



# **UV-Visible Spectrophotometric Method for Quantitative Estimation of Sulfamethoxazole with BM Reagent**

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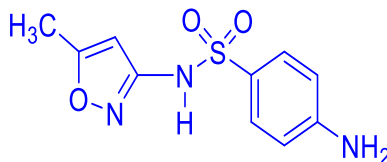
**ABSTRACT:** A simple, sensitive, and selective rapid spectrophotometric method has been developed for the determination of Sulfamethoxazole in both pure form and pharmaceutical formulations. The method is based on an oxidative coupling reaction with Bratton–Marshall (BM) reagent at pH 4.0, producing a chromogen that is extractable and measurable at 469nm. Beer's law is obeyed over the concentration range of 5–30 µg/mL. The proposed method was successfully applied for the direct and convenient analysis of pharmaceutical formulations. The relative standard deviation (RSD) was found to be 0.25%, and the recovery was 99.96%, indicating good precision and accuracy. The method was fully validated and demonstrated to be rugged. No interference from excipients or other formulation ingredients was observed. The repeatability and reliability of the method were confirmed through statistical evaluation and recovery studies.

**KEYWORDS:** UV-Visible Spectrophotometric Method, Sulfamethoxazole, Quantitative Estimation, Bratton–Marshall (BM) reagent, Robustness and Ruggedness.

## **I. INTRODUCTION**

Sulfamethoxazole (SMX) is a sulfonamide-class antibacterial agent chemically designated as 4-amino-N-(5-methyl-1,2-oxazol-3-yl) benzenesulfonamide. It exhibits broad-spectrum antibacterial activity against both Gram-positive and Gram-negative microorganisms and is widely used due to its efficacy, affordability, and well-established clinical profile. SMX is most commonly administered in combination with trimethoprim (TRI), forming the synergistic formulation known as co-trimoxazole, which is extensively prescribed for the treatment of urinary tract infections, respiratory tract infections, and gastrointestinal infections (1–3). In addition, SMX has demonstrated therapeutic utility in combination with primary amines for the treatment of chloroquine-resistant *Plasmodium falciparum* malaria.

The antibacterial activity of SMX is attributed to its ability to competitively inhibit the enzyme dihydropteroate synthase (DHPS), thereby disrupting the bacterial folic acid biosynthetic pathway. This inhibition prevents the formation of dihydrofolic acid, an essential precursor required for nucleic acid synthesis, ultimately leading to the suppression of bacterial growth and proliferation (4). Several analytical methods have been reported for the quantitative determination of SMX in pharmaceutical formulations and biological matrices. These include charge-transfer complexation techniques (5), UV–Visible spectrophotometric methods (6–13), rapid ultra-performance liquid chromatography (UPLC) methods (15), flow injection analysis coupled with potentiometric detection (17), and colorimetric sensor-based approaches (18,19), which offer advantages such as simplicity and visual detectability. However, despite these reported techniques, no visible spectrophotometric method based on diazotization and coupling reactions using *o*-phenylenediamine (OPDA) has been documented to date. In view of this gap, the present study aims to develop and validate a simple, sensitive, and cost-effective UV–Visible spectrophotometric method for the quantitative determination of SMX in bulk drug and pharmaceutical dosage forms. The proposed method employs OPDA as a derivatizing reagent, providing an efficient and reliable analytical alternative suitable for routine quality control analysis.

**Fig.1.**Chemical structure of Sulfamethoxazole

## II. MATERIALS AND METHODS

### Instrumentation

Spectral measurements were performed using a Thermo Scientific GENESYS 10S UV–Visible spectrophotometer, and data acquisition was carried out using Thermo Fisher Scientific software. Quartz cuvettes with a path length of 10 mm were used throughout the analysis. Sonication of standard and sample solutions was carried out using an Ultrasonicator (1.3 L capacity). Weighing of standard and sample materials was performed using an ML-T Analytical Balance (RS232) with appropriate accuracy.

