Spectrophotometric determination of sulfanilic acid and sulfonamides in pharmaceutical samples with potassium 1,2-naphthoquinone-4-sulfonate

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An accurate and simple method is proposed for the determination of sulfanilic acid in the presence of sulfonamides. This method is based on measuring the intensity of the red colour that develops when sulfanilic acid is allowed to react with potassium 1,2-naphthoquinone-4-sulphonate (NS) in a chloroaceticchloroacetate buffer at pH 3.4. Colour development reaches completion after 2 h, allowing sulfanilic acid to be quantified spectrophotometrically at 470 nm ($\epsilon = 4.7 \times 10^3$ L mol^{-1} cm^{-1}). The main product causing colour formation, potassium 1,2-naphthoquinone-4-(N-aminophenylen-4-sulphonate) (NSSA), was isolated and characterized. When samples also contain sulfonamides an extraction into chloroform must be performed. Sulfanilic acid in binary mixtures with sulfanilamide, sulfacetamide, or sulfathiazole can be determined either by direct measurement of the aqueous phase after extraction at 470 nm or by subtracting from the absorbance of the aqueous phase before extraction the absorbance of sulfonamide as determined by measuring the extracted chloroform phase at 345 nm. Sulfadiazine, sulfamethoxipyridazine, and sulfamethoxazole interferences are prevented by their extraction into chloroform at pH 7.2; these species cannot be determined. The effects of pH, reagent concentration, time, and temperature on colour formation were investigated. In all cases the standard addition method gave more accurate results. The method was applied to several pharmaceutical samples.

Key words: spectrophotometry, sulfanilic acid, sulfonamides, potassium 1,2-naphthoquinone-4-sulphonate.

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On propose une méthode précise et simple pour déterminer l'acide sulfanilique en présence de sulfonamides. La méthode est basée sur la mesure de l'intensité de la couleur rouge qui se développe lorsqu'on fait réagir de l'acide sulfanilique avec le 1,2-naphtoquinone-4-sulfonate de potassium (NS) dans un tampon d'acide chloroacétique-chloroacétate, à un pH de 3,4. La couleur est complètement développée après deux heures et l'on peut alors quantifier l'acide sulfanilique d'une façon spectrophotométrique à 470 nm ($\epsilon = 4{,}7 \times 10^3$ L mol^{-1} cm^{-1}). On a isolé et caractérisé le produit principal responsable de la couleur, le 1,2-naphtoquinone-4-(N-aminophénylène-4-sulfonate) de potassium (NSSA). Lorsque les échantillons contiennent aussi des sulfonamides, on doit procéder à une extraction au chloroforme. L'acide sulfanilique en mélanges binaires avec la sulfanilamide, la sulfacétamide ou le sulfathiazole peut être déterminée soit par une mesure directe de la phase aqueuse à 470 nm après extraction soit en soustrayant l'absorbance de la sulfonamide, telle que déterminée par une mesure à 345 nm de la phase chloroformique extraite, de l'absorbance de la phase aqueuse avant extraction. On peut prévenir les interférences de la part de la sulfadiazine, de la sulfaméthoxipyridazine et du sulfaméthoxazole en procédant à leur extraction dans le chloroforme à un pH de 7,2; on ne peut déterminer ces espèces. On a examiné les effets du pH, de la concentration des réactifs, du temps et de la température sur la formation de la couleur. Dans tous les cas, la méthode standard d'addition a fourni les meilleurs résultats. On a appliqué la méthode à de nombreux échantillions pharmaceutiques.

Mots clés : spectrophotométrie, acide sulfanilique, sulfonamides, 1,2-naphtoquinone-4-sulfonate.

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Introduction

Sulfonamides are pharmaceuticals used extensively as antibacterial compounds. They have, in general, very high chemotherapeutic coefficients, so that overdosage can cause digestive or renal accidents. Sulfonamides can rupture under the action of mineral acids at the S—N bond, producing sulfanilic acid (1). Sulfanilic acid exhibits a still greater toxicity than sulfonamides. It is therefore of interest to detect its presence in drugs.

The voltammetric behaviour of sulfanilic acid and sulfonamides has been studied, and selected mixtures have been analyzed by differential pulse voltammetry (2). It is difficult to obtain quantitative results using this approach, however, since the peaks of different compounds are not well resolved. Therefore, the method is proposed only for detection after high-performance liquid chromatography. On the other hand, the spectrophotometric determination of primary arylamines, including sulfonamides, sulfanilic acid, and aniline derivatives, with metol in the presence of *N*-bromosuccinimide has been proposed (3). Nevertheless, these compounds are usually determined by colourimetry based on the Bratton–Marshal procedure (4), which involves diazotation of the amine and coupling to a coloured dye. Since these methods are tedious, it is of interest to find less complicated procedures.

The present study is a method for the determination of sulfanilic acid, sulfonamides, and binary mixtures of the two based on condensation with potassium 1,2-naphthoquinone-4-sulphonate (NS) and separation of the resulting sulfonamide adducts by extraction into chloroform. The derivative of sulfanilic acid remains in the aqueous phase owing to its higher polarity. A few publications on the use of NS for detecting sulfonamides have appeared (5–7), but the proposed methods have been applied only to the pure amines, with no attempt to resolve mixtures in real samples.

