Available online at www.sciencedirect.com
SCIENCE DIRECT®
Il Farmaco 58 (2003) 1295–1300
IL FARMACO
www.elsevier.com/locate/farmac

3-Aminophenol as a novel coupling agent for the spectrophotometric determination of sulfonamide derivatives

P. Nagaraja *, H.S. Yathirajan, C.R. Raju, R.A. Vasantha, P. Nagendra, M.S. Hemantha Kumar

Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570006, India

Received 9 August 2002; accepted 29 April 2003

Abstract

A rapid, simple and sensitive spectrophotometric method for the determination of some sulfa drugs is described. The method is based on the formation of orange yellow colored azo product by the diazotization of sulfonamides, viz., dapsone (DAP), sulfathiazole (SFT), sulfadiazine (SFD), sulfacetamide (SFA), sulfamethoxazole (SFMx), sulfamerazine (SFMr), sulfaguanidine (SFG) and sulfadimidine (SFDd) followed by a coupling reaction with 3-aminophenol in aqueous medium. Absorbance of the resulting orange yellow product is measured at 460 nm and is stable for 6 days at 27 °C. Beer's law is obeyed in the concentration range of 0.05–8.0 μg/ml at the wavelength of maximum absorption. The method is successfully employed for the determination of sulfonamides in various pharmaceutical preparations and common excipients used as additives in pharmaceuticals do not interfere in the proposed method. Plausible reaction mechanism is proposed for the formation of the azo product.

Keywords: Sulfa drugs; 3-Aminophenol; Diazotization; Spectrophotometry; Pharmaceuticals

1. Introduction

Sulfonamides are an important class of antibacterial drugs used in medicine and veterinary practice. Sulfa drugs are widely used in the treatment of infections, especially for patients intolerant to antibiotics. The vast commercial success of these medicinal agents has made the chemistry of sulfonamides to become a major area of research and an important branch of commercial importance in pharmaceutical sciences. An excellent review of pharmacology and therapeutic use of dapsone (DAP) is given by Uetrecht [1]. Comprehensive descriptions on the analytical aspects of some important sulfa drugs have been reviewed [2–6]. The official method of British Pharmacopoeia [7] and United States Pharmacopoeia [8] describe nitrite titration method for the analysis of sulfa drugs. A survey of literature reveals that there are numerous methods available for the determination of sulfonamide derivatives. These methods have been summarized recently by various workers including from our laboratories [9–16].

The solution of 3-aminophenol is colorless and stable. 3 Aminophenol is used as a dye intermediate, used in the manufacture of *p*-aminosalicylic acid and also used earlier as a coupling agent for the spectrophotometric determination of phenols [17], nimesulide [18] and folic acid [19]. The purpose of this work is to introduce 3-aminophenol as a novel coupling agent, which is economically cheaper and fairly sensitive than most of the widely used coupling agents, viz., *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NEDA), 3-methylbenzothiazoline hydrazone hydrochloride (MBTH) and primaquine phosphate (PP). In continuation of our work on the spectrophotometric determination of some organic compounds of biological interest and pharmaceutical importance [20–22], the present paper describes a simple and sensitive spectrophotometric method for the determination of sulfa drugs. In the present investigations, the diazotized sulfa drug is coupled with 3-aminophenol in aqueous medium to give an orange yellow product which is stable for 6 days.

* Corresponding author.
E-mail address: nagarajap@mailcity.com (P. Nagaraja).

0014-827X/03/$ - see front matter © 2003 Published by Éditions scientifiques et médicales Elsevier SAS.
doi:10.1016/S0014-827X(03)00093-4

2. Experimental

2.1. Instrument

A JASCO Model UVIDEC-610 UV–Vis spectrophotometer with 1.0 cm matched cells was used for electronic spectral measurements.

2.2. Reagents

Sulfonamide derivatives were all purchased from Sigma (USA) and were used without further purification. 3-Aminophenol (Sigma), sodium nitrite (BDH), HCl (AR) and all other reagents and solvents were of analytical grade. Commercial dosage forms were purchased from local sources.