### Chemicals and Reagents

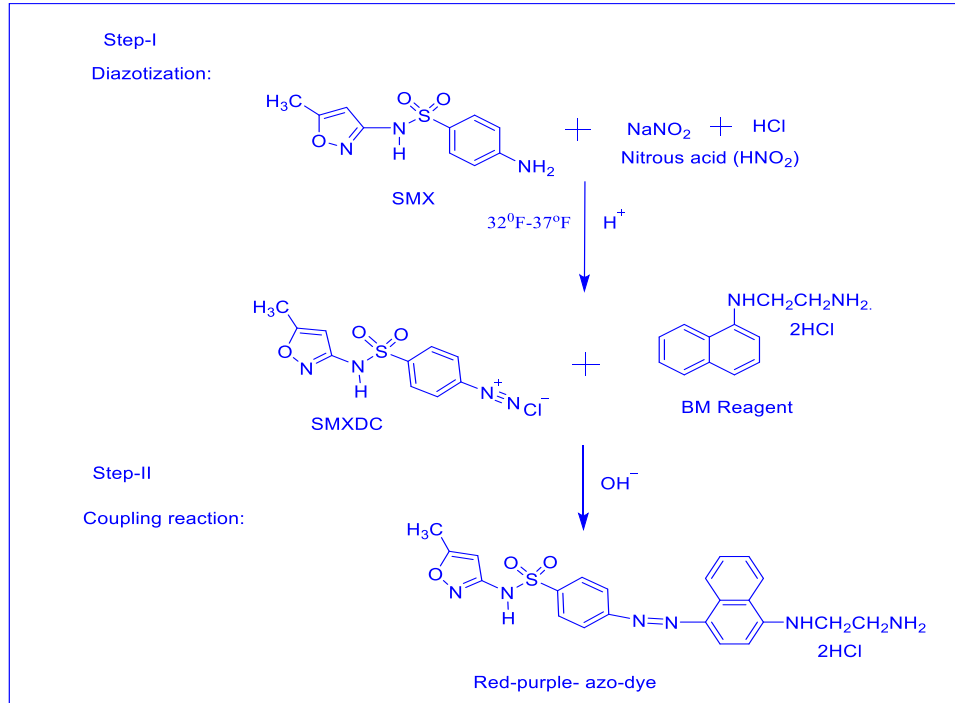
A reference standard of Sulfamethoxazole (SMX) was kindly provided by Analog Lab, Hyderabad. The marketed formulation GANTANOL (SMX 400 mg) was procured from a local pharmacy. Bratton–Marshall (BM) reagent and distilled water (used as solvent) were obtained from Brass Scientific Pvt. Ltd., Andhra Pradesh, India. All reagents and chemicals used were of analytical reagent grade.

### Preparation of Standard Stock Solution

An accurately weighed 100 mg of SMX reference standard was transferred into a 100 mL volumetric flask and dissolved in approximately 50 mL of distilled water. The solution was sonicated for 5 minutes to ensure complete dissolution and then diluted to volume with the same solvent to obtain a stock solution of 1000 µg/mL. From this stock, 10 mL was transferred into a 100 mL volumetric flask and diluted with distilled water to obtain a working standard solution of 100 µg/mL. Further dilutions were prepared as required for analysis.

### Selection of Analytical Wavelength

Appropriate dilutions of the standard stock solution were prepared using Milli-Q water and scanned in the wavelength range of 200–800 nm against a reagent blank using a 1 cm quartz cuvette. The overlain absorption spectra revealed a well-defined maximum absorbance ( $\lambda_{max}$ ) at 525 nm for sulfamethoxazole. This wavelength was selected as optimal for further spectrophotometric analysis.



