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Development Green Spectrophotometric Method for Determination of Sulfamethoxazole in Pure and Pharmaceutical Formulations

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Abstract

Green analytical chemistry is considered as a branch of the green chemistry and the main its goal is to achieve more green analysis in quality control laboratories through different direction, such as replacing or minimizing toxic reagents and modify or replace analytical techniques or methods with safer ones. We develop a simple, accurate and sensitive spectrophotometric procedure for determination of Sulfamethoxazole (SMX) using phenoxazine (PNZ) as green analytical reagent. The method is based on oxidation in aqueous mildly acidic medium primary amine group of SMX and coupling with PNZ in the prescient iron (III) to form a stable color having maximum absorption at 520 nm. Beer's law was obeyed in the range concentration of 0.1-6 mg/l and molar absorptivity 6.105 × 10^4 $L.mol^{-1}.cm^{-1}$. Sandell's sensitivity 0.003 $\mu g.cm^{-2}$ and detection limit (DL) 0.021 ppm. The direct determination of SMX in pure form and in its pharmaceutical formulations method successfully applied with very good recoveries 98.70%-101.5%. Green spectrophotometric analytical analyses become unique when the pharmaceutical drugs reagents combine with low concentration to determinate or estimate pharmaceutical drugs.

Keywords: Sulfamethoxazole; Phenoxazine; Spectrophotometric; Green reagent; Pharmaceutical formulations

Introduction

The green analytical chemistry was first mentioned by Paul Anastas in 1999 [1]. Analytical methodologies have continuously been improved by their accuracy, sensitivity and precision to monitor pollutants from environmental and food samples. In addition, improvements may be done in terms of reducing or eliminating the use of reagent/solvents in a sample preparation and determination, however, not always mentioning the concept of green analytical chemistry. Green analytical chemistry is not about monitoring of environmental pollutants, but rather "greening of methodologies". Out of the 12 Paul Anastas principles, can be extracted 7 principles of green analytical chemistry.

We have to consider the method to be environmentally sustainable as possible as without effecting accurate, precise and sensitive of analytical method in this direction Sulfa drugs have attracted special attention from their therapeutic importance [2]. Sulfamethoxazole (SMX) belongs to Sulfa drugs and Its chemical name 4-amino-N-(5-methyl-1, 2-oxazol-3-yl)-benzenesulfonamide.[3]. Because of its low cost and high efficiency against many gram-positive and gram-negative bacteria, they are used mainly for the treatment of urinary infectious diseases [4,5]. The official method is based on diazo-coupling reaction with N-(1-naphthyl) ethylenediamine dihydrochloride resulting in dye formation and this is characterized by high sensitivity but often has drawbacks of pH dependence, diazotization temperature and coupling time. Besides, these procedures often use large sample volumes of carcinogenic reagent(s), which makes it excluded out of the standards of green analytical chemistry [6].

The main objectives of green analytical chemistry (GAC) in our study are to obtain new analytical spectrophotometric to make the method greener by avoiding diazotization reaction and use less amounts and save analytical reagent. For this reason, utilizing the non-toxic chemicals phenoxazine (pharmaceutical drugs) as a new and novel green analytical reagent and novel oxidative coupling reactions was used to develop a rapid, simple and accurate spectrophotometric method for determination of Sulfamethoxazole in bulk drug and pharmaceutical formulations. Utilization of pharmaceutical drugs to replace chemical indicators/complexing agents has been interesting. However, the use of low cost and environmentally friendly reagent would be more attractive which could facilitate the implantation of this method in a fast routine analysis. Spectrophotometric parameters and statistical analysis of data were established for standardization of this method.

Materials and Method

Reagents and solutions

Sulfamethoxazole (Figure 1A, Modern Pharma Company) stock solutions (100 mg/L) weighed accurately 100 mg of Sulfamethoxazole in 20 ml of 2 N HCl acid and diluted with water in a 100 ml volumetric flask and phenoxazine (0.025%, w/v) (Figure 1B, (Aldrich) solutions 25 mg weighed accurately in 100 ml with ethyl alcohol the purity greater than 98%. A 0.01 M iron(III) sigma, solution was prepared by accurately equivalent weighed from ammonium iron(III) sulphate dodecahydrate in 0.01 M hydrochloric acid.