In this paper we also report a study of the pure derivative of sulfanilic acid with NS (NSSA) (8) by UV–visible, IR, ^{1}H- and ^{13}C-NMR, and mass spectroscopy including the determination of its acid dissociation contant.

Experimental

Apparatus

Perkin Elmer 1310 and Varian XL 200 spectrometers were used for recording IR and NMR spectra. A Perkin Elmer 554-UV–visible spectrophotometer with matched glass cells of 1.00 cm path was employed. The mass spectrum was obtained with a Kratos MS8RFA apparatus under 70 eV as ionisation voltage and 300°C as the temperature of the source. A Beckman model-70 pH-meter was used.

Chemicals and pharmaceuticals

Amberlite CG-50 I cation exchange resin (Merck) was digested with

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TABLE 1. Infrared spectral data for NSSA*

Bands (cm^{-1})	Assignments
3450 s	ν_{O-H}
3330 m	$\nu_{as\ N-H}$
1670 m	$\nu_{C=O}$ (quinone)
1620 vs	$\nu_{C=N}$
1185 s	ν_{C-O} (phenol)
1155 m	$\nu_{as\ SO_2}$
1010 m	$\nu_{s\ SO_2}$
810–750 w	δ_{Ph-H} (out-of-plane)

*Abbreviations: s, strong; m, medium; w, weak; v, very.

1 *M* KOH, and then placed in a 1-cm diameter ion exchange column to a depth of 10 cm. The column was washed with water until the pH of the eluate was 5 to 6. Sulfanilic acid was analytical grade (Merck-Schuchard), and 1,2-naphthoquinone-4-sulfonic acid, potassium salt (NS) was technical grade (Ega-Chemie). Sulfamethoxazole, sulfamethoxipirydazine, sulfadiazine, sulfanilamide (sodium salt), sulfacetamide, and sulfathiazole were pharmaceutical grade (Acofarma Tarrasa, Barcelona), and were utilized as working standards as received. Distilled-deionised water and spectroscopic grade chloroform were used throughout. Abactrim was an antibacterial chemotherapeutic suspension (Roche) containing 40 mg of trimetoprime and 200 mg of sulfamethoxazole per 5 mL; Bio Exazol Simple was an antibacterial tablet (Andreu–Barcelona) containing 125 mg of erythromycine base, 125 mg of sulfamethoxipyridazine total, and 150 mg of dimethylchlorotetracycline chlorhydrate per tablet; Amidrin was an antibacterial antiseptic solution (Fardi–Barcelona) containing 4 mg of sulfanilamide, 8 mg chlorhydrate of efedrine, and 5 mg of chlorobutanol per mL; and colirio Oculos was a sedative antiseptic solution (Frumtost-Zyma S.A. Barcelona) containing 100 mg of sulfacetamide sodium salt, 4 mg of tetracaine chlorhydrate, 6 mg of carbamide, and 0.4 mg of naphazoline per mL.

Synthesis and purification of NSSA

A solution of sulfanilic acid (0.7 g) in 60% ethanol–water (v:v) was added to an aqueous solution of 1,2-naphtoquinone-4-sulphonate (NS) (1.0 g). The mixture was refluxed for 30 min, then cooled and passed through an Amberlite CG-50 I column at a rate not exceeding 3 mL min^{-1}. The resin was washed with 200 mL of water and the eluates (about 400 mL) concentrated to approximately 40 mL. The solution upon cooling yielded a deep red violet product. The product when recrystallized from water showed only one spot in TLC tests on silica gel (R_f = 0.72 for chloroform–ethanol 75:25 and R_f = 0.30 for acetone–methanol 90:10). Melting point >300°C. *Anal.* Calcd. for $C_{16}H_{10}NSO_5K$: C 52.30, H 2.74, N 3.81, S 8.72; found: C 51.89, H 2.78, N 3.76, S 8.45. The assignments made for the IR, ^1H- and ^{13}C-NMR spectra of the product are showed in Tables 1 to 3. The calculated values of the ionization constant of the product are collected in Table 4. The molecular peak in the mass spectrum at m/z 368 is close to the calculated molecular weight of the compound (367.1 g mol^{-1}).

Reagent solutions

Sulfanilic acid and sulfonamide stock solutions

Dissolve in 250 mL of water an accurately weighed each of the following: for sulfanilic acid about 39.5 mg, sulfanilamide sodium salt about 44 mg, sulfacetamide about 49 mg, sulfathiazole about 49 mg, sulfamethoxipyridazine about 49 mg, sulfamethoxazole about 231 mg, and sulfadiazine about 200 mg. Working standard solutions are prepared by diluting with water as necessary.

NS stock solution

Dissolve 0.2012 g of potassium 1,2-naphthoquinone-4-sulphonate (Ega-Chemie) in 50 mL of water and dilute to 100 mL with water. This NS solution must be prepared daily.

Chloroacetic acid – chloroacetate (sodium salt) buffer, 0.1 *M* (total concentration), pH = 3.42.

Imidazole – hydrochloric acid buffer, 0.1 *M* (total concentration), pH = 7.20.