Scheme 4.1: Reaction mechanism of sulfamethoxazole with -BM Reagent:

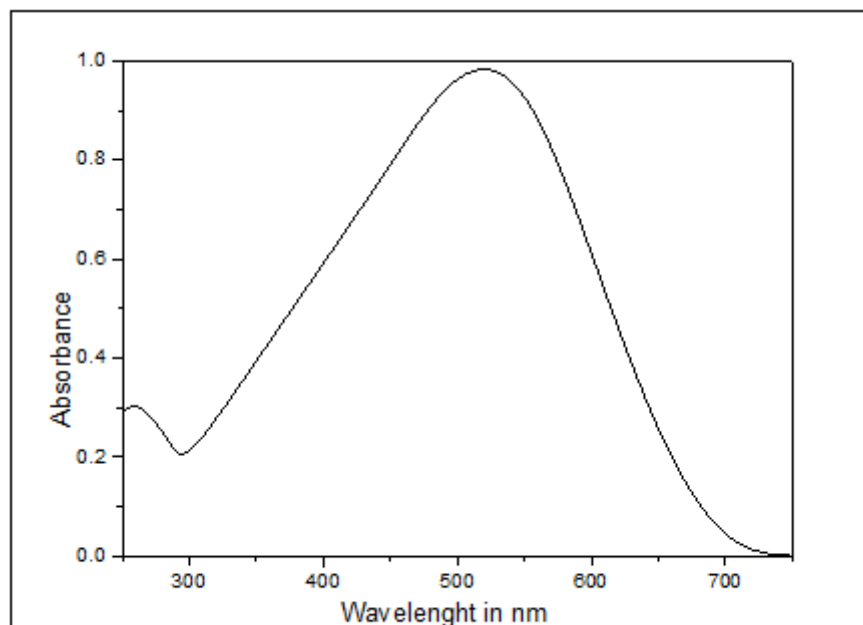


Fig.4.1. Absorption spectrum of Sulfamethoxazole treated with B.M reagent

### Linearity

The linearity of the proposed spectrophotometric method was evaluated by analyzing sulfamethoxazole solutions over a concentration range of 5–30 $\mu\text{g/mL}$  at the selected wavelength of 525 nm. A calibration curve was constructed by plotting absorbance versus corresponding concentration, which demonstrated a strong linear relationship within the

studied range. The regression analysis confirmed excellent linearity, indicating proportionality between absorbance and concentration of sulfamethoxazole. The linearity data are summarized in Table 1, and the corresponding calibration curve is depicted in Fig. 3.

Table.1. Linearity results for Sulfamethoxazole

S.No	Concentration in $\mu\text{gml}^{-1}$	Absorbance
1	5	0.197±0.004
2	10	0.394±0.008
3	15	0.592±0.003
4	20	0.789±0.007
5	25	0.987±0.002
6	30	1.184±0.006

