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Spectrophotometric Determination of Sulphamethazine by the Diazotisation-Coupling Method with m-Aminophenol as the Coupling Agent-Application to Pharamaceutical

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ABSTRACT

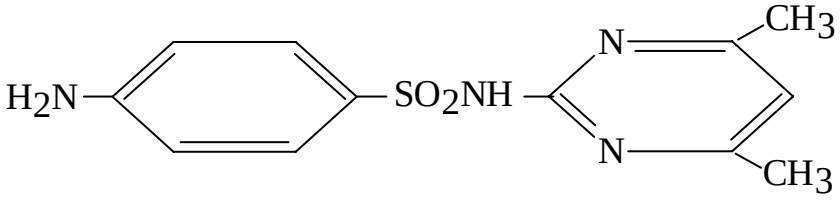
A spectrophotometric method for the trace determination of sulphamethazine has been worked out. It is based on the reaction of sulphamethazine with sodium nitrite in acidic medium to form the corresponding diazonium salt. After removal of excess nitrite with sulphamic acid, the diazotised sulphamethazine is coupled with m-aminophenol fo form an intensely-yellow coloured, water-soluble and stable azo dye which shows maximum absorption at 457 nm. The plot of absorbance versus determinand concentration shows that Beer's law is obeyed over the concentration range 5-150 µg sulphamethazine/25 ml final volume, i.e., 0.2-6 ppm with a molar absorptivity of $4.8X10^4$ $l.mol^{-1}.cm^{-1}$. The method has been applied to the assay of the drug in a tablet.

الملخص

تم في هذا البحث تطوير طريقة طيفية لتقدير كميات اثرية من السلفاميتازين . وتعتمد الطريقة على تفاعل السلفاميتازين مع نتريت الصوديوم في محيط حامضي لتكوين ملح الدايزونيوم المقابل . وبعد ازالة الزيادة من النتريت بواسطة حامض السلفاميك ، تم اقتران ملح الدايزونيوم مع ميتا – امينوفينول لتكوين صبغة ازوية ذات لون اصفر غامق مستقرة وذائبة في الماء لها اعلى امتصاص عند 457 نانومتر. وعند رسم الامتصاص مقابل تركيز المادة المعنية ، تبين ان قانون بير كان معمولا به ضمن مدى التركيز 5-150 مايكروغرام سلفاميثازين/25 مل حجم نهائي، أي: 0.2-6 جزء/مليون بمعامل امتصاصية مولارية $4.8X10^4$ لتر.مول $^{-1}$.سم$^{-1}$، تم تطبيق الطريقة بتقدير الدواء في قرص دوائي.

INTRODUCTION

Sulphamethazine (also called sulphadimidine) is a sulpha drug, the name of which is given to [N-(4,6-dimethyl-2-pyrimidinyl)-sulphanilamide] and has the following structure (Aldrich, 1990-1991):



Sulpha drugs are active against bacteria and are used for the treatment of infections. From the analytical point of view, this has resulted in a wide variety of methods suggested for the determination of sulpha drugs. These methods can vary from the classical gravimetric ones to the modern instrumental methods, including chromatography
(Al-Talib, 1995).

To our knowledge, the recent method for the assay of sulphamethazine, for example, is based on the oxidative coupling reaction of the intended compound with phenothiazine, 4-amino-N,N-diethylaniline or with 4-amino,-N,N-dimethylaniline in the presence of oxidising agents such as ceric ion, benzoyl peroxide or dichromate. The methods are not enough satisfactory although they have been applied to various samples (Al-Talib, 1995).

The method described in this paper for the spectrophotometric determination of sulphamethazine involves the formation of an intensely-coloured azo dye after the diazo-coupling reactions. The applicability of the method has been evolved by determining the drug in a tablet.

EXPERIMENTAL

Apparatus: All spectrophometric measurements have been carried out using Shimadzu UV-VIS 160 recording spectrophometer with 1-cm matched silica cells.

pH readings are made on Philips PW 9420 pH meter supplied with aglass-combined electrode.

Reagents: All chemicals used are of the highest purity available.

Working sulphamethazine solution, 100 Mg/ml. A 0.0100 g amount of sulphamethazine (Sigma) is dissolved in 6 ml of ethanol and then the volume is diluted to 100 ml with distilled water in a volumetric flask. This solution is kept in a brown bottle.

Acetic acid solution, 1N. This solution is prepared by appropriate dilution of the concentrated (BDH) acid with distilled water.

Sodium nitrite solution, 1%. This solution is prepared by dissolving 1g of sodium nitrite in 100 ml distilled water. This solution is stable for at least one week when kept in a brown bottle.

Sulphamic acid solution, 3%. This solution is prepared by dissolving 3g of the compound in 100 ml distilled water. This solution is stable for at least one week when kept in a brown bottle.

m-Aminophenol solution, 0.5%. This solution is prepared by dissolving 0.5g of m-aminophenol (Hopkin and Williams) in 100 ml of distilled water in a volumetric flask. This solution is kept in a brown bottle.

