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Research paper

Iminodibenzyl as a novel coupling agent for the spectrophotometric determination of sulfonamide derivatives

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Abstract

A rapid, selective and simple spectrophotometric method for the determination of sulfa-drugs is described. The method is based on the formation of violet colored azo product by the diazotization of sulfonamides, viz. sulfathiazole (SFT), sulfadiazine (SFD), sulfacetamide (SFA), sulfamethoxazole (SFMx), sulfamerazine (SFMr), sulfaguanidine (SFG) and sulfadimidine (SFDd) followed by a coupling reaction with iminodibenzyl in alcohol medium. Absorbance of the resulting violet azo product is measured at 570–580 nm and is stable for 24 h at 27°C. Beer's law is obeyed in the concentration range of 0.05–6.0 μg ml^{-1} 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. The method offers the advantages of simplicity, rapidity and sensitivity without the need for extraction or heating. A reaction mechanism is proposed for the formation of the violet azo product.

Keywords: Sulfa-drugs; Iminodibenzyl; Diazotization; Spectrophotometry; Pharmaceuticals

1. Introduction

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. A survey of literature reveals that there are various methods available for the determination of sulfonamide derivatives, which suffer in one way or the other. The official method of British Pharmacopoeia [1] and United States Pharmacopoeia [2] describes nitrite titration method for the analysis of sulfa-drugs. The other methods include gas chromatography [3], high-performance liquid chromatography (HPLC) [4,5], HPTLC [6] electroanalytical methods [7–10], potentiometry [11], immunochemical assay [12], volumetric method [13,14], and spectrofluorimetry [15]. The most important spectrophotometric methods, which have already been reported [16–29], for the determination of sulfa-drugs suffer from disadvantages like lack of sensitivity, involves heating or extraction, requires long time for the completion of reaction, narrow detection limit etc. A comparison of some spectrophotometric methods for the analysis of sulfonamide derivatives are presented in Table 1.

The purpose of this work is to introduce iminodibenzyl as a new coupling agent for the first time, which is economically cheaper and sensitive than the most widely used coupling agents, viz. *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NEDA) and 3-methyl-benzothiazoline hydrazone hydrochloride (MBTH), which are used for the determination of various organic compounds, i.e. phenols, polyhydroxy compounds, aromatic and aliphatic amines, alicyclic amines, bilirubin etc.

In the present investigations, the diazotized sulfa-drug is coupled with iminodibenzyl in alcohol to give a violet azo product which is stable for 24 h.

2. Materials and methods

2.1. Instrument

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

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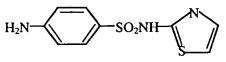
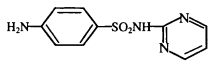
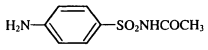
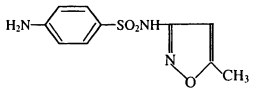
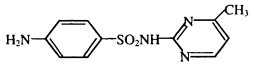
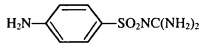
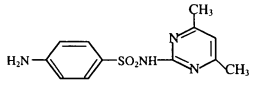
Table 1
Comparison of some spectrophotometric methods for the analysis of sulfonamide derivatives[a]