In tablets –10 tablets were grinded and mixed well. Equivalent 100 mg Powder tablets of the drug weighed and dissolved in 20 ml of 2 HCl acid in a 100 ml volumetric flask and solution was shaken swirled, the solution diluted with water up to the mark to get final concentration

(1000 µg ml^{-1}). To avoid any suspended or un-dissolved material the solutions were filtered through Whatman filter paper No. 41, the final concentration of Sulfamethoxazole was brought to 100 µg ml^{-1} with water.

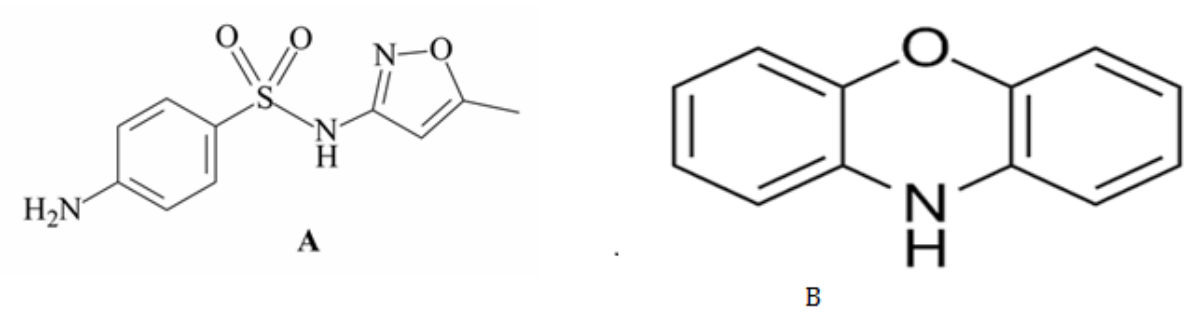

Figure 1: Chemical structure of (A) Sulfamethoxazole and (B) Phenoxazine.

Apparatus

The absorption spectra and absorbance were recorded and obtained by using computerized UV-Visible spectrophotometer Shimadzu-1800 with 1 cm quartz and glass cells for product analysis and using quartz cell.

General recommended procedure

After optimizing the instrumental parameters for the spectrophotometric method, the analytical curves (n=3) were constructed by addition of aliquots of different volumes of the stock solution of SMX into a 25 ml flasks 2 ml of PNZ (0.025%) w/v, 1 ml of HCl (2 N) and 1 ml 0.01 M of iron (III) finally the mixture was left for 2 min and make up with water.

Parameter	Value
Linear rang (mg/L)	0.10-6.0
Stability (h)	2
Maximum wavelength (nm)	520
Molar absorptivity(L.mol^{-1}.cm^{-1})* 104	6.105
Sandell sensitivity (µg cm^{-2})	0.003
Detection limit (mg/L)	0.021
Limit of detection(µg ml^{-1})	0.004
Limit of quantification (µg ml^{-1})	0.018
Regression equation*	
Intercept (a)	-.0141
Slop (m)	0.0452
Correlation coefficient (r)	0.9995
Reaction time (min)	2
* y=mx+a where x is the concentration of SMX in µgml^{-1}	

Table 1: Performance data for spectrophotometric of sulphamethozaole with phenoxazine reagent.

The absorbance of each solution was measured at 520 nm against blank. The performance data for spectrophotometric determination of SMX using PNZ was detailed in Table 1.

All measurements were carried out in three times for each concentration (n=3). According to IUPAC the limits of detection (LOD=$3S_B$/b) and quantification (LOQ=$10S_B$/b) were estimated [7] where SB is the standard deviation of the blank (n=10) and b is the slope of the calibration curve.

Results and Discussion

Green analytical chemistry may be defined in light of the 12 principles of green chemistry [8] as a collaboration of efforts to develop more clean or eco-friendly technologies characterized by elimination or at least minimizing emissions of pollutants into the environment without compromising efficiency even at low concentration of analyte in samples with a complex matrix composition. Such definition can lead to the development of new technologies or modification of current analytical methods.