Hydrochloric acid solution, 1N. The solution is prepared by the appropriate dilution of the standardised concentrated acid solution.

Sulphadimidine tablet solution. A 0.0100g amount of the 2.5g powdered-made tablet is dissolved in 6 ml ethanol and the volume is made to 100 ml, with distilled water, in a volumetric flask.

Procedure and calibration curve

To a series of 25-ml volumetric flasks, aliquots of sulpha-methazine solution to contain 5-150 μg of the compound are transferred. 5 ml of distilled water, 1 ml of 1N acetic acid and 0.3 ml of 1% sodium nitrite are then added. After the mixtures are shaken for 2 minutes, 0.5 ml of 3% sulphamic acid is added. After shaking for 5 minutes, 3 ml of 0.5% m-aminophenol and 3 ml of 0.5 N hydrochloric acid and dilution to the mark with distilled water are then made. The absorbances of the resulting solutions are measured against the corresponding blank at 457 nm using 1-cm cells. The apparent molar absorptivity at the wavelength of measurement is $4.8X10^4$ $l.mol^{-1}.cm^{-1}$.

RESULTS AND DISCUSSION

For the subsequent experiments, 50 μg of sulphamethazine is taken and the final volumes are 25 ml.

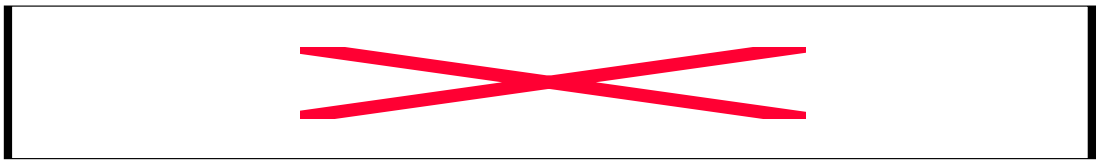
Study of the optimum reaction conditions

The effect of various parameters affecting and related to the colour intensity of the formed dye is studied and optimum reaction conditions have been selected.

Principle of the colour reaction

The colour reaction involved in this study include the followings :

1. Sulphamethazine reacts, in acidic medium, with excess nitrite to form the corresponding diazonium salt:



2. After removal of excess nitrite with sulphamic acid (nitrite is converted into nitrous acid in acidic medium):

$$HNO_2 + H_2N - SO_3H \rightarrow \uparrow N_2 + H_2SO_4 + H_2O$$

3. The diazotised sulphamethazine is coupled with the amine to give the intensely-coloured azo dye:

from the above, it can be observed that the intensity of the finally-formed coloured dye is a parameter of various factors that have to be studied and optimised.

Effect of diazotisation acid

The effect of weak and strong acids on the diazotization process of sulphamethazine, in the presence of excess nitrite, has been investigated. The results show that full colour development is achieved in the presence of 1.0-7.0 ml of each of 1N HCl, HNO_3, CH_3COOH or H_2SO_4. Diazotisation still occurs, but to smaller extent, when no acid is present. This has been attributed to the presence of sulphamic acid (added in the removal of excess nitrite) (Norwitz and Keliher, 1981). A 1 ml of 1N CH_3COOH is selected for the diazotisation process of sulphamethazine. Acetic acid can be obtained in a more pure state than HCl or H_2SO_4 and is more available.

Effect of nitrite amount and time

The absorbance of the coloured dye increases when 0.1-0.5 ml of 1% $NaNO_2$ are used. The time of reaction of nitrite with the sulpha drug decreases as the amount of nitrite added is increased. A 0.3 ml of 1% $NaNO_2$ solution is used to diazotise sulphamethazine for 2-minutes interval time.

Effect of sulphamic acid and time

Excess unreacted nitrite remaining in the previous step should be removed because of high blank values. The experimental data reveal that 0.5 ml of 3% sulphamic acid solution when allowed to react for 5-minutes period is optimum and the blank value is only 0.003.

Effect of m-aminophenol amount

Keeping the above optimum conditions constant, the effect of m-aminophenol amount on the intensity of the coloured dye has next been investigated. The results are given in Table 1.

Table 1: Effect of m-aminophenol amount on absorbance.

Ml of 0.5% m-aminophenol solution	Absorbance/μg of sulphamethazine						Correlation coefficient, r
	4	10	30	50	70	100	
1.0	0.006	0.059	0.099	0.169	0.234	0.333	0.9976
3.0	0.019	0.041	0.100	0.169	0.225	0.333	0.9987
5.0	0.017	0.035	0.108	0.183	0.278	0.383	0.9976
7.0	0.065	0.071	0.147	0.190	0.257	0.343	0.9968
10.0	0.022	0.046	0.179	0.179	0.239	0.353	0.0084

The above data indicate that the absorbance of the resulting azo dye is independent of the amount of m-aminophenol in the range (1.0-10.0) ml of 0.5% solution. A 3 ml of 0.5% m-aminophenol solution is selected for the recommended procedure.

