos 9 and 12}} = (\text{mol wt})\, M \left(\frac{V_2 A}{w_2} - \frac{V_3 A}{w_3}\right) \quad (7)$$

where V_3 is volume of chloramine-T required in the titration of Portion 3 (w_3 mg) and the other symbols have the same meaning as before. Compounds Nos 7 and 10 are calculated using equation (5).

(iii) Sample No. IX. Again, two analyses were required using two separate portions of the powdered sample.

Portion 1 was treated with 10 ml of 5% sulphuric acid, 0.5 g of potassium bromide, 25 ml of methanol and two or three drops of methyl red, then titrated with 0.02 M chloramine-T.

Portion 2 was mixed with 10 ml of 10% sulphuric acid and gently boiled for 15 min (to hydrolyse the acylated sulphonamide). The cooled solution was combined with 0.5 g of potassium bromide, 25 ml of methanol and two or three drops of methyl red, then titrated with 0.02 M chloramine-T.

$$\text{Compound No. 19 (mg/tablet)} = \left(\frac{\text{mol wt}}{2}\right) M\left(\frac{V_1 A}{w_1}\right) \quad (8)$$

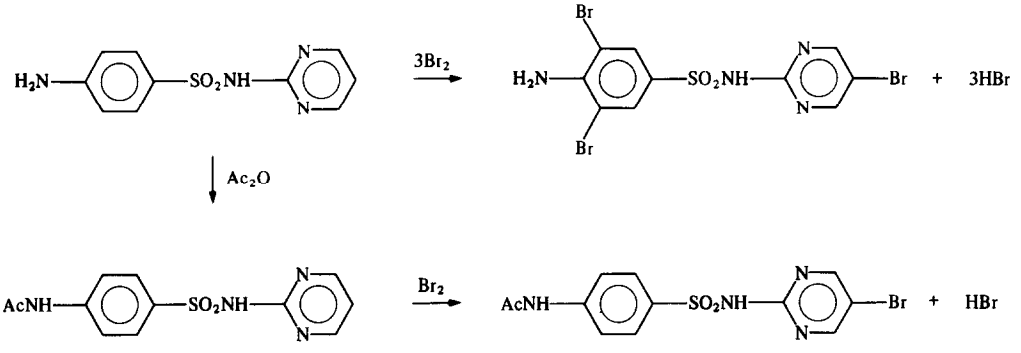
$$\text{Compound No. 20 (mg/tablet)} = \left(\frac{\text{mol wt}}{2}\right) M\left(\frac{V_2 A}{w_2} - \frac{V_1 A}{w_1}\right) \quad (9)$$

where the symbols have the same meaning as before.

Results and Discussion

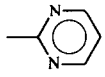
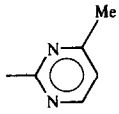
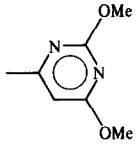
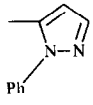
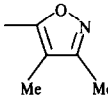
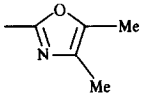
Upon bromination sulphonamides consume four atoms of bromine. However, certain substituents on the sulphonamide side-chain, as shown in Table 1, require two additional atoms of bromine as these substituents also are capable of undergoing bromination. From the examination of the structures of such substituents [26], it has been concluded that any non-substituted position that is β to the nitrogen atom or α to the sulphur atom in the heterocyclic nucleus will undergo electrophilic substitution. That the β-carbon undergoes bromination is confirmed by the observation that sulphamethoxydiazine, which already has a substituent at the relevant position, consumed only four atoms of bromine. The mechanism of the reaction with sulphafurazole and sulphamoxole is not clear. However, bromination via 1,4-addition–elimination steps is suggested for oxazole and similar heterocyclic compounds [26].