Prepared pharmaceutical samples

To accurately weighed amounts of Abactrim, Bio Exazol Simple, Amidrin, and Colirio Oculos an accurately weighed amount of sulfanilic acid was added. The samples were then dissolved in water and diluted to give the compositions shown in Table 5. The samples so prepared were starting materials for the analyses.

Assay procedure

Transfer 5 mL of a prepared pharmaceutical sample solution into a 25-mL calibrated flask and add 0, 1, 3, or 5 mL of sulfanilic acid or sulfonamide working standard solution to construct a standard additions graph (see Table 6). Add 5 mL of chloroaceticchloroacetate solution buffer and 5 mL of NS solution. Mix thoroughly and dilute to volume with water. Let stand for 2 h, then measure the absorbance at 470 nm (A_0) in a 1-cm cell against a blank. Subsequently carry out the separation of sulfonamides into chloroform using either technique A or B.

(A) For samples containing sulfamethoxipyridazine, sulfamethoxazole, or sulfadiazine

Adjust the pH of a 10-mL aliquot of prepared solution to 7.2 by adding 10 mL of imidazole–HCl buffer. Transfer 10 mL of this sample into a separatory funnel and extract twice for 2 min each time with 5 mL of chloroform. Discard the extracts and measure the absorbance of the aqueous phase at 470 nm (A_a) against blanks prepared under the same conditions, but using 5 mL of water instead of sample solution.

The intercept value in a plot of A_a vs. mg of added sulfanilic acid gives the sulfanilic acid concentration in the prepared pharmaceutical sample.

(B) For samples containing sulfanilamide, sulfacetamide or sulfathiazole

Place a 5-mL aliquot of prepared solution from the 25-mL calibrated flask in a separatory funnel and extract twice for 2 min each time with 5 mL of chloroform. Collect the extracts and measure the absorbance of the chloroform phase at 345 nm (A_b) against a blank.

The intercept value of a plot of $(A_0 - \epsilon_{470}/\epsilon_{345} \times A_b)$ vs. mg of added sulfanilic acid gives the sulfanilic acid concentration in the pharmaceutical sample. In the above expression ϵ_{470} and ϵ_{345} are the apparent molar absorptivities for the sulfonamide in the aqueous phase (λ_{max} 470 nm) and chloroform phase (λ_{max} 345 nm), respectively.

The intercept value of the graphic representation of A_b vs. mg of added sulfonamides gives the sulfonamide concentration in the prepared pharmaceutical sample.

Construction of calibration graphs

The calibration graphs to determine the molar absorptivities were not prepared from pure substances, but with binary mixtures of sulfanilic acid and sulfonamides, prepared by diluting and mixing aliquots of the stock solutions to give a series of suitable concentrations. Linearity is maintained over the range 7 to 28 μg mL^{-1} for sulfanilic acid; 7 to 15 μg mL^{-1} for sulfanilamide, sulfacetamide, or sulfathiazole; and 0 to 45 μg mL^{-1} for sulfamethoxipyridazine, sulfamethoxazole, or sulfadiazine. Sulfamethoxipyridazine, sulfamethoxazole, and sulfadiazine are not determinable because they develop little colour in the organic phase; their interference with the sulfanilic acid determination is eliminated by extraction at pH 7.20.

Determination of the stoichiometry of the sulfanilic acid – NS species

Job's method of continuous variations in equimolar (9) and in non-equimolar (10) conditions was employed. Standard aqueous solutions of sulfanilic acid (1.09×10^{-3} *M* to 2.30×10^{-3} *M*) and NS (2.20×10^{-3} *M* to 0.82×10^{-3} *M*) were used. A series of standard solutions of sulfanilic acid and NS in complementary proportions totalling 12 mL (from 0 to 12 and 12 to 0 inclusive) were prepared in 25-mL calibrated flasks. To each was added 5 mL of chloroacetic acid – sodium chloroacetate solution buffer. After 2 h the absorbance of each solution was measured at 470 nm against blanks prepared under the same conditions, but replacing the sulfanilic acid with an equal volume of water.

Yoe and Jones' method of molar ratios was also applied (11). Into a

TABLE 2. ^{1}H-nmr spectral data[a] (δ ppm)

Proton number	Sulfanilic acid	Compound III	Compound IV	NSSA Signal	NSSA Integration
3	—	5.87(s)	[b]	6.18(s)	1
5	—	8.06(m)	8.09(m)	7.96(m)	
6	—	7.76(m)	7.88(m)	7.81(m)	
7	—	7.82(m)	7.88(m)	7.86(m)	4
8	—	8.33(m)	8.05(m)	8.10(m)	
2′, 6′	7.30(m)[c]	7.24(m)	—	7.39(m)	4
3′, 5′	7.76(m)[c]	7.32(m)	—	7.65(m)	
4′	—	7.51(m)	—	—	
2(—OH)	—	—	11.50(s)	9.29	1
4(—NH—)	—	8.50(s)	—		

[a]Concentration of the compounds about 40 mg mL^{-1} in d_6-dimethylsulfoxyde, s = singlet, m = multiplet.
[b]Not comparable chemical environment.
[c]Assignments may be reversed.