S. No.	Reagent	Coloured product	λ_{max} (nm)	Derivative studied	Beer's law range ($\mu g\ ml^{-1}$)	Remarks	Reference
1.	8-Hydroxyquinoline-5-sulfonic acid	Azo dye	530	SFT	50–250	Low sensitivity	[16]
2.	Chloramine-T	Yellow chromogen	525	SFAn, SFA, SFD, SFSo	Up to 8000 for SFAn and SFA, 6000 for other two	Requires very long time (4 h at room temperature or 1 h at 50°C for colour development and method is applicable only for higher concentration	[17]
3.	Hypochlorite and phenol	Green-yellow chromogen	450	SFG	Not reported	Involves heating at 45°C for 10 min. Colour is highly unstable	[18]
4.	Bindone	Charge transfer complex	480	SFMm and SFDm	12–48	Involves non-aqueous (1,4-dioxan) medium and heating at 100°C for 15 min	[19]
5.	Ammonium vanadate	Blue coloured complex	800	SFG and SFTu	400–1100 and 76–760	Less sensitive and involves heating at 100°C for 5 min	[20]
6.	*o*-Chloranil	Charge transfer complex	525	SFA	10–70	Require 20 min for completion of reaction and product is stable for only 1 h	[21]
7.	Metol-periodate	Purple chromogen	520	SFDo and SFL	4000–44 000	Very low sensitivity and require 20 min for completion of reaction	[22]
8.	*N*-[3-chloro-4-oxo-1(4H)-napthylidine] benzene sulfonamide	Ion-pair complex	502	SFT	Not reported	Involves non-aqueous medium (DMSO) and heating for 5 min at 100°C	[23]
9.	*p*-Benzoquinone	Charge transfer complex	500	SFA	5–70	Involves heating for 10 min at 90°C	[24]
10.	4-Dimethylamine–cinnamaldehyde	Red coloured chromogen	545	SFMx and SFD	0.4–4.8	Requires 15 min for completion of reaction and has narrow detection limit	[26,27]
11.	*p*-Benzoquinone	Charge transfer complex	500	SFMx and SFD	Up to 55	Involves heating for 1 h at 65°C	[28]
12.	Acetylacetone–formaldehyde	Charge transfer complex	400	SFA, SFDmd, SFD and SFT	4–80	Preliminary heating on water bath for 5 min and later at 40°C for 25 min	[29]
13.	Iminodibenzyl	Coupling product	570–580	SFD, SFA, SFT, SFMx, SFG, SFMr and SFDd	0.05–6	Highly sensitive, involves no heating or extraction and reaction is rapid	Present method

[a] SFAn = Sulfanilamide, Sulfis = Sulfisoxazole, SFMm = Sulfamonomethoxine, SFDm = Sulfadimethoxine, SFTu = Sulfathiourea, SFDo = Sulfadoxine, SFL = sulfolene.

Table 2
Sulfa-drugs studied

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

2.2. Reagents

Sulfonamide derivatives were all purchased from Sigma (USA) and were used without further purification. Iminodibenzyl (Sigma, USA), sodium nitrite (BDH), sulfuric acid (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^{-1}) were prepared by dissolving 100 mg of each sulfonamide in 5.0 ml of 10 mol dm^{-3} H_2SO_4 and then diluting to the mark in a 100 ml standard flask. A working standard solution of each sulfonamide containing 25 μg ml^{-1} was prepared by further dilution and standardized by the USP method [2]. A 1% of solution of $NaNO_2$ in water, 0.5% alcoholic solution of iminodibenzyl and aqueous sulfamic acid were used for the experiment.

2.4. Recommended procedure

Sulfonamide derivative solutions (1.25–150 μg) were transferred into each of the series of 25 ml standard flasks and 5 ml of 10 M H_2SO_4 were added to each. 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, 3 ml of 0.5% iminodibenzyl in alcohol was added. The solution was made up to the mark with alcohol, mixed thoroughly and after 10 min, the absorbance was measured at 570–580 nm against the corresponding reagent blank and calibration graphs were constructed.

2.5. Procedure for 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 10 M H_2SO_4 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 eye drops, an accurately measured volume was appropriately diluted with 5 ml of 10 M H_2SO_4 and 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 iminodibenzyl in alcohol to produce a violet azo product. Sulfa-drugs studied in the present investigation are given in Table 2.

3.1. Spectral characteristics

Absorption spectrum of the violet azo product (for SFMx as a model compound) with maximum absorption at 580 nm is shown in Fig. 1. The colourless reagent blank has practically negligible absorption at this wavelength. The optical characteristics and precision data for all the seven sulfa-drugs are given in Table 3.

