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Short communication

Simultaneous spectrophotometric determination of sulfadoxine and pyrimethamine in pharmaceutical formulations

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Abstract

This study was carried out to simultaneously determine quantitatively sulfadoxine and pyrimethamine in four brands of anti-malarial formulations. The reaction principle was based on the complexation reaction between the drugs (π-donors) and chloranilic acid (π-acceptor) giving rise to colour formation. The complexes of sulfadoxine and pyrimethamine absorbed maximally at 500 and 520 nm, respectively. The limits of detection of these complexes were 0.005 mg/ml for pyrimethamine and 0.010 mg/ml for sulfadoxine. Calibration graphs were linear at 0.015 mg/ml for pyrimethamine and 0.020 mg/ml for sulfadoxine. Quantitative recovery experiments gave percentage range between 94.79 ± RSD 3.85% and 98.04 ± 2.21% for sulfadoxine and 93.75 ± RSD 0.89% to 103 ± 1.04% for pyrimethamine. Analysis by the Official method similarly gave percentages range of between 97.9 ± 2.3% and 100.1 ± 3.1% for sulfadoxine; 97.8 ± 1.9% and 99.6 ± 2.5% for pyrimethamine. Comparison of the two methods by Students *t*-test did not reflect any statistical difference ($P > 0.05$). These figures show that these brands of anti-malarial meet the Pharmacopoeia standard of 95–105%. We found this technique suitable for quality assurance of these drugs. The sensitivity, accuracy, simplicity of this technique also commends it for field studies.

Keywords: Pyrimethamine; Sulfadoxine; Donor/acceptor complexation; Spectrophotometric determination

1. Introduction

The phenomenon of donor–acceptor interactions between organic molecules has been applied successfully to the analysis of pharmaceutical products [1–3]. These interactions usually give rise to intensely coloured complexes that absorb in the visible region of the spectrum [4,5]. Physical methods of vibrational spectra and nuclear magnetic resonance spectroscopy have been employed in the study of the nature of these complexes [5]. Drug molecules with functional groups like amines, hydroxyl, methoxy and thio that possess excess electrons (donors) can engage in charge-transfer interactions with acceptor molecules like iodine, chloranil, tetracyanoethylene [6,7]. Charge-transfer interactions as applied to phar-

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maceutical analysis although have proved very useful, are not without some limitations. For instance, the possible formation of isomeric and termolecular complexes, interference of one specie with another, specific solvation of donor–acceptor molecules all could be factors that affect empirically determined optical absorption due any of the complexes formed [5]. This situation has been observed most often in complexes involving π-donors and π-acceptors; where only a small fraction of either component may actually be involved with complexation. The major drawbacks of charge-transfer technique are that no direct evidence is available to show the nature of interaction other than the colour change. The Benesi–Hildebrand condition of a large excess of donor over the acceptor concentrations places a constraint on the interpretation of data. The stability of the complex determines the suitability for analytical purposes since as noted earlier only a small fraction of the reactants may be in equilibrium condition. Despite these constraints the study of charge-transfer interactions are of scientific value because they may help to explain intramolecular interaction across space that may be involved in the maintenance of structure of some biological macromolecules, or they may explain drug–drug and drug–receptor interactions.

Pharmaceutical analysis of sulfadoxine or N^1-(5,6-dimethoxypyrimidin-4-yl)-sulfanilamide and pyrimethamine or 2,4-diamino-5(5*p*-chlorophenyl)-6-ethylpyimidine has been very well reported [8–16]. The classical methods of analysis have been adapted in the chromatographic (HPLC) detection and quantitation of these drugs in plasma, whole blood and urine [17–21]. Thin layer chromatography has similarly been reported for the quantitative determination of sulfadoxine and pyrimethamine [22].

Sulfadoxine and pyrimethamine will continue to be of interest to analyst because of their prominent position in the chemotherapy of malaria disease. The recent widespread appearance and massive circulation of counterfeit or fake sulfadoxine and pyrimethamine in developing countries in particular pose a serious threat to malaria chemotherapy in these regions. In developing countries the dearth of research funds and the high-technology analytical instruments or even the availability of most reagents recommended in official compendia has forced Scientist to develop alternative and sensitive methods of analysis of these and other drugs. It is in this regard that we attempt to develop a simple, rapid, inexpensive and sensitive colorimetric method of authentication of these tablets.