2.3. Solutions

Deionized water was used to prepare all solutions. Standard solutions of sulfonamides (1000 μg/ml) were prepared by dissolving 100 mg of each sulfonamide in 5.0 ml of 5 M HCl and then diluting to the mark in a 100-ml standard flask. A working standard solution of each sulfonamide containing 25 μg/ml was prepared by further dilution and standardized by the USP method [8]. A 1% solution of $NaNO_2$ in water, 2% aqueous solution of 3-amino phenol and 2% aqueous sulfamic acid were used for the experiment. Solution of 3-aminophenol was stable for a week at room temperature and stable for a month, if refrigerated.

2.4. Recommended procedure

Sulfonamide derivative solutions (2.5–125 μg; 1.25–125 μg for DAP; 2.5–200 μg for sulfamethoxazole, SFMx) were transferred into each of the series of 25-ml standard flasks and 1.0 ml of 5 M HCl was added to each flask. After cooling in an ice bath, 1 ml of 1% $NaNO_2$ solution was added with swirling. The solutions were allowed to stand for 5 min and then 2 ml of 2% sulfamic acid solution was added. The solutions were swirled and allowed to stand for 5 min. Then, 2.0 ml of 2% 3-aminophenol solution was added, heated in a boiling water bath for 5 min and cooled. The solution was made up to the mark with water, mixed thoroughly and after 5 min, the absorbance was measured at 460 nm against the corresponding reagent blank and calibration graphs were constructed.

2.5. Procedure for the assay of sulfonamide derivatives in commercial samples

Twenty tablets were weighed and finely powdered. The powder amount equivalent to 50 mg was dissolved in 5 ml of 5 M HCl and filtered. The filtrate was made upto 100 ml and appropriate aliquots of the tablet solutions were treated as described above in the recommended procedure for the pure drug. For eye drops, an accurately measured volume was appropriately diluted with 5 ml of 5 M HCl, made upto 100 ml and the recommended procedure was followed.

3. Results and discussion

The method involves the diazotization of the sulfonamide derivatives followed by coupling with 3-aminophenol in water to produce an orange yellow azo product. Sulfa drugs studied in the present investigation are given in Table 1.

3.1. Spectral characteristics

Absorption spectra of the azo products with all the eight sulfa drugs indicate that the maximum absorption occurs at 460 nm. The colorless reagent blank in each case has practically negligible absorption at this wavelength. The spectral characteristics and precision data for all the eight sulfa drugs are given in Table 2.

3.2. Optimum reagents concentration

It was found that a 5 mol/dm^3 solution of HCl in the range of 0.5–3.0 ml, 1% solution of sodium nitrite in the range of 0.5–3.0 ml, 2% solution of sulfamic acid in the range of 1.0–4.0 and 1.0–4.0 ml of 2% 3-aminophenol were necessary to achieve maximum color intensity. Hence, 1.0 ml of HCl, 1.0 ml of $NaNO_2$, 2.0 ml of sulfamic acid were used for diazotization and 2.0 ml of 3-aminophenol was sufficient to produce the azo product. The excess of nitrite during diazotization could be removed by the addition of sulfamic acid solution and an excess of sulfamic acid has no effect on the color intensity. The use of water for dilution was found to give better results when compared to hydrochloric acid in terms of color development and stability of the product.

3.3. Reaction sequence

For the diazotization process, sulfa drug could be readily diazotized in acidic medium and that the diazonium cation would then react with a molecule of 3-aminophenol by electrophilic substitution at the para-position to the amino group of the coupling agent to produce an orange yellow dye. Investigation of the continuous molar variation [23] of the sulfathiazole (SFT) and 3-AP showed that the diazotized SFT interacts with 3-AP in the ratio of 1:1 (For DAP and 3-AP, the diazotized DAP interacts with 3-AP in the ratio of 1:2). Similar results have been observed with the mole ratio method [24]. A reaction mechanism based on the above results is shown in Scheme 1.