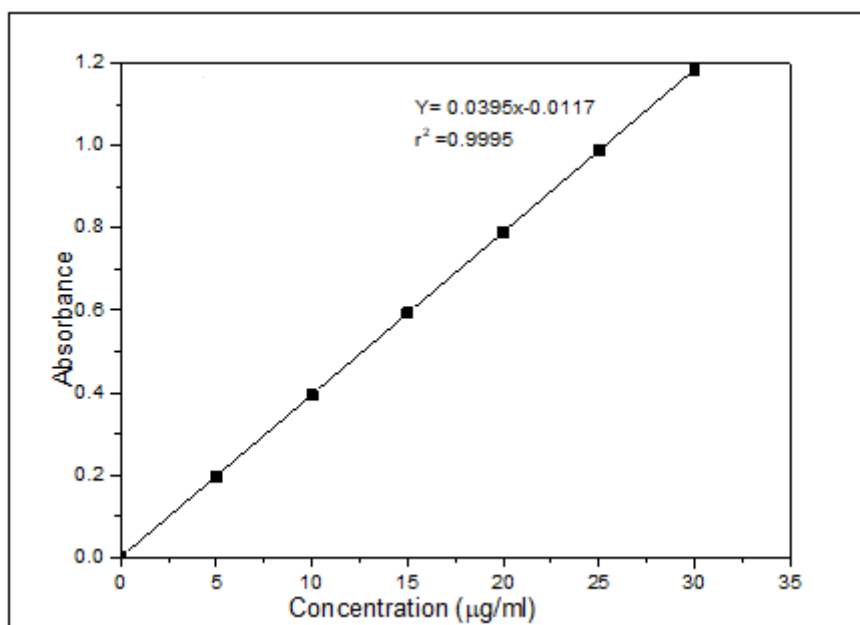


Fig3. Linearity graph for Sulfamethoxazole

#### Accuracy (Recovery Studies)

Accuracy of the proposed method was evaluated through recovery studies conducted in accordance with standard analytical guidelines. Recovery experiments were performed at three concentration levels 10%, 20%, and 30% by spiking known amounts of sulfamethoxazole into the pre-analyzed sample matrix. The resulting solutions were analyzed at the selected wavelength, and the absorbance values obtained were used to calculate the percentage recovery and percentage relative standard deviation (%RSD) for each concentration level. The recovery results ranged between 98–102%, with %RSD values below 2%, demonstrating the accuracy, reliability, and reproducibility of the developed method. The detailed recovery data for sulfamethoxazole are presented in Table 2.

Table2. Results obtained from recovery studies

S.No	Amount added $\mu\text{gml}^{-1}$	Amount found* $\mu\text{gml}^{-1}$	% Recovery
1	5	4.97± 0.04	98.40±0.213
2	10	9.89 ± 0.01	98.90±0.422
3	15	14.86 ± 0.03	99.00±0.613
4	20	19.92±0.06	99.60±0.300
5	25	24.88±0.02	99.52±0.101
6	30	29.99±0.01	99.96±0.124

### Precision

The precision of the developed analytical method was evaluated in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision) and expressed as percentage relative standard deviation (%RSD). A test solution containing 10  $\mu\text{g/mL}$  of sulfamethoxazole was analyzed in six replicate measurements. Intra-day precision was assessed by analyzing the six replicates within the same day, whereas inter-day precision was determined by repeating the analysis over three consecutive days under identical experimental conditions. The %RSD values obtained were 0.678% for intra-day precision and 0.785% for inter-day precision, demonstrating excellent method precision and reproducibility. The precision data are summarized in Table 3, confirming that the proposed method meets acceptable analytical performance criteria.

Table3. Precision Results for Sulfamethoxazole

S.No	Intra Day	Inter Day
1	0.394±0.002	0.388±0.006
2	0.395±0.004	0.385±0.007
3	0.397±0.006	0.384±0.002
4	0.399±0.001	0.381±0.003
5	0.401±0.003	0.379±0.001
6	0.402±0.005	0.377±0.004
7	% RSD=0.678	% RSD=0.785

### Ruggedness and Robustness

The ruggedness of the proposed analytical method was evaluated by assessing its reproducibility under normal operating conditions when performed by different analysts. The analysis was carried out using the same experimental procedure, and the percent relative standard deviation (%RSD) was calculated from three replicate absorbance measurements for sulfamethoxazole. The obtained %RSD value of 0.253 indicates minimal variability between analysts, thereby confirming the ruggedness of the method. The corresponding results are presented in Table 4. Robustness was evaluated by deliberately introducing small, controlled variations in experimental conditions to assess their effect on method performance. The results demonstrated that such minor changes did not significantly influence the analytical outcome, confirming the robustness of the developed method. The robustness data are summarized in Table 5.