TABLE 3. ^{13}C-nmr spectra[a] (δ ppm)

Carbon number	Sulfanilic acid	Compound IV	NSSA
1	—	181.3[c]	182.6[c]
2	—	159.7	152.6[c]
3	—	111.1	102.4
4	—	84.7[b,c]	168.4[c]
5	—	133.3	132.6
6	—	126.0	126.1
7	—	125.5	125.3
8	—	134.5	134.9
9	—	130.6[c]	130.4[c]
10	—	131.9[c]	132.5[c]
1′	147.0	—	—
2′, 6′	122.3	—	—
3′, 5′	127.0	—	—
4′	132.0	—	—

[a]Concentration of the compounds about 40 mg mL^{-1} in d_6-dimethylsulfoxide.
[b]Chemical environment of carbon not comparable.
[c]Quaternary carbon atom confirmed by Attached Proton Test (APT).

TABLE 4. Determination of pK_{a2}

Method	pK_{a2}[a,b]	ϵ_{HR^-}(470 nm)[c] (L mol^{-1} cm^{-1})	$\epsilon_{R^{2-}}$(530 nm)[b] (L mol^{-1} cm^{-1})
Maroni–Calmon			
Parallel straight lines	11.31	4480	3680
Concurrent straight lines	11.31	4480	3700
Romain–Colleter	11.26	4480	—
Potentiometric	11.62	—	—

[a]Mean value, 11.37.
[b]Obtained by corresponding method.
[c]Experimental value.

series of 25-mL calibrated flasks were placed 5 mL of chloroacetic acid – sodium chloroacetate solution buffer, 4 mL of sulfanilic acid (1.09×10^{-3} *M*) and variable amounts of NS solution (6.20×10^{-3} *M*). After 2 h the absorbances at 470 nm were measured against blanks.

Results and discussion

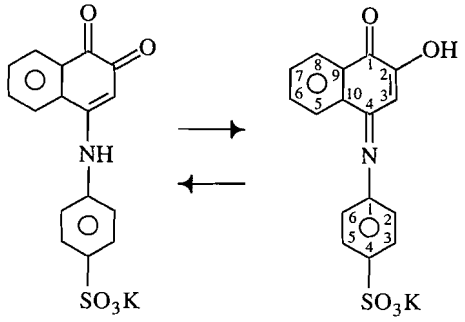
(A) Study of the NSSA adduct

Infrared, NMR, and UV–visible spectra

The use of several spectroscopic techniques permits one to establish the nature of the adduct formed between sulfanilic acid and 1,2-naphthoquinone-4-sulphonate (potassium salt). Two tautomers could be present (**I**, 4-arylamino-1,2-naphthoquinone; and **II**, 4-arylamino-2-hydroxy-naphthoquinone).

The infrared spectrum of the derivative (potassium bromide disk) is complicated; the main bands and their assignments are given in Table 1. The characteristic strong C=N band at 1620 cm^{-1} and the presence of a C=O band at 1670 cm^{-1} confirm the coupling of sulfanilic acid at position 4 of the NS molecule.

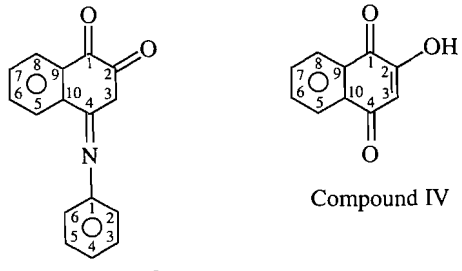
The most relevant features of ^{1}H- and ^{13}C-NMR spectra in d_6-dimethylsulphoxide are summarized in Tables 2 and 3. For



Tautomer I Tautomer II

comparison we synthesized substances **III** and **IV** by the method of Fieser (12, 13).

The most characteristic signal for this type of substance in the ^{1}H-NMR spectrum (Table 2) is a singlet at δ = 6.2 ppm



Compound III Compound IV

TALE 5. Composition of synthetic pharmaceutical samples

Pharmaceutical sample	Sulfonamide contained	Concentration in sample solutions (mg mL^{-1})	
		Sulfonamide content	Sulfanilic acid added
Abactrim	Sulfamethoxazole	400	73
Bio exazol simple	Sulfamethoxipyridazine	415	73
Amidrin	Sulfanilamide	40	73
Colirio oculos	Sulfacetamide (sodium salt)	36	55

TABLE 6. Concentration of used working standard solutions to be added to the prepared pharmaceutical samples in the standard addition graph

Sample	Concentration of sulfonamides ($\mu g\ mL^{-1}$)	Concentration of sulfanilic acid ($\mu g\ mL^{-1}$)
Abactrim	—	111
Bio exazol simple	—	55
Amidrin	39 (sulfanilamide) (sodium salt)	55
Colirio oculos	132 (sulfacetamide)	111

corresponding to the proton attached at C_3. The peak at 9.29 ppm disappears on deuteration. The δ-value lies between those of —NH in **III** and —OH in **IV**, suggesting the tautomeric character of this proton.