Table 1

Sulfa drugs studied

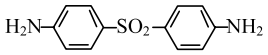
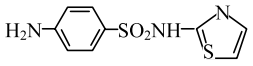
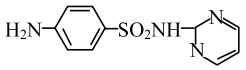
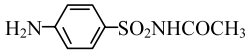
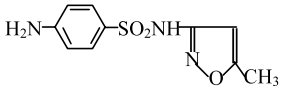
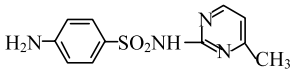
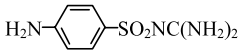
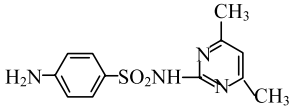
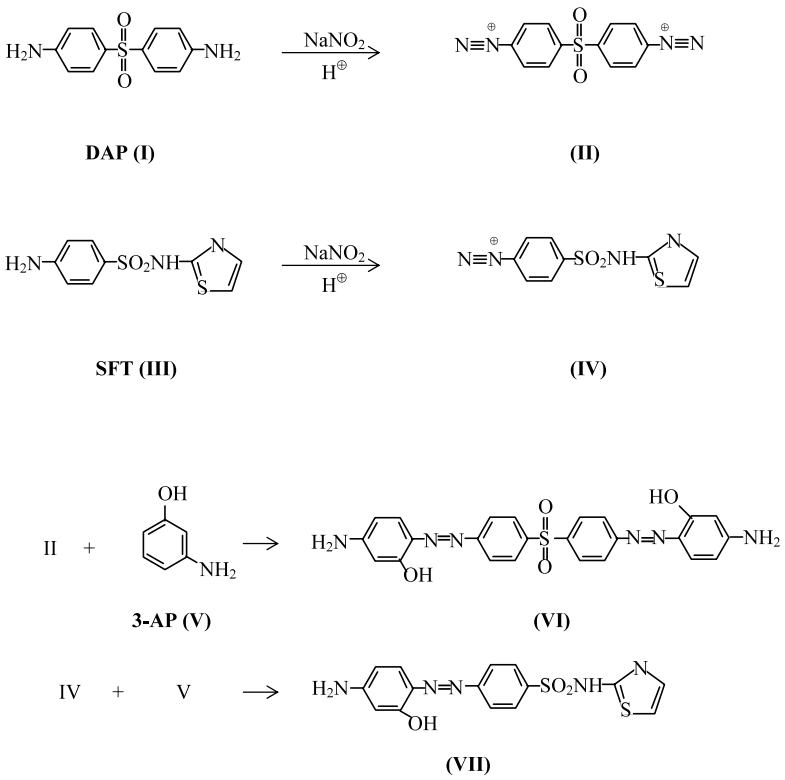
Sl. No.	Name of the derivative	Abbreviation	Structure
1.	Dapsone	DAP	
2.	Sulfathiazole	SFT	
3.	Sulfadiazine	SFD	
4.	Sulfacetamide	SFA	
5.	Sulfamethoxazole	SFMx	
6.	Sulfamerazine	SFMr	
7.	Sulfaguanidine	SFG	
8.	Sulfadimidine	SFDd	