Table4. Ruggedness of the experimental studies

S.No	Variation	(10 $\mu\text{gml}^{-1}$ )
1	Actual	0.394±0.001
2	Analyst to Analyst	0.393±0.004
3	Instrument to instrument	0.397±0.006
4	%RSD	0.253

Values are given in the table are mean SD of three replicate experiment

Table5. Robustness study of (15 µgml<sup>-1</sup>)

S. No	Change in wave length (+2 nm)	Absorbance	Change in Temp. (0 <sup>0</sup> c)	Absorbance
1	520	0.591±0.001	At room temp.	0.529±0.004
2	522	0.591±0.003	Sun light at Morning	0.595±0.002
3	524	0.589±0.004	Refrigerator	0.599±0.003

Values are given in the table are mean SD of three replicate experiment

### Sensitivity

The sensitivity of the developed analytical method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ) in accordance with the International Council for Harmonisation (ICH) guidelines. A standard solution was prepared, and its absorbance was measured under optimized experimental conditions. The LOD values for sulfamethoxazole were found to be 0.167 µg/mL and 0.250 µg/mL, indicating the method's ability to detect the analyte at very low concentration levels. Similarly, the LOQ values were determined to be 0.506 µg/mL and 0.759 µg/mL, demonstrating that the method provides reliable and precise quantification even at trace concentrations. These results confirm the high sensitivity of the proposed spectrophotometric method, supporting its suitability for routine analysis and quality control of sulfamethoxazole. The detailed results are presented in Table 6.

Table6. Results of Sensitivity studies (LOD and LOQ)

S.No	Parameter	Present method
	<b>Intraday</b>	
1	LOD	0.167
2	LOQ	0.506
	<b>Interday</b>	
3	LOD	0.250
4	LOQ	0.759

### Formulation Analysis

The absorbance of the tablet sample solution was measured at 525 nm under optimized experimental conditions. The formulation analysis demonstrated that the developed method could accurately and reliably quantify sulfamethoxazole in the pharmaceutical dosage form. The assay results indicated that more than 98% of the labeled amount of the active ingredient was recovered, with an observed assay value of 99.85%, confirming excellent agreement with the labeled claim. The analytical results obtained for the marketed formulation are presented in Table 7, while a comprehensive summary of the analytical performance of the proposed method is provided in Table 8. These findings confirm the suitability of the method for routine quality control analysis of sulfamethoxazole in pharmaceutical formulations.

Table7. Formulation results for Sulfamethoxazole (SMX)

S. No	Drug	Brand name	Label claim	Amount prepared	Amount found	%assay
1.	SMX	GANTANOL	400 mg	40 mg	39.94 ±0.004	99.85

Table 8. Analytical performance data for the proposed methods

S.No	Parameter	Present method
1	Wavelength(nm)	525
2	Color	Red-Purple
3	Linearity ( $\mu\text{gml}^{-1}$ )	5-30
4	Molar absorptivity $\text{l}^{-1} \text{mole}^{-1} \text{cm}^{-1}$	$1.502 \times 10^4$
5	Shandell's sensitivity ( $\mu\text{gcm}^{-1}$ )	0.0168
6	Regression Equation( $y=mx+c$ )	$Y=0.0395x-0.0003$
7	Slope (m)	0.0395
8	Intercept (c)	0.0003
9	Correlation Coefficient ( $r^2$ )	0.9995
10	Intraday Precession	
	%RSD	0.678
11	Interday Precession	
	% RSD	0.785
12	Intraday	
	LOD( $\mu\text{gml}^{-1}$ )	0.167
	LOQ( $\mu\text{gml}^{-1}$ )	0.506
13	Interday	
	LOD( $\mu\text{gml}^{-1}$ )	0.250
	LOQ( $\mu\text{gml}^{-1}$ )	0.759

### Procedure for Formulation Analysis

#### Procedure

Twenty pre-weighed, coated tablets containing sulfamethoxazole (SMX) were finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of SMX was transferred into a 100 mL volumetric flask and dissolved in ethanol with continuous stirring for approximately 5 minutes. The resulting solution was filtered, and the filtrate was evaporated to dryness. The residue was reconstituted with ethanol and diluted to volume in a 100 mL volumetric flask to obtain Stock Solution. For further dilution, 10 mL aliquots of Stock Solution were transferred into separate 100 mL volumetric flasks and diluted to volume with ethanol to obtain the working solution. Serial dilutions were subsequently prepared in the concentration range of 5–30  $\mu\text{g/mL}$ . The absorbance of these solutions was measured at 525nm, and the obtained results were statistically evaluated. The reaction mechanism of sulfamethoxazole with ortho-phenylenediamine is illustrated in Scheme 1.