The number of ^{13}C-NMR signals, Table 3, are 16 as expected and their positions agree well with those of corresponding carbons in the comparison compounds. The APT technique (14) shows no inversion for the C_1, C_2, and C_4 signals, in accordance with the quaternary character of these atoms. The presence in the NSSA spectrum of a single signal around 182 ppm (assigned to C=O) and one signal for C=N at 168.4 ppm is consistent with the tautomer **II** structure. On the other hand, for product **IV** two signals for C=O (C_1 and C_4) appear but the C=N signal is not present.

Ultraviolet–visible spectra at various pH values in water (Fig. 1) show a band at 270 nm and a shoulder at 340 nm, both assigned to $\pi \rightarrow \pi^*$ transitions, and a wide band with a maximum at 470 nm characteristic of 1,2-naphthoquinone nuclei bounded with arylamines at position 4. The chromophoric system changes beyond pH 11 and the maximum at 470 nm shifts to 525 nm. This may be explained by considering that in alkaline medium the 2-keto group is converted to a —OH group (transformation of form **I** to form **II**, producing maxima at 253 and 280 nm, typical for 1,4-naphthoquinones) which then deprotonates go give the corresponding anion. An analogous change in the molecular structure of other NS arylamine derivatives does not appear in alkaline media, but only in strong acids (pH ≤ 0.8) due to protonation of the 2-keto group (15). Weakly acidic or alkaline solutions contain 4-arylamino-1,2-naphthoquinone as the more important form. The fact that NSSA in strong acids does not undergo transformation from form **I** to form **II** could be attributable to the presence of the $—SO_3^-$ group, which precludes protonation of the 2-keto group.

Equilibria of NSSA with protons

The spectra of aqueous solutions of NSSA are pH-dependent

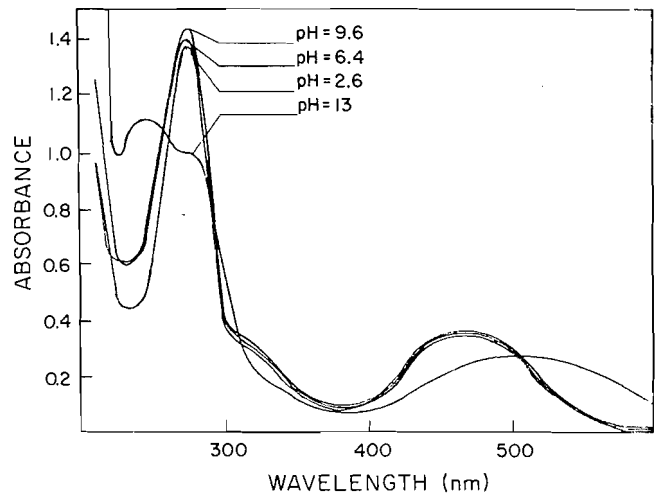


FIG. 1. Ultraviolet absorption spectra of NSSA ($c = 6.4 \times 10^{-5}$ mol L^{-1}) at different pH values.

(Fig. 2). The NSSA spectrum over pH 1 to 11 is attributed to the charged species RH^-, with an absorption maximum in the visible region at 470 nm which in strong acids may be converted to a fully protonated species (RH_2). We did not study this pK_a because we found that protonation of the $—SO_3^-$ group, if it occurs, is not reflected by any change in the spectrum of NSSA when a large amount of acid is present. Moreover, this pK_a lacks practical interest. The major species above pH 11 is the anion R^{2-}, which has an absorption maximum in the visible region at 525 nm.

Graphical analysis of A vs. pH curves (Fig. 2*A*) over the appropriate wavelengths yielded a pK_{a2} value. The Maroni–Calmon and Romain–Colleter methods were used. Deprotonation is shown by the absorbance step that appears for each wavelength in the basic region, with an inflection point dividing the steep part of the graph into two sections.

Maroni and Calmon (15) developed two techniques which permit one to obtain the pK_{a2} value and the molar absorptivity $\epsilon_{R^{2-}}$. In the first technique (method of parallel straight lines) A vs. $|A_{HR^-} - A|[H^+] \times 10^{12}$ is plotted (Fig. 2*B*); in the second one $1/|A - A_{HR^-}|$ vs. $[H^+] \times 10^{12}$ is plotted (Fig. 2*C*). In both cases the inverse of the slope yields the pK_{a2} value.

The Romain–Colleter method (16) was started with an initial pK_{a2} value of 10.3 (estimated from Maroni–Calmon's method) to obtain a more refined value. Budesinky's technique (17) to calculate the number of protons implicated in the ionization reaction was applied to the A vs. pH curves. It was found that only one proton participated in the reaction.