Table 2
Optical characteristics and precision data

Parameters/Characteristics	DAP	SFT	SFD	SFA	SFMx	SFMr	SFG	SFDd
Color	orange yellow	orange yellow	orange yellow	orange yellow	orange yellow	orange yellow	orange yellow	orange yellow
λ_{max} (nm)	460	460	460	460	460	460	460	460
Stability (in days)	06	06	06	06	06	06	06	06
Beer's law range (μg/ml)	0.05–5.0	0.1–5.0	0.1–5.0	0.1–5.0	0.1–8	0.1–5.0	0.1–5.0	0.1–5.0
LOD (μg/ml)	0.0366	0.0232	0.0504	0.0415	0.0472	0.0458	0.0302	0.0450
LOQ (μg/ml)	0.1210	0.0766	0.166	0.137	0.156	0.151	0.0997	0.149
Molar absorptivity (l/mol/cm)	4.64×10^4	4.23×10^4	4.47×10^4	4.33×10^4	4.32×10^4	3.59×10^4	3.87×10^4	3.78×10^4
Sandell's sensitivity (μg/cm^2)	0.0053	0.0063	0.0056	0.0049	0.0074	0.0073	0.0055	0.0074
Optimum photometric range (μg/ml)	1–4	0.5–4	0.5–4.5	0.5–4	0.5–6	0.5–4	0.5–4	0.5–4
Regression equation (Y) [a] Slope (b)	0.1427	0.1687	0.1555	0.1889	0.1727	0.1427	0.1730	0.1645
Intercept (a)	0.0096	−0.0065	0.0091	0.0051	−0.0035	0.005	0.0043	−0.0173
Correlation coefficient (r)	0.9996	0.9998	0.9996	0.9999	0.9997	0.9993	0.9999	0.9998
Relative standard deviation [b] (%)	0.326	0.330	0.350	0.298	0.330	0.295	0.420	0.375
Range of error	±0.665	±0.553	±0.585	±0.662	±0.685	±0.625	±0.625	±0.550

[a] $Y = bx + a$, where x is the concentration of sulfonamide in μg/ml.
[b] Five replicates.



Scheme 1. Reaction sequence for the formation of orange yellow azo products.

3.4. Stability of the products

The orange yellow azo products were stable for more than 6 days in all the cases. The stability of the azo products resulting from the suggested method was studied in the temperature range of 20–50 °C. The products were found to be stable for more than 12 h at 50 °C and the results were reproducible. However, the absorbance readings were recorded in the temperature range of 20–30 °C.

3.5. Interference

The extent of interference by common ions were determined by measuring the absorbance of a solution containing 2.5 μg/ml of sulfonamide derivatives and various amounts of diverse species. Majority of the common ions do not interfere. An error of 2.0% in the absorbance readings was considered tolerable. Some of the common excipients, which often accompany the pharmaceutical preparations do not interfere in the present method. The results are given in Table 3 for DAP. The actual DAP to excipient ratio is nearly 10- to 20-fold with respect to the excipient in pharmaceutical preparations. But in Table 3, the conditions under which the experiments were carried out, viz., the ratio of DAP to excipient is nearly equal to 20 000 with respect to excipient. That means, the excipients were taken in very large excess for the experiment. Under the diazotization reaction conditions used, other amines such as morpholine, aniline, piperidine, etc. gave a positive reaction.

3.6. Quantification, accuracy and precision

The validity of the proposed procedure for the determination of the studied sulfa drugs in their pure state and in their pharmaceutical formulations was tested by analyzing these drugs using the proposed procedure and the official methods [7,8]. The results obtained for pure drugs (Table 2) were reproducible with low RSD (0.29–0.42%) and the mean recoveries were comparable to those obtained using the official

Table 3
Determination of DAP [a] in presence of excipients

Excipients	Amount (mg)	% Recovery of DAP ± SD [b]
Talc	50	99.8 ± 0.7
Gumacacia	50	99.5 ± 0.65
Starch	40	99.6 ± 0.7
Sodium chloride	50	100.1 ± 0.6
Dextrose	30	100.5 ± 0.7
Glucose	30	100.3 ± 0.7
Lactose	30	100.8 ± 0.65
Sodium alginate	40	99.0 ± 0.55
Carboxy methylcellulose	50	99.3 ± 0.60
Magnesium stearate	40	99.0 ± 0.70

[a] 2.5 μg/ml of DAP taken.
[b] Average of five determinations.