Acetylation of the aromatic amino group prevents *ortho*-substitution but the reaction of side-chain substituents remains unaffected. Thus, substances with reactive side groups can be determined by using acetylation as a prereaction when they are formulated alone or in admixture with other sulphonamides (Tables 1 and 2). The following reactions occur with sulphadiazine as a representative example:



Prevention of *ortho*-bromination also occurs by prediazotization of the amino group. Consequently, diazotized sulphaphenazole, sulphafurazole and sulphamoxole do not consume any bromine. Hydrolysis of acylated sulphonamides, e.g. phthalylsulphacetamide, liberates the free amino group of sulphacetamide which then undergoes *ortho*-bromination. Suitable combinations of these prereactions allow the analysis of mixtures of sulphonamides (Tables 3 and 4). The reliability of the present methods was

Table 1
Assay of certain sulphonamides after acetylation

H_2N—C$_6$H$_4$—SO_2NH—R	R	Amount (mg) Taken*	Found†	% RSD‡
Sulphadiazine		4.56	4.58	0.4
		7.24	7.19	
		10.15[a]	10.05	0.6
		12.68[b]	12.75	0.5
Sulphamerazine		5.24	5.29	
		7.80[c]	7.72	0.5
		9.95	10.03	0.4
		12.55[d]	12.62	0.5
Sulphadimethoxine		6.82[e]	6.89	0.6
		8.07	8.15	0.5
		10.23[a]	10.36	0.6
		11.98	12.08	
Sulphathiazole		4.23	4.18	0.5
		6.51	6.43	
		9.22[f]	9.32	0.7
		11.35	11.46	0.6
Sulphaphenazole		5.92	6.01	0.5
		7.34[g]	7.43	
		10.26[b]	10.04	0.6
		12.52	12.68	0.7
Sulphafurazole		3.82[b]	3.92	0.4
		6.15	6.22	
		8.29[h]	8.15	0.6
		10.37	10.28	0.6
Sulphamoxole		4.03[a]	4.13	0.5
		5.81	5.76	0.5
		8.82[h]	8.73	0.6
		10.04	10.18	0.7

*Quantity of first four sulphonamides determined by nitrite titration [9], and of the rest by nonaqueous titration [14]. Test material mixed with (a) sulphanilamide (10 mg), (b) sulphacetamide (15 mg), (c) sulphamethoxydiazine (6 mg), (d) sulphamethoxypyridazine (12 mg), (e) sulphamethazine (8 mg), (f) sulphamethizole (10 mg), (g) sulphaproxyline (5 mg) and (h) sulphamethoxazole (10 mg).

†Average of six replicates.

‡Relative standard duration.

Table 2
Assay of certain sulphonamides in drug formulations after acetylation

Sulpha drug* formulation	Sulphonamide present	Amount (mg) Label claim	Present method†	% RSD	Comparison method‡
Diastrep[a]	Sulphadiazine	100	96	0.4	NA
Sulfuno	Sulphamoxole	500	519	0.5	522[d]
Orisul	Sulphaphenazole	500	504	0.6	508[d]
Cibazol	Sulphathiazole	500	520	0.4	525[e]
Lasibon	Sulphadimethoxine	500	496	0.5	493[e]
Furoquinol[b]	Sulphafurazole	350	341	0.6	NA
Proxymer[c]	Sulphamerazine	375	380	0.6	NA

*Other substances present include (a) chloramphenicol (125 mg) and streptomycin sulphate (125 mg); (b) diiodohydroxyquinoline (200 mg), chloroquine phosphate (50 mg) and oxyphenonium bromide (2 mg); and (c) sulphaproxyline (375 mg).

†Average of six replicates.

‡(NA) The comparison methods could not be used for this sample; (d) nonaqueous alkalimetry [14]; and (e) nitrite titration [9].