Potentiometric titration of a NSSA solution (0.0016 mol / L)

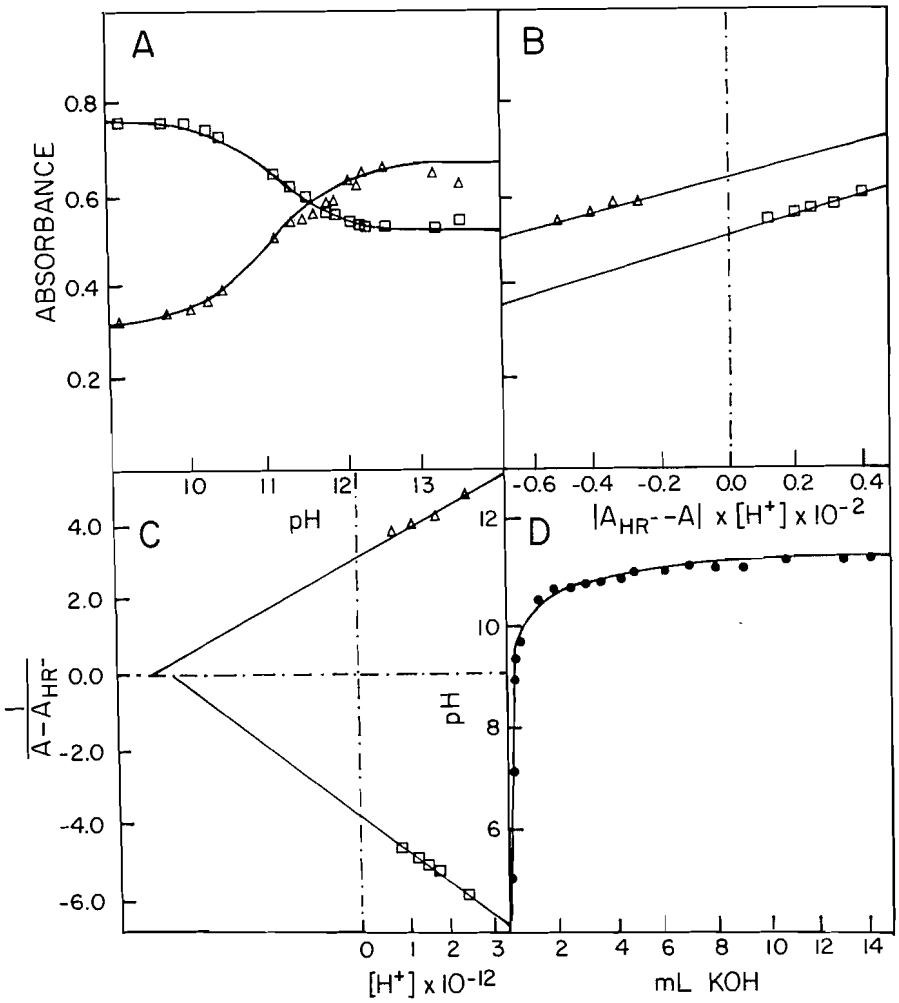


FIG. 2. Determination of pK_{a2} of NSSA: (*A*) absorbance vs. pH curves; (*B*) parallel straight lines method; (*C*) concurrent straight lines method; (*D*) potentiometric method.

with 0.0185 mol / L KOH in water under nitrogen while keeping temperature and ionic strength constant was also carried out (Fig. 2*D*). A pK_{a2} determination from the potentiometric measurements was made using Schwarzenbach's method (18).

The pK_{a2} and arithmetic mean values obtained by all these methods are summarized in Table 4.

Stability and behaviour against reducing and oxidizing agents

NSSA is very stable in the pH 4 to 9 range, but out of this range it suffers rapid decomposition. Both reducing and oxidizing agents at 1% (w / v) concentration alter the absorption spectra of NSSA in water, the material being more unstable against oxidizing than reducing agents. The decomposition products were not identified.

(B) Reaction of sulfanilic acid with NS and determination of sulfanilic acid and sulfonamides

A characteristic red colour with an absorption maximum at 470 nm develops when sulfanilic acid reacts with NS in the chloroacetic acid – chloroacetate (sodium salt) buffer. The reaction can be used to determine this acid. NS is yellow in aqueous solution, but its color does not affect sulfanilic acid determintions if blanks with the same concentration of NS are used.

The reaction of NS with six sulfonamides was studied. Sulfanilamide, sulfacetamide, and sulfathiazole also show intense red colouration when treated with NS in the presence of chloroaceticchloroacetate buffer, both in aqueous medium and after extraction into chloroform. In chloroform quantitative measurements can be undertaken.

Preliminary studies revealed the additivity of the absorbance caused by sulfanilic acid and by the other sulfonamides studied. Hence, we attempted to resolve the several binary mixtures by mathematical analysis of the spectroscopic data, as well as by derivative spectroscopy. The results were not satisfactory, however, because the absorption maxima and molar absorptivities were very close. Extraction into chloroform to prevent the interference of sulfanilamide, sulfacetamide, and sulfathiazole with the sulfanilic acid determination was, however, successful. Moreover, simultaneous determinations are possible. To determine sulfanilic acid in these mixtures a simple absorbance measurement of the aqueous layer at 470 nm can be employed, but results are more precise if the equation described under the Assay Procedure is used. This equation corrects the total absorbance of the aqueous phase before extraction by subtracting the sulfonamide contribution (obtained from measurement of the chloroform phase at 345 nm). Sulfamethoxiazole, sulfamethoxipyridazine, and sulfadiazine form so little colour with NS that they cannot be quantitatively determined; instead they are extracted into chloroform from an imidazole buffer to eliminate their slight interference in the aqueous phase.