Table 4
Determination of sulfonamide derivatives in pharmaceutical preparations

Commercial formulations	Label claim (mg)	Amount of drug found [a] (mg)				
		Proposed method	B.P. method [7]	Literature method [11]	t-value	F-value
Tablets						
Septran [b] (SFMx)	400	399.0±0.65	398.5±0.70	399.3±0.65	1.78	1.69
Sulfadiazine [c] (SFD)	500	498.0±0.70	498.0±0.70	498.5±0.65	1.87	1.54
DAP [a]	25	24.9±0.60	24.85±0.60	24.9±0.55	0.58	1.36
	50	49.3±0.65	49.0±0.65	49.3±0.60	0.68	1.44
	100	99.0±0.70	99.0±0.70	99.3±0.65	0.63	1.34
Eye drops						
Albucid [d] (SFA)	10/ml	9.93±0.20	9.90±0.20	9.90±0.15	1.56	1.42
Locula [e] (SFA)	10/ml	9.90±0.15	9.85±0.15	9.93±0.15	1.48	1.79

[a] Average of five determinations±SD.
[b] Marketed by Burroughs Wellcome.
[c] Marketed by Rhone–Poulenc.
[d] Marketed by Nicholas Piramal India Ltd.
[e] Marketed by East India Ltd.

methods for each of the studied drugs. Limit of quantification (LOQ) is determined by taking the ratio of SD (σ) of the blank with respect to water and the slope of calibration curve (s) multiplied by a factor 10. This means that LOQ is $\approx$3.3 times limit of detection (LOD). Naturally, the LOQ slightly crosses the lower limit of Beer's law range. However, LOD is well below the lower limit of Beer's law range. The upper limit of Beer–Lambert range is determined by a plot of absorbance against concentration at the value of λ_{max}. Beyond this limit, the correlation results were strongly affected. Hence, the measurements were excluded above these limits to keep the relationship linear.

3.7. Application

The reproducibility of the method was checked by five replicate determination of 2 μg/ml level of sulfa drug and the RSD (%) was found to vary between 0.29 and 0.42. The present method has been applied for the analysis of certain sulfa drugs in pharmaceutical preparations. The results of the analysis of tablets and eye drops are given in Table 4 and compare favorably with those of the official method [7] and literature method [11]. The performance of the proposed method was compared statistically in terms of student's t-test and the variance ratio F-test. At 95% confidence level, the calculated t-values and F-values do not exceed the theoretical values for all the sulfa drugs studied. The theoretical t-value was 2.776 (for $n = 5$) and F-value was 6.39 (for $n = 5$). Therefore, there is no significant difference between the proposed method and the official method [7], indicating that the proposed method is as accurate and precise as the official method.

4. Conclusion

The proposed method is found to be simple, rapid and economical and will compete with most of the spectrophotometric methods available in literature. The method is advantageous over many spectrophotometric methods with special reference to stability and sensitivity. The method does not make use of any oxidant or organic dye or catalyst, thereby avoiding possible errors in the determination of sulfa drugs. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. The recommended procedure is well-suited for the assay and evaluation of drugs in pharmaceutical preparations to assure high standard of quality control.

Acknowledgements

One of the authors (C.R.R) thank the University Grants Commission, New Delhi, for a Teacher Fellowship under FIP and University of Mysore for the support of this research work.

References

[1] J. Uetrecht, Review of pharmacology and therapeutic use, Clin. Dermatol. 7 (1989) 111.