Effect of acid and base amount

The present coupling reaction can take place in acid medium and basic medium, since the coupling agent has the –OH and $–NH_2$ in its nucleus at the coupling agent has the –OH and $–NH_2$ in its nucleus at the same time. The orientation effect of the hydroxyl group will be operative in basic medium and of the amino group in neutral and acidic media (Norwitz and Keliher, 1981).

The effect of different amounts of different acids and bases on the colour intensity of the dye has been tested for optimal results which are shown in Table 2.

Table 2: Effect of acid and base on absorbance.

0.5 N electrolyte solution added	Absorbance/ml of electrolyte added			$\lambda_{Max/nm}$
	1	3	5	
H_2SO_4	0.325	0.318	0.303	452-456
HCl	0.342	0.327	0.316	453-458
CH_3COOH	0.323	0.302	0.296	442-443
CH_3COONa	0.297	0.298	0.296	439-440
Na_2CO_3	0.294	0.302	0.292	439-441
NaOH	0.283	0.177	0.180	440-480

From the above results, it can be observed that performing coupling reaction in acid medium is more favourable than in basic medium because of higher sensitivity and probably higher selectivity. All results before Table 2 are obtained by introducing 2.5 ml of 0.5 N NaOH solution for the coupling reaction. For the subsequent experiments, 3 ml of 0.5 N HCl solution is selected.

Effect of surfactants

Sometimes, the introduction of surfactants in the mixture solution of the diazotisation coupling reaction leads to bathochromic and hyperchronic effects (Ramos et al., 1989). The effect of different amounts of different types of surfactants (cationic, anionic and neutral) has been investigated. Sodium dodecyl sulphate, cetylpyridinium chloride and triton x-100 are selected and examined for their effects. The experimental results show that none of the surfactants could have the expected effects. Therefore, they are excluded in the subsequent investigations.

Effect of time

A study of the effect of time on the absorbance of the coloured dye, produced from various amounts of sulphamethazine, has shown that the colour develops practically completely immediately and remains maximum and constant for at least 50 minutes. The results are given in Table 3.

Table 3: Effect of time and concentration on absorbance.

Time, min	Absorbance/μg sulphamethazine present			
	5	50	100	150
0	0.051	0.349	0.667	0.970
10	0.054	0.349	0.665	0.971
30	0.054	0.346	0.658	0.972
50	0.054	0.344	0.652	0.971
60	---	0.343	---	0.970

Absorption spectra

When a dilute aqueous solution of sulphamethazine is treated according to the optimum experimental conditions, a yellow coloured dye forms immediately. This yellow-coloured dye shows maximum abosrption at 457 nm, in contrast to the colourless reagent blank which shows slight absorption at the wavelength of maximum absorption.

Figure (1) shows the absorption spectra of the coloured dye and the corresponding reagent blank. The wavelength of maximum absorption at 457 nm has been adapted for the subsequent work.

Fig. 1: Absorption spectra of 50μg of sulphamethazine / 25 ml treated according to the recommended procedure and measured against (A) blank, (B) measured against distilled water.

Nature of the dye

The composition of the coloured azo dye has been established using Job’s method of continuos variations. The experimental data indicate that the azo dye has been formed by a 1:1 combining ratio of diazotised sulphamethazine to m-aminophenol, revealing a mono azo dye. Diazotised sulphamethazine couples with m-aminophenol at the 4-position and the structure of the azo dye can be written as follows :

The apparent stability constant has been calculated and is found to be 5.2×10^4, in comparison with similar azo dye (Younis and Bashir, 1995).

Application of the method

Sulphadimidine is used for the treatment of infection diseases of calf, sheep and goat. The only drug that has been available is a sulphadimidine tablet. The determination of sulphadimidine content of the tablet by the present proposed method and literature method (Norwitz and Keliher, 1982) is given in Table 4.

Table 4: Determination of sulphadimidine content in tablet.

Drug	Sulphadimidine content, %	
	Present method	Literature method
Sulphadimldine tablet*	70	74.5

* Weight of the tablet is 2.5 g.

Comparison of method

The present method is compared with the literature method (Norwitz and Keliher, 1982) which is also based on the diazotisation-coupling reaction but using N-NED as the coupling agent. The comparison is given in Table 5.

Table 5: Comparison of m-aminophenol and N-NED methods for determination of sulphamethazine.

Property	m-Aminophenol	N-NED
Purity of commercial reagent	Better purity (Aldrich, 1990-1991)	Good purity (ibid)
Expense of reagent	Less cost-effective	10-times more expensive
Stability of reagent	Stable when kept in a brown bottle	3 days
Colour of dye time for colour	Yellowish-orange	Violet
Development	Immediately	75 min
Sensitivity	4.8×10^4 l.mol^{-1}.cm^{-1}	5×10^4 l.mol^{-1}.cm^{-1}.

From our point of view, the present method is more favourable for the routine determination of the drug.

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