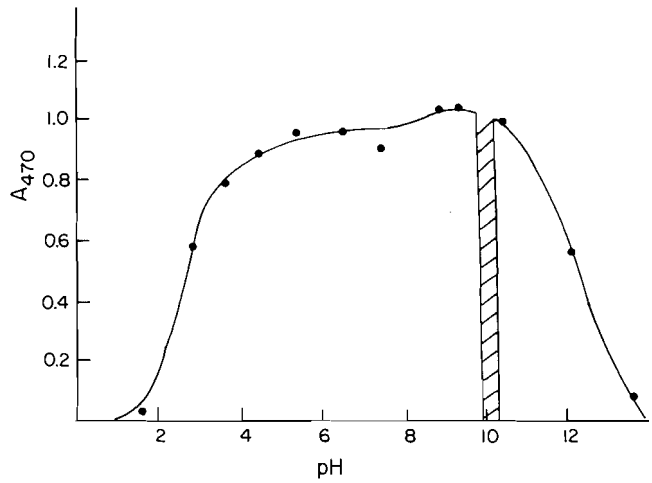


FIG. 3. Absorption intensity of the chromogen as a function of pH. Concentration of sulfanilic acid 43 μg mL^{-1}. The cross-hatched zone represents precipitation of NS in the samples.

Optimisation of variables

The optimisation of several variables influencing the reaction between sulfanilic acid and NS was studied. Maximum colour intensity was obtained after 90 min at 20 ± 5°C. The colour was stable for a further 24 h. The optimum pH range using an equimolar solution of both compounds is 3.3 to 8.0 (Fig. 3). A pH value of 3.5, adjusted with chloroacetic acid – sodium chloroacetate buffer, is recommended. The optimum concentration of NS leading to maximum intensity of colour was found to be 15-fold that of the sulfanilic acid concentration in the final solution. The amount of buffer solution, ionic strength, addition order, and temperature did not affect the results. A modified simplex method (19) was used to optimize pH and relative amount of NS reagent to sulfanilic acid in addition to the classical approach for optimisation. The result obtained by the simplex method was the same as that obtained by the classical one.

Quantification, linearity of Beer's law plot, accuracy, and precision

A linear correlation ($r = 0.9997$) was found between absorbance at 470 nm and concentration of sulfanilic acid in the range 3.1 to 31.0 μg mL^{-1}. The apparent molar absorptivity was found to be 4.7×10^3 L mol^{-1} cm^{-1}. The reproducibility of the procedure was determined by running replicate samples, each containing 15.8 μg mL^{-1} of sulfanilic acid in the final test solution. At this concentration level the relative standard deviation did not exceed 0.4% for twelve separate samples.

Sensitivity and specificity

The optimum range for measurement of sulfanilic acid with NS was determined by Ringbom's method (20) to be in the range from 8 to 25 μg mL^{-1}. This indicates good sensitivity for the reaction, and thus the present method can be recommended for the detection of trace amounts of sulfanilic acid in drugs. The influence of numerous cations and some amines on sulfanilic acid quantification was tested. Cations do not interfere except for Cu(II) and Al(III) at molar ratios higher than 1:10 (sulfanilic acid:cation). Hydrazides, semicarbazides, and some aminopyridines interfer by forming precipitates with NS. Aniline and sulfonamides give coloured products with NS in aqueous medium, but can be separated by extraction into organic solvents.

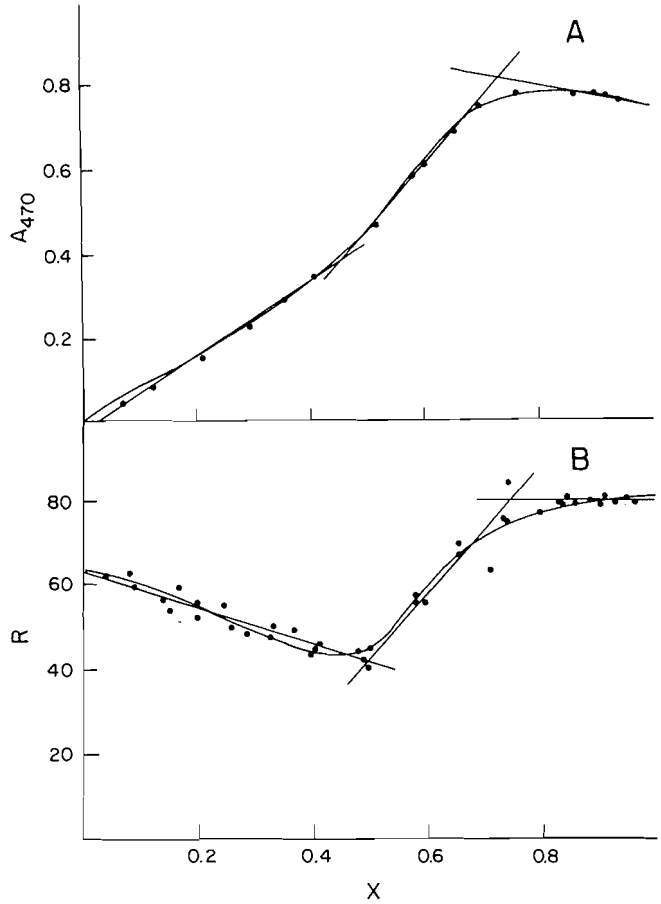


FIG. 4. Sulfanilic acid to NS ratio in the chromogen: (*A*) molar ratio method; (*B*) plot of data from investigation in non-equimolar conditions. *X* is the molar fraction of NS $C_{NS} / (C_{NS} + C_S)$.