3.2. Optimum reagents concentration

It was found that 10 M solution of H_2SO_4 in the range of

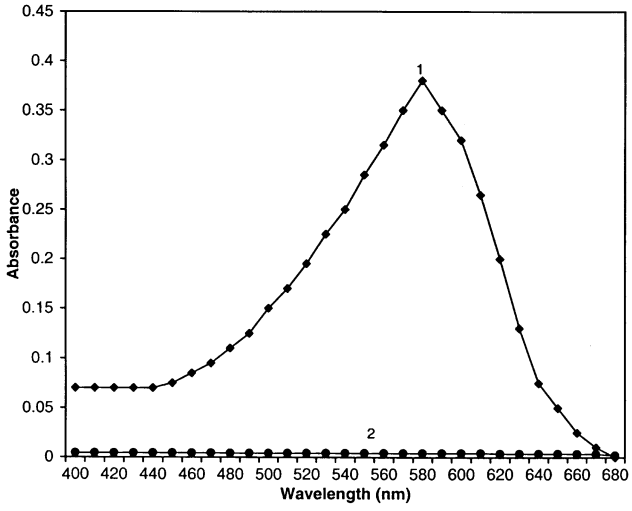


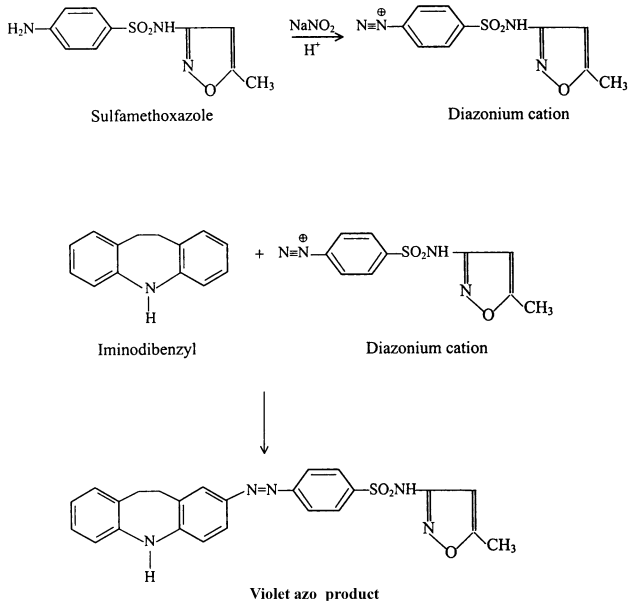
Fig. 1. Absorption spectra of the reaction product (1) of sulfamethoxazole with iminodibenzyl. Final drug concentration = 2 μg ml^{-1}. (2) Reagent blank.

Table 3
Optical characteristics and precision data

Parameters/characteristics	SFT	SFD	SFA	SFMx	SFMr	SFG	SFDd
Colour	Violet	Violet	Violet	Violet	Violet	Violet	Violet
λ_{max} (nm)	580	580	570	580	580	570	570
Stability (in days)	01	01	01	01	01	01	01
Beer's law range ($\mu g\ ml^{-1}$)	0.1–4.0	0.1–6.0	0.1–5.0	0.05–4.0	0.1–4.0	0.1–5.0	0.1–4.5
Least of detection ($\mu g\ ml^{-1}$)	0.0357	0.0872	0.0454	0.0335	0.0330	0.0596	0.0358
Least of quantification ($\mu g\ ml^{-1}$)	0.1189	0.2906	0.1513	0.1115	0.1103	0.1988	0.1195
Molar absorptivity ($1\ mol^{-1}\ cm^{-1}$)	4.23×10^4	2.14×10^4	4.33×10^4	4.79×10^4	3.84×10^4	2.58×10^4	4.36×10^4
Sandell's sensitivity ($\mu g\ cm^{-2}$)	0.0060	0.0117	0.0068	0.0053	0.0069	0.0083	0.0064
Optimum photometric range ($\mu g\ ml^{-1}$)	0.5–3.5	0.5–7.0	0.5–4.5	0.2–3.4	0.5–3.5	0.5–4.5	0.5–4.0
Regression equation (Y)[a]							
Slope (b)	0.1830	0.0749	0.1439	0.1952	0.1975	0.1095	0.1822
Intercept (a)	−0.0122	0.0016	−0.0076	−0.0075	−0.0442	0.0189	−0.0273
Correlation coefficient (r)	0.9995	0.9972	0.9996	0.9998	0.9982	0.9966	0.9981
Relative standard deviation (%)[b]	0.3991	0.4561	0.3607	0.2315	0.2814	0.3912	0.3056
Range of error	±0.554	±0.6331	±0.5006	±0.3213	±0.3906	±0.5429	±0.4242

[a] $Y = bx + a$, where x is the concentration in $\mu g\ ml^{-1}$.
[b] Ten replicates.