The test is based on the reaction between chloranilic acid, a typical π-acceptor molecule and sulfadoxine and pyrimethamine both of which are donors. This author has not cited any report in the literature concerning the quantitative determination of sulfadoxine and pyrimethamine using π-acceptors. Preliminary studies in our laboratory [23] on the thermodynamics of complexation of these drugs with chloranilic acid has revealed high and negative equilibrium constants and high free energy changes, suggesting highly stable complexes.

The objective of this study therefore is to develop a method based on the observed stability of the complexes to the quantitative determination of these drugs. The result of this study will assist in determining whether this technique can be used in the quality assurance and stability-indicating assay of the drugs.

2. Experimental

2.1. Materials and methods

Four brands of malarial drugs containing sulfadoxine and pyrimethamine in combination were purchased from the Pharmacy Department of Jos University Teaching Hospital, Jos, Nigeria. Chloranilic acid was purchased from Riedel de Haem (Germany) and was recrystallized from acetone (BDH Chemicals, England). 1,4-Dioxan (BDH Chemicals) was used without further purification. Pure samples of sulfadoxine and pyrimethamine were kindly donated by Europharm Laboratories (PLC) Jos, Nigeria. All other reagents used were of analytical grade and were freshly prepared each time.

2.2. Determination of absorption maxima for chloranilic acid and its complexes with sulfadoxine and pyrimethamine

Chloranilic acid solution (4.5×10^{-3} M) was prepared in 1,4-dioxan and its absorption maximum determined using spectrophotometer (Pye-Unicam SP 800, England). Solutions of sulfadoxine (1.613×10^{-2} M) and pyrimethamine (1.004×10^{-2} M) were also prepared in 1,4-dioxan. One milliliter each of sulfadoxine and pyrimethamine solutions were transferred into 10-ml volumetric flasks and 1.0 ml of chloranilic acid added. The volumes were brought up to mark with more 1,4-dioxan solvent. The solutions were mixed thoroughly and allowed to stand for 30 min for the colour to be fully developed. The solutions were then scanned on a double-beam UV/visible spectrophotometer (Pye-Unicam Sp 800).

2.3. Determination of extinction coefficient

Standard solutions of sulfadoxine and pyrimethamine equivalent to 2.5 mg/ml were prepared in 1,4-dioxan. Serial volumes of 1.0, 2.0 to 5.0 ml were transferred to 10-ml volumetric flasks and 1.0 ml of chloranilic acid added to each flask. The solutions were brought up to mark with more 1,4-dioxan. Triplicate preparations were made, the contents were thoroughly mixed and their absorbances measured after 30 min. The absorbances were further monitored for another 9 h at regular intervals of 1 h. The means of the absorbances were calculated and plotted against the concentrations. The curves were regressed by the method of least squares [15]. The slopes gives the measure of the extinction coefficient.

2.4. Optimization of the analytical technique

The optimization of the technique was established first by determining the limit of detection or sensitivity of the spectrophotometer by serially diluting the stock solutions of the complexes until a minimum detection was recorded. The minimum concentrations of the drugs that gave reproducible deflections with chloranilic acid as the complexing agents were similarly determined. The concentrations of the analytes were further varied randomly and their absorbances measured. Various solutions were prepared and analyzed for accuracy, precision and selectivity of the technique using standard protocols.

2.5. Construction of calibration curves and their validations

Pure sulfadoxine and pyrimethamine were used to prepare stock solutions containing 1.613×10^{-2} M and 1.004×10^{-2} M, respectively. Serial volumes of the sulfadoxine stock solution, i.e. 1.0, 2.0 to 5.0 ml were accurately transferred to 10-ml volumetric flasks and 1.0 ml of chloranilic acid (4.5×10^{-3} M) each added Triplicate preparations for each volume of sulfadoxine were measured. The contents were thoroughly mixed and the mixture brought up to mark with dioxan. The solutions were left to stand for 30 min before their absorbances were measured. The same procedure was followed for pyrimethamine. These solutions were further left to stand under the laboratory conditions and their absorbances monitored at regular intervals of 24 h for 96 h.