Accordingly, greener analytical process may be achieved through; avoidance of toxic reagents; reagents and solvents should eliminate or reduce. The presented spectrophotometric method was established to enhance the determination of SMX through optimize the best chemical and physical condition with good sensitivity and precision. The influence of various analytical parameters including acid solution, reagent amount, coexisting ions, reaction time and sample volume were investigated [9,10].

Absorbance spectra of the colored reagent

Absorption spectrum was scanned on spectrophotometer in the wavelength region of 200 to 700 nm against reagent blank and maximum of absorption at 520 nm.

Hence, at this wavelength all measurements were made against reagent blank.

Effect of phenoxazine concentration

The effect of PNZ concentration greatly enhances the reaction and production color day. The effects were tested from 1–5 ml of 0.025% w/v phenoxazine.

2 ml give maximum color intensity and was chosen as the best volume for the reaction (Table 2).

Volume of PNZ	Absorbance
0.5	0.398
1.0	0.457
2.0	0.534
3.0	0.501
4.0	0.503
5.0	0.496

Table 2: Effect of Volume of PNZ on the absorbance (2 μgml^{-1} SMX).

Effect of temperature

The temperature effect on reaction was studied. The absorbance was measured at 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C and 80°C temperatures under optimum conditions. The highest absorbance was determined at 30°C. Therefore, room temperature was considered as an optimal for the reaction. The intensity of the color decreased when the temperature increases this may be due to the dissociation of complex.

Effect of time

The optimum reaction time was determined by monitoring the color developed at room temperature the development was attained after 2 min and remained stable for at least 2 h.

Effect of acid

The effect of different acids sulphuric, hydrochloric, phosphoric or acetic acid showed that hydrochloric acid was the best and give maximum intensity of color (Table 3). The volume of HCl of 2 M was tested from 1–5 ml HCl. Therefore, 1 ml gives maximum color intensity and was chosen as the best volume for the reaction.

Acid	Absorbance
Sulphuric acid	0.398
Hydrochloric acid	0.502
Phosphoric acid	0.352
Acetic acid	0.211

Table 3: Effect of different acid on the absorbance.

Linearity (Bear's law application)

The linearity of the spectrophotometric method for the determination of SMX was evaluated under optimum conditions. The regression calibration equation obtained under optimum conditions for SMX with PNZ was: Y=0.0452X+-0.0141; r=0. 0.9995 and n=7, where Y is the absorbance and X is the SMX concentration as μg ml^{-1}. The calibration curve was linear over the range 0.10 – 6.0 μg ml^{-1} (Figure 2).

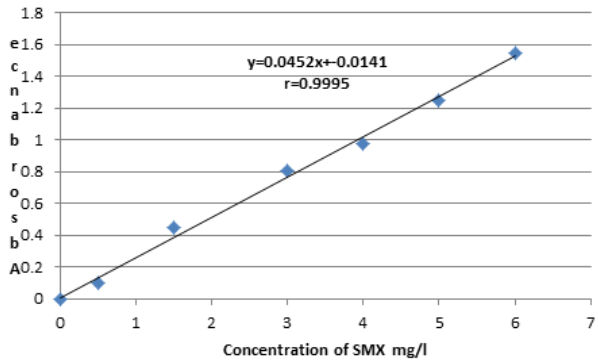

Figure 2: Calibration curve for SMX using PNZ.

Precision and accuracy

The precision and accuracy of the method were determined as documented by the BP (3). The precision and accuracy data are summarized in Table 4. The precision (repeatability) of the proposed method was calculated from a series of three solutions of 1, 3 and 5 ppm of SMX on the same day analyses. The relative standard deviation (RSD) for three analyses was 0.95, 0.66 and 0.86% and accuracy was 101.05%, 100.89% and 99.59% respectively. The day to day precisions were obtained by the repeated analyses of 1, 3 and 5 ppm of SMX (three analyses) over one week. The results showed that the inter-day RSD was 0.64, 0.81 and 0.51% and accuracy was 98.92%, 99.91% and 99.60% respectively.