[2] B.C. Rudy, B.Z. Senkowski, Anal. Profiles Drug Subs. 2 (1973) 467.
[3] C.E. Orzech, Anal. Profiles Drug Subs. 5 (1976) 87.
[4] R.D.G. Woolfenden, Anal. Profiles Drug Subs. 6 (1977) 515.
[5] C. Papostephanou, M. Frantz, Anal. Profiles Drug Subs. 7 (1978) 401.
[6] H. Stober, W. Dewitte, Anal. Profiles Drug Subs. 11 (1982) 523.
[7] British Pharmacopoeia, HMSO, vol. I, London, 1998, pp. 1620, 1952.
[8] United States Pharmacopoeia XXIV, USP Convention Inc., Rockville, MD 20852 2000, pp. 496, 1560.
[9] M.E. Metwally, Primaquine phosphate as a promising substitute for *N*-(1-naphthyl)ethylenediamine: II. Analysis of sulfa drugs in pharmaceutical dosage forms and biological samples, Anal. Sci. 15 (1999) 979.
[10] P. Nagaraja, K.R. Sunitha, R.A. Vasantha, H.S. Yathirajan, A sensitive method for the spectrophotmetric determination of dapsone, Ind. Drugs 38 (2001) 489.
[11] P. Nagaraja, K.R. Sunitha, R.A. Vasantha, H.S. Yathirajan, Iminodibenzyl as a novel coupling agent for the spectrophotometric determination of sulfonamide derivatives, Eur. J. Pharm. Biopharm. 53 (2002) 187.
[12] P. Nagaraja, H.S. Yathirajan, K.R. Sunitha, R.A. Vasantha, A new sensitive and rapid spectrophotometric method for the determination of sulfa drugs, J. Assoc. Off. Anal. Chem. 85 (2002) 869.
[13] N.D. Dinesh, P. Nagaraja, K.S. Rangappa, A facile and highly sensitive spectrophotometric determination of sulfonamides in pure and dosage forms, Proc. Nat. Acad. Sci. India 72A (2002) 231.
[14] P. Nagaraja, H.S. Yathirajan, K.R. Sunitha, R.A. Vasantha, Novel methods for the rapid spectrophotometric determination of dapsone, Anal. Lett. 35 (2002) 1531.
[15] P. Nagaraja, K.R. Sunitha, R.A. Vasantha, H.S. Yathirajan, Rapid spectrophotometric determination of sulphonamide derivatives with resorcinol, Ind. J. Pharm. Sci. 64 (2002) 391.
[16] P. Nagaraja, K.R. Sunitha, H.S. Yathirajan, R.A. Vasantha, Spectrophotometric determination of 4,4′-sulphonyldianiline, Ind. J. Pharm. Sci. 65 (2003) 82.
[17] C.S.P. Sastry, K.E. Rao, U.V. Prasad, Spectrophotometric determination of some phenols with sodium metaperiodate and aminophenols, Talanta 29 (1982) 917.
[18] P. Nagaraja, H.S. Yathirajan, H.R. Arunkumar, R.A. Vasantha, Novel coupling reagents for the sensitive spectrophotometric determination of nimesulide in pharmaceutical preparations, J. Pharm. Biomed. Anal. 29 (2002) 277.
[19] P. Nagaraja, R.A. Vasantha, H.S. Yathirajan, Spectrophotometric determination of folic acid in pharmaceutical preparations by coupling reactions with iminodibenzyl or 3-aminophenol or sodium molybdate-pyrocatechol, Anal. Biochem. 307 (2002) 396.
[20] P. Nagaraja, H.R. Arunkumar, R.A. Vasantha, H.S. Yathirajan, Novel reagents for the sensitive spectrophotometric determination of flutamide, an anticancer drug in pharmaceutical preparations, Int. J. Pharm. 235 (2002) 113.
[21] P. Nagaraja, R.A. Vasantha, H.S. Yathirajan, Spectrophotometric methods for the rapid determination of menadione and menadione sodium bisulfite and their application in pharmaceutical preparations, J. Pharm. Biomed. Anal. 28 (2002) 161.
[22] P. Nagaraja, K.R. Sunitha, R.A. Vasantha, H.S. Yathirajan, Spectrophotometric determination of metronidazole and tinidazole in pharmaceutical preparations, J. Pharm. Biomed. Anal. 28 (2002) 527.
[23] W.C. Vosburgh, G.R. Cooper, Complex ions I. The identification of complex ions in solution by spectrophotometric method, J. Am. Chem. Soc. 63 (1941) 437.
[24] J.H. Yoe, A.L. Jones, Colorimetric determination of iron with disodium 1,2-dihydroxybenzene-3,5-disulfonate, Ind. Eng. Chem. Anal. Ed. 16 (1944) 111.