Table 3
Stoichiometry of the reaction of sulphonamides after pretreatment

Sample No.	Sulphonamides	Mol. wt	Equivalents of Br/Pretreatment method None	Acetylation	Diazotization	Hydrolysis
I	Sulphathiazole	255	6	2		
	Phthalylsulphathiazole	403	2	2		
II	Sulphaphenazole	314	6	2		
	Sulphacetamide	214	4	0		
III	Sulphadiazine	250	6	2		
	Sulphamethoxazole	253	4	0		
IV	Sulphaguanidine	214	4	0	0	
	Sulphamerazine	264	6	2	2	
	Sulphafurazole	267	6	2	0	
V	Sulphaproxyline	334	4	0	0	
	Sulphadiazine	250	6	2	2	
	Sulphamoxole	267	6	2	0	
VI	Sulphamerazine	264	6	2		
	Succinylsulphathiazole	355	2	2		
VII	Sulphamerazine	264	6	2		
	Phthalylsulphathiazole	403	2	2		
VIII	Sulphadiazine	250	6	2		
	Phthalylsulphathiazole	403	2	2		
IX	Sulphaguanidine	214	4	—	—	4
	Phthalylsulphacetamide	362	0	—	—	4
X	Sulphadiazine	250	6	2		
	Sulphadimidine	264	4	0		

Table 4
Assay of mixtures of sulphonamides

Sample No.	Sulpha drug* formulation	Compound No.	Sulphonamides	Amount (mg) Label	Present method†	% RSD
I	Laboratory-made tablet	1	Sulphathiazole	200	202	0.5
		2	Phthalylsulphathiazole	150	155	0.6
II	Laboratory-made tablet	3	Sulphaphenazole	150	146	0.4
		4	Sulphacetamide	100	103	0.3
III	Laboratory-made tablet	5	Sulphadiazine	200	208	0.5
		6	Sulphamethoxazole	200	196	0.8
IV	Laboratory-made tablet	7	Sulphaguanidine	200	198	0.4
		8	Sulphamerazine	250	253	0.6
		9	Sulphafurazole	200	205	1.2
V	Laboratory-made tablet	10	Sulphaproxyline	150	148	0.4
		11	Sulphadiazine	250	256	0.4
		12	Sulphamoxole	150	152	1.0
VI	Laboratory-made tablet	13	Sulphamerazine	150	151	0.5
		14	Succinylsulphathiazole	100	99	0.6
VII	Chlorosulf[a]	15	Sulphamerazine	50	55	0.7
		16	Phthalylsulphathiazole	150	146	0.8
VIII	Enteromycetin sulfa[b]	17	Sulphadiazine	200	209	0.5
		18	Phthalylsulphathiazole	150	158	0.6
IX	Entrothalazyme[c]	19	Sulphaguanidine	200	212	0.4
		20	Phthalylsulphacetamide	200	220	0.8
X	Streptriad	21	Sulphadiazine	150	143	0.6
		22	Sulphadimidine	150	156	0.6

*Other substances present include (a) chloramphenicol (100 mg), (b) chloramphenicol (150 mg), and (c) quiniodichlor (250 mg).
†Average of six replicates.

checked by analysing laboratory-made tablets spiked with known amounts of sulphonamides.

Chloramine-T and bromine (produced *in situ*) are general oxidimetric reagents and may react with a number of substances [25]. Indirect determinations carried out by adding an excess of chloramine-T and then titrating the excess reagent have not been used for sulphonamides because of possible interferences due to even weakly reducing substances that may be present [9], and unstable stoichiometry [22, 25]. A study on the likely interferences in the present methods has revealed that the decolorization of methyl red is faster than most secondary reactions. Substances that do not affect the results when present in up to a 20-fold molar excess of the analyte include lactose, citric, tartaric, fumaric, glutamic and folic acids, thiamine hydrochloride, phenacetin, trimethoprim, pyrimethamine, chloramphenicol, streptomycin sulphate, chloroquine phosphate and diiodohydroxyquinoline. Substances that interfere when present in even

small amounts are barbituric acid, isoniazid, vitamin C, methionine, thymol and penicillins.

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