### III. RESULTS AND DISCUSSION

A UV–Visible spectrophotometric method was successfully developed and validated for the quantitative determination of sulfamethoxazole in tablet dosage forms in accordance with the International Council for Harmonisation (ICH) guidelines (ICH Q2(R1), 2005). Purified water was used as the solvent system, and absorbance measurements were recorded at 525 nm. Calibration curves were constructed for this method, and excellent linearity was observed over the concentration ranges of 5–30 µg/mL. The calculated absorptivity values confirmed the sensitivity of the developed methods within the selected wavelength regions. Accuracy was evaluated through recovery studies, which yielded recovery values ranging from 98.90–99.96%. These values fall within the acceptable range of 98–102%, with relative standard deviation (%RSD) values below 2%, confirming the accuracy and reliability of the proposed methods. Precision was assessed in terms of repeatability (intra-day) and intermediate precision (inter-day). The %RSD values for this were 0.678% (intra-day) and 0.785% (inter-day), respectively. These low %RSD values demonstrate excellent repeatability and reproducibility of the analytical procedures. Ruggedness studies further confirmed the robustness of the methods, with %RSD value is of 0.25 across six replicate measurements performed by different analysts. The results indicate that minor variations in analytical conditions do not significantly affect method performance. Overall, the developed spectrophotometric methods were found to be rapid, accurate, precise, and reproducible, making them suitable for routine quantitative analysis of sulfamethoxazole in pharmaceutical formulations. The validation parameters confirmed the reliability of the methods with respect to linearity, accuracy, precision, robustness, and reproducibility.

### IV. CONCLUSION

A simple, sensitive, and reliable UV–visible spectrophotometric method has been successfully developed and validated for the quantitative determination of sulfamethoxazole in pharmaceutical dosage forms using ethanol as the solvent. The proposed methods offer several advantages, including simplicity, cost-effectiveness, minimal sample preparation, high precision, and excellent accuracy. The validated methods demonstrated compliance with ICH guidelines and were proven to be suitable for routine quality control analysis in pharmaceutical laboratories. Owing to their robustness, reproducibility, and analytical efficiency, the proposed methods can be effectively employed for the routine estimation of sulfamethoxazole in bulk drugs and marketed formulations.

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### REFERENCES

1. Sohrabi MR, Fathabadi A, Nour AH. Simultaneous spectrophotometric determination of sulfamethoxazole and trimethoprim in pharmaceutical preparations using multivariate calibration methods. *J Appl Chem Res.* 2010; 3:47–52.
2. Yu W, Liu Z, Gao S, Cui S, Yang X. Determination of sulfonamides in blood using acetonitrile–salt aqueous two-phase extraction coupled with high-performance liquid chromatography and liquid chromatography–tandem mass spectrometry. *Anal Methods.* 2013; 5:5983–5989.
3. Nebo C, Regal P, Miranda JM, Fuente C, Cepeda A. Rapid method for quantification of nine sulfonamides in bovine milk using HPLC–MS/MS without solid-phase extraction. *Food Chem.* 2013; 141:2294–2299.
4. Takagishi Y, Ohsuga K, Ohama S. Suppository containing sulfamethoxazole/trimethoprim complex and process for preparing the same. U.S. Patent US4461765; 1975.
5. Olajide AA, Chinedum PB, Olayinka AK, Oyakhire O. Simultaneous spectrophotometric determination of trimethoprim and sulfamethoxazole following charge-transfer complexation with chloranilic acid. *Arab J Chem.* 2017;10: S3848–S3860.
6. Islam T, Ferdous S, Jain P, Reza HM. Method development and validation of baclofen mouth dissolving tablets by UV spectroscopy. *Eur J Appl Sci.* 2013; 5:7–11.
7. Bana T, Yadav G, Dudhe R. Development and validation of oseltamivir phosphate API by UV spectrophotometric method. *Glob J Pharmacol.* 2013; 7:294–297.