	Drug concentration (μg ml^{-1})	Mean found concentration (μg ml^{-1})	Accuracy%	Precision %
Intra-day(n=3)	1	1.05	101.05 ± 0.75	0.95
	3	2.98	100.89 ± 0.52	0.66
	5	5.06	99.59 ± 0.83	0.86
Inter-day(n=3)	1	1.09	98.92 ± 0.73	0.64
	3	3.03	99.91 ± 0.96	0.81
	5	5.07	99.60 ± 1.09	0.51

Table 4: Precision and accuracy of the method for determination of SMX in standard solutions.

Stoichiometry of the reaction

It was Job's method of continuous variation used for determining the molar ratio of SMX to each of the analytical reagents employed in the oxidative coupling reactions. These ratios were 1:1 in all cases. This indicates that only one dye products are formed is possible for the formation of the complex have stability constant 3.23 × 108 M^{-1}.

Interference study

In order to estimate the selectivity performance of the method for determination of SMX, the effect of tolerable coexisting common excipients include lactose, menthol, sucrose, acacia, microcrystalline

cellulose, talc, starch and magnesium stearate which my found with SMX drug in formulations were studied. For this purpose, 10-fold excess of different excipient added to solutions (2 ppm) of SMX were treated conferring to the proposed procedure (Table 5).

Excipients	Amount taken (mg)	%Recovery ± RSD*
Menthol	20	99.85 ± 0.56
Sucrose	20	99.18 ± 0.56
Acacia	20	101.5 ± 0.78
Microcrystalline Cellulose	20	101.1 ± 0.46
Talc	30	100.8 ± 0.78
Magnesium Stearate	25	98.70 ± 0.52
Starch	30	100.9 ± 0.75

Table 5: Determination SMX (2 ppm) in the presence of excipients by proposed method.

Pharmaceutical applications

In order to validate the applicability and reliability of the developed method for four different dosage forms containing SMX. Measurement for each pharmaceutical formula was taken in three replicate for three different concentrations of Table 6. The values of recoveries have assured the validity of the proposed method 98.70%-101.5%. Finally, the accuracy using F- and t-test analysis that there is no significant difference between the developed method and the official method [3] (Table 6).

Tablet name[a]	Label claim (mg/ tablet)	% Recovery found ± RSD[b]		F-test[d]	t-test[c]
		Proposed method	Official method [3]		
Glotrim	200	99.79 ± 0.35	100.1 ± 0.72	2.12	3.05
Cotrix	400	101.10 ± 0.83	99.59 ± 0.56	1.98	2.13
Parathrim	400	100.9 ± 0.63	99.91 ± 0.56	0.99	2.56
Parathrim Fort	800	99.10 ± 0.33	101.21 ± 0.56	2.05	1.67

[a]Tablets from different Manufacturers, [b]Mean of six determinations, [c]Tabulated t-value at 95% confidence level is 2.78.[d] Tabulated F-value at 95% confidence level is 6.39

Table 6: Determination of accuracy of the proposed method for determination SMX in Formulations.

Conclusion

Analytical methods are developing rapidly and introducing Phenoxazine as novel spectrophotometric reagent for the direct determination of SMX. There is a strong driving force that is concerned about this reagent (PNZ) whish exhibit better sensitivity, safety of the environment and higher reproducibility. So, the public needs confirmation that chemical products and processes are safe in this way the use of mild acidic medium and choice available with the reagents make the procedure versatile and cost-effective. On the other hand, when choosing the proper analytical method waste prevention must become a part of the decision process, pharmaceutical chemistry will reduce of toxic waste as consequence result in exploitation of less or non-toxic reagents. The new direct method has special advantages of simplicity, reproducibility, sensitivity and mainly use and produce lower amounts of toxic. Finally the replacement of old reagent by an attractive oxidative electrophilic reaction by taking the advantage of available plenty molecules in the area of pharmaceutical chemistry increases utilization in pharmaceutical drugs.

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