4–6 ml, 1% solution of sodium nitrite in the range 0.5–2 ml, 2% solution of sulfamic acid in the range 1–4 ml and 2–4 ml of 0.5% solution of iminodibenzyl in alcohol were necessary to achieve maximum colour intensity. Hence, 5 ml of H_2SO_4, 1 ml of sodium nitrite, 2 ml of sulfamic acid were used for diazotization and 3 ml of iminodibenzyl 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 colour intensity. The use of sulfuric acid as the reaction medium was found to give better results than hydrochloric acid in terms of colour development and stability of the product.



Scheme 1. Reaction mechanism for the formation of violet azo 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 iminodibenzyl by electrophilic substitution at the 4-position of the coupling agent to produce a violet azo dye. Investigation of the continuous molar variation of the SFMx and IDB showed that the diazotized SFMx interacts with IDB in the ratio of 1:1. Similar results have been observed with the mole ratio method. A reaction mechanism based on the above results is shown in Scheme 1.

3.4. Stability of the product

The violet azo product formed was stable for more than 1 day in all the cases. The stability of the azo product resulting from the suggested method was studied in the temperature

Table 4
Determination of sulfamethoxazole[a] in presence of excipients

Excipients	Amount (mg)	% Recovery of sulfamethoxazole ± % RSD[b]
Dextrose	50	99.8 ± 0.40
Glucose	40	100.4 ± 0.35
Lactose	35	99.0 ± 0.30
Talc	40	99.5 ± 0.32
Starch	50	100.2 ± 0.26
Stearic acid	40	100.2 ± 0.38
Sodium alginate	35	101.4 ± 0.30
Carboxy methylcellulose	35	99.5 ± 0.20
Magnesium stearate	30	100.2 ± 0.38
Vitamin-B_6	30	100.2 ± 0.40
Gumacacia	40	99.0 ± 0.40

[a] 4 $\mu g\ ml^{-1}$ of sulfamethoxazole taken.
[b] Average of five determinations.

Table 5
Determination of sulfonamide derivatives in pharmaceutical preparations

Commercial formulations analysed	Label claim	Amount of drug found[a] (mg)		
		BP method [1]	Reported method [29]	Proposed method
Tablets				
Septran[b] (SFMx)	400 mg	398.3 ± 0.65	399.0 ± 0.70	399.3 ± 0.50
Sulfadiazine[c] (SFD)	500 mg	498.4 ± 0.50	498.0 ± 0.40	499.0 ± 0.60
Eye drops				
Albucid[d] (SFA)	10 mg ml^{-1}	9.75 ± 0.15	9.80 ± 0.20	9.90 ± 0.10
Locula[e] (SFA)	10 mg ml^{-1}	9.85 ± 0.08	9.80 ± 0.10	9.93 ± 0.10

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

range of 20–50°C. The product was found to be stable for more than 12 h at 50°C and the results were reproducible. However, temperature range of 20–30°C is preferred for the coupling reaction.

3.5. Interference

The extent of interference by common ions were determined by measuring the absorption of a solution containing 2 μg ml^{-1} 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 4.

3.6. Application

The reproducibility of the method was checked by 10 replicate determinations at 2 μg ml^{-1} level of sulfa-drug and the relative standard deviation (%) was found to vary between 0.2 and 0.4. 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 5 and compare favorably with those of the official method [1] and reported method [29].

4. Conclusion

The proposed method is found to be simple, rapid, selective and highly sensitive than most of the spectrophotometric methods available in literature. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. Thus the method can be adopted as an alternative to the existing spectrophotometric methods. The recommended procedure is well-suited for the assay and evaluation of drugs in pharmaceutical preparations to assure high standard of quality control.

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