8. Frag EY, Mohamed GG. Electronic, infrared, mass, and <sup>1</sup>H NMR spectral studies of charge-transfer complexes of sulphonamide drugs with  $\pi$ -acceptors in acetonitrile. *J Mol Struct.* 2010; 979:46–55.
9. Pokala RV, Kumari K, Bollikola HB. UV spectrophotometric method development and validation of sulfadiazine and trimethoprim in combined dosage form. *Int J Pharm Sci.* 2018; 10:103–107.
10. Goran S, Elizabeth DS, Marija S, Romel V. Optimization, validation, and application of UV–visible spectrophotometric colorimetric methods for determination of trimethoprim in pharmaceutical products. *Maced Vet Rev.* 2016; 39:65–76.
11. Zaman SM. Analytical method development for the spectrophotometric determination of sulfamethoxazole in bulk drug and pharmaceutical preparations. *J Chem Biochem.* 2015; 3:63–74.
12. Azeez MD, Kiran KB, Manoranjan V, Venkateshwarlu G, Maninder B, Naresh Y. Simultaneous determination of sulfamethoxazole and trimethoprim using UV–Visible spectrophotometry. *Int J Pharm Sci Rev Res.* 2013; 23:224–227.
13. Shamsa F, Amani L. Determination of sulfamethoxazole and trimethoprim in pharmaceuticals by visible and UV spectrophotometry. *Iran J Pharm Res.* 2016; 1:31–36.
14. Srinivasa RJ, Vejendla RK, Ravali N, Varnalatha K, Kalpana K. Rapid UPLC method for simultaneous estimation of sulfamethoxazole and trimethoprim in pharmaceutical dosage forms. *Indo Am J Pharm Res.* 2014; 4:896–904.
15. Wang JC, Zhang Q, Cai DF. Stability-indicating validated HPLC method for analysis of berberine hydrochloride and trimethoprim in pharmaceutical dosage forms. *J Chem.* 2013; 2013:1–9.
16. Madhulika CH, Anton Smith A, Seetaramaiah K, Kottaimuthu A, Manavalan R. Spectrophotometric determination of sulfamethoxazole in pharmaceutical formulations. *Int J Pharm Sci Rev.* 2011; 7:198–202.
17. Sabriye P, Ozkuruçuklu I, Yücel Şahin I, Güleren Aİ. Determination of sulfamethoxazole in pharmaceutical formulations by flow injection analysis coupled with potentiometric detection using a polypyrrole electrode. *J Braz Chem Soc.* 2011; 22:2171–2177.
18. Vijayalakshmi R, Sowjanya D, Archana S, Dhanaraju MD. Application of extractive spectrophotometric methods for the determination of flucloxacillin and trandolapril using bromocresol green. *Asian J Pharm Clin Res.* 2014; 7:216–218.
19. Genting N, Silalahi J, Pardede TR, Sudarmi S, Nerdy N. Effect of harvesting time on nitrate levels in spinach (*Amaranthus tricolor* L.) and mustard (*Brassica rapa* L.). *Asian J Pharm Clin Res.* 2018; 11:110–113.
20. International Conference on Harmonisation (ICH). *Validation of Analytical Procedures: Text and Methodology (Q2(R1))*. Geneva, Switzerland; 2005.



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