Sulfanilic acid to NS ratio in the chromogen

A continuous molar variation study of sulfanilic acid and NS showed that interaction between them does not occur on a equimolar basis. Hence we applied the continuous variation method in non-equimolecular solutions according to Ramanathan and Walvekar (10) to a set of experimental curves obtained at different molarities of the reactants. This study suggested the possible presence of more than a single complex if a large excess of NS is present, even though the isolated adduct obtained under experimental conditions of equal molar amounts of both reactants corresponds clearly to a 1:1 stoichiometry.

To evaluate more precisely the combining ratios of sulfanilic acid and NS, we used the molar ratio method (11) with a fixed amount of sulfanilic acid (Fig. 4*A*), and also the data from an investigation under non-equimolar conditions (Fig. 4*B*).

Plots of *R* (relative percentage of adduct to total amount of minor component in sulfanilic–NS mixtures) against *X* (molar fraction of NS in the sulfanilic acid – NS mixtures) are shown in Fig. 4*B*. *R* is calculated on the assumption that the absorbance limit at each end of the studied range corresponds to different species involving sulfanilic acid and NS. If these species are called **I** and **II**, $C_{\mathbf{I}}$ and $C_{\mathbf{II}}$ are calculated from $\epsilon_{\mathbf{I}}$ and $\epsilon_{\mathbf{II}}$ at each point and thus situation (*A*) occurs when $C_S \geq C_{NS}$, $R = (C_{\mathbf{I}} / C_{NS}) \times 100$, and (*B*) when $C_S \leq C_{NS}$, $R = (C_{\mathbf{II}} / C_S) \times 100$. In the above expressions C_S and C_{NS} are the concentrations of sulfanilic acid and NS in the samples. Situation *A* reflects predominance of sulfanilic acid, and *B* when NS predominates.

TABLE 7. Assay of sulfanilic acid and some sulfonamides in pharmaceuticals by the NS method

Sample	Sulfonamide assay: Amount present (mg mL^{-1}) I[b]	II[c]	Recovery, %[a] I	II	Sulfanilic acid spike recovery: Amount added (mg mL^{-1})	Recovery, %[a]
Abactrim	—	—	—	—	7	93±1.5
Bio exazol simple	—	—	—	—	22[d]	109±1.3
Amidrin	4	—	98±2.1	—	7	96±1.3
Colirio oculos	—	100	—	89±2.0	152	113±1.2

[a]All averages are based on the three determinations; uncertainties are one standard deviation. Theoretical recovery is 100%.
[b]**I** = sulfanilamide.
[c]**II** = sulfacetamide.
[d]In mg/tablet.

The graph R vs. X should be monotonic if only one complex is involved, but the presence of two points of discontinuity demonstrates that, in agreement with the molar ratio method, there is an initial combining ratio of 1:1 (sulfanilic acid – NS) and a second above a 1:3 ratio.

The first ratio indicates formation of the equimolar adduct that was isolated and characterised in this work. The second inflection point is not easy to interpret. We conclude that the red colour produced when sulfanilic acid reacts with a large excess of NS is due to a mixture of a 1:1 complex and a second complex of undefined higher stoichiometry.

Application to pharmaceuticals

The suggested method was applied to the quantitative determination of spiked sulfanilic acid, and to sulfonamides in pharmaceuticals. The data in Table 7 indicate the suitability of the method for routine quality control analysis.

Conclusions

The nature of the species formed between sulfanilic acid and 1,2-naphthoquinone-4-sulphonate has been established by elemental and spectroscopic analysis. The adduct has two tautomers: 4-arylamino-1,2-naphthoquinone (form **I**) and 4-arylimine-1,4-naphtoquinone (form **II**). Form **I** is present in acidic and slightly basic aqueous solutions. Deprotonation of this form leads to form **II** (pK_a = 11.3).

Although several methods are described in the literature for the determination of sulfonamides, all have serious limitations. The photometric Bratton–Marshal method for amines, which uses diazotation and coupling of the formed azo dye, is tedious. Acid–base titrimetry in dimethylformamide with tetramethylammonium hydroxide of the sulfonamide group (—SO_2—NH—) presents problems from interferences by other substances in the sample. Moreover, relatively high concentrations are needed. In this study we propose a new simple procedure for the determination of sulfanilic acid and sulfonamides. Furthermore, binary mixtures can be resolved. Sulfanilic acid can be determined in the presence of a two-fold molar excess of sulfanilamide, sulfacetamide, and sulfathiazole and vice versa. The method also tolerates sulfamethoxazole, sulfamethoxipyridazine, and sulfadiazine at molar concentrations five- to eight-fold greater than sulfanilic acid, but these sulfonamides could not be quantified in the mixtures.

Although direct analysis is feasible, use of a standard additions method is recommended to obtain more precise results. When the amines are alone the direct procedure is more advantageous.

Results of application of the method to several samples are in general satisfactory and deviations from theoretical recovery of 100.0% are minimal (Table 7).

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