The means of the absorbances were calculated and plotted against the concentrations. The curves were regressed by the method of least squares [25]. Employing the same analytical procedure further validated the calibration curves generated above except the concentrations of sulfadoxine and pyrimethamine used were changed. The mean absorbances for each concentration was determined and then converted to the corresponding concentration using the calibration curve.

The percent recoveries and their errors with respect to the calibration curves were determined by comparing the concentrations obtained from the calibration curve with the actual concentration of the drugs that were spiked. The relative standard deviation (RSD) for each concentration was calculated using the following model equation:

$$\%\ \mathrm{RSD} = \mathrm{S.D.}/n \times 100$$

where n = number of observations.

2.6. Simultaneous assay of sulfadoxine and pyrimethamine in their dosage forms

Four different brands of pharmaceutical tablets containing sulfadoxine (500 mg) and pyrimethamine (25 mg) equivalent to 0.5 g of sulfadoxine and 0.025 g of pyrimethamine were extracted into 100-ml volumetric flasks using 1,4-dioxan as described previously. The volumes were made up to mark. Serial volumes of 1.0, 2.0 up to 5.0 ml were transferred in triplicates into 10-ml volumetric flasks and 1.0 ml chloranilic acid (4.5×10^{-2} M) added to each flask. The contents were again brought up to mark and after 30 min; their absorbances were simultaneously read at their absorption maxima, i.e. 500 nm for sulfadoxine and 520 nm for pyrimethamine. With the aid of the standard curves, the percent recovery for sulfadoxine and pyrimethamine were individually calculated. Since these drugs were isolated from their pharmaceutical recipients by the inert 1,4-dioxan, no further tests were carried out for the formulation characteristics and potential interfering substances. Non-aqueous method of analysis was carried out according to official methods [24].

3. Result and discussion

The complexes formed between chloranilic acid and sulfadoxine and pyrimethamine appeared purple with absorption maxima at 500 and 520 nm, respectively. The slight yellow solution of chloranilic acid in 1,4-dioxan absorbs maximally at 420 nm (Fig. 1). The observed colour change suggests complex formation between chloranilic acid (π-acceptor) and the drugs (π-donors). The purple colour was stable over a period exceeding 24 h, therefore, we assume that the analyte (complex) was also stable throughout the duration of the assay (45 min). Solutions of these drugs in the inert solvent did not show any evidence of decomposition over a period of 24 h suggesting their stability in solution. Further work is in progress to establish the suitability of this method in stability-indicating analysis.

Empirical considerations of the nature of charge-transfer interactions have suggested that there could be no more evidence for the existence of the complex than the observed colour change [4,5]. This observation is associated most with π-donors and π-acceptor interaction as is the case in this investigation. In fact, in this investigation, the infra-red spectra of the complexes of these drugs did not show any shifts in absorption bands, indicating that the association did not produce any detectable electronic shifts. The precision of the analytical technique gave a RSD of 1.04%, thus suggesting high degree of reproducibility and accuracy was determined to be between 95 and 99%. Since the solvent used for the analysis did not dissolve any part of the excipient, selectivity for the complexation could not be established unequivocally.

The limit of detection ranged from 0.005 to 0.010 mg/ml for both drugs and that of quantitations were between 0.015 and 0.020 mg/ml for both drugs. The calibration curves were linear from 0.020 mg/ml; the various plots were subjected to linear regression by the method of least squares [25]. The following equations satisfied the mathematical model developed for the linearity of the range of concentrations employed.

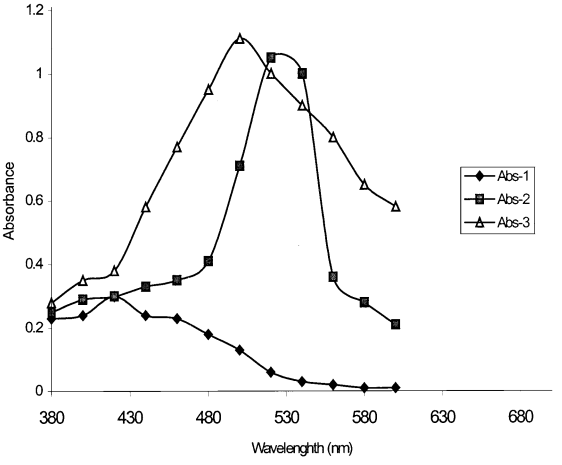

Fig. 1. Absorption maxima for (1) chloranilic acid, (2) pyrimethamine and (3) sulfadoxine.

Table 1
Summary of recovery experiments

Brands	Sulfadoxine (500 mg)[a]		Pyrimethamine (25 mg)[a]	
	% Recovery $n = 5$	% RSD	% Recovery $n = 5$	% RSD
Charge-transfer method				
A	98.04	2.21	103.5	1.04
B	96.55	3.41	98.05	1.08
C	95.44	3.17	93.75	0.89
D	94.79	3.85	95.33	1.15
Non-aqueous method				
A	100.1	3.1	99.6	2.5
B	98.5	2.8	97.8	1.9
C	98.7	1.8	99.1	2.1
D	97.9	2.3	98.3	2.7

[a] Label claim.

$$A_1 = 0.193x_1 + 0.00952 \qquad \text{(for sulfadoxine)}$$

and

$$A_2 = 0.25x_2 + 0.00619 \qquad \text{(for pyrimethamine)}$$

where A are absorbances; m, slope; x_1 and x_2, concentrations of the drugs in the final assay mixture in moles per litre, the reciprocal of which give the extinction coefficient. The regression coefficients for sulfadoxine and pyrimethamine were calculated to be 0.98 and 0.94, respectively and extinction coefficient of 10 504 for sulfadoxine and 16 155 for pyrimethamine. The RSD for the slope and intercept for sulfadoxine was 1.03 and 0.55%, respectively while that for pyrimethamine was 1.6 and 0.35%, respectively.

The observation that these complexation interactions follow Beer-Lambert Law suggests that termolecular or isometric complexes were not formed. The choice of 1,4-dioxan, a solvent with low dielectric constant severely reduced the capacity for solvation of the analyte molecules and the complexes, also the molecular sizes of the drugs did not favour solvation activity. The optimization step in which various concentrations of the analyte and chloranilic acid were investigated without significant ($P > 0.01$) variation in the standard deviations (RSD = 1.04%) and linear regression coefficients further confirm the absence of these isomeric complexes in the course of this analytical study.

Quantitative recovery experiments were carried out on four different brands of antimalarial drugs containing sulfadoxine and pyrimethamine in combination, out of many brands that are commercially available in the Nigerian market. The Nigerian market situation is typical of what obtains in developing countries in terms of large variety of branded names and the prevalence of fake, adulterated and substandard drugs.

The recovery experiments are presented in Table 1. These result reveals that all the brands studied meet the pharmaceutical standards of 95–105% for therapeutic use [24]. The low values of RSDs are an indication of the precision and accuracy of the measurements. Comparison of charge-transfer technique with the Official method of non-aqueous titration was carried out by the application of the Student t-test at the level of 95%. Result shows the calculated t-value to be 2.94 (theoretical value is 2.7) indicating non-significant difference.

It is our suggestion that donor–acceptor technique could be used in the quality assurance and a possible stability-indicating assay of these drugs. The sensitivity, simplicity and accuracy of this technique can be utilized in field studies and laboratory conditions for rapid screening of drugs.

4. Conclusion

Non-aqueous titration as an analytical tool is largely empirical particularly with respect to very

weak bases such as drugs. The sharpness of colour change at the equivalence point depends largely on the concentration of the base being titrated and the effect of the amount of acid bound by the indicator. In addition to these is the observation that most often perchloric acid is not completely dissociated during the reaction. These limitations in non-aqueous titrations result in lack of high degree of reproducibility and accuracy. The apparently high recovery values reported in this study may be ascribed to these factors. Spectrophotometric analysis by charge-transfer technique is constrained by the nature of interactions that take place between the donor and acceptor molecules, influence of solvent and stability of the complex formed. Despite these limitations charge-transfer technique still represents a more sensitive and accurate approach than the non-aqueous method. Molecules that absorb in the visible region, usually absorb very strongly so that absorbances can readily and accurately be determined.

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