

## Determination of trimethoprim by various analytical techniques- A- review

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### Abstract

Trimethoprim is one of the representative drugs within the pharmaceutical and personal care products (PPCPs) group and one of the main pollutants in pharmaceutical wastewater, mainly used in synergistic antibacterial activities. Trimethoprim found with sulfamethoxazole combination have a synergetic effect, due to the inhibitor effect that occurs in more than one step during the obligatory sequence of enzymatic reactions in the bacteria. It is used individually in the treatment of respiratory tract infections, urinary tract infections, intestinal infections, and other diseases. Therefore, it is important to develop rapid, simple and low cost analytical methods for the simultaneous quantification of these compounds for a good quality control. A considerable number of analytical papers for determination of association in commercial formulations and biological samples have been reported in the literature, these include mainly spectrophotometric, chromatography, ion selective electrodes.

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### 1. Introduction

Trimethoprim chemically its 5-(3, 4, 5-Trimethoxybenzyl) pyrimidine -2, 4-diamine, is C<sub>14</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub>, representing a molecular weight of 290.3 g/mole. White or yellowish-white powder, very slightly soluble in water, slightly soluble in ethanol. Dihydrofolate eductase [1]. Few analytical techniques were used for determination trimethoprim which studied Concentration range, correlation coefficient, detection limit, recovery, relative standard deviation, type of column, mobile phase, flow rate,

slope, response time and the range of PH for trimethoprim or sulfamethoxazole. The results of this study were listed in Tables 1, 2, 3. In addition for tables 1, 2, 3 there were another analytical method for determination of trimethoprim such as: square wave voltammetry [3], Photo-Fenton Oxidation Technology [4], charge transfer complexes formation [5].

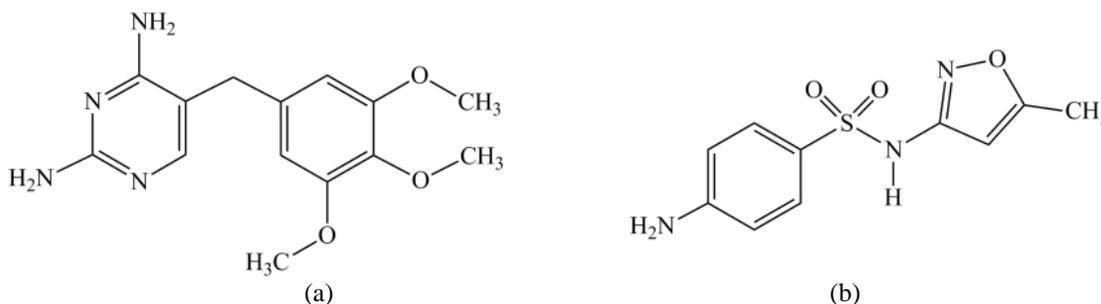


Figure 1: Chemical structures of (a) trimethoprim (b) sulfamethoxazole [2]

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Table 1: Spectrophotometric methods for determination of Trimethoprim

Name of Method	Results	Ref. No
Extractive spectrophotometric	Sample : trimethoprim $\lambda_{\max} = 422,418 \text{ nm}$ Concentration Range= 4.0-24 and 5.0-25, $\mu\text{g/ml}$ $r^2=0.9969,0.9973$ %Re=99.95,99.93 Slope=0.000391,0.000572 %RSD=1.600,0.8593	[6]
Simultaneous spectrophotometric	Sample : trimethoprim–sulphamethoxazole $\lambda_{\max} = 520,440 \text{ nm}$ Beer's law limits= 7.5–60 ,2–10 $\mu\text{g/ml}$ Limit of detection= 2.69 ,0.589 $\mu\text{g/ml}$ Slope= $0.0048 \pm 7.5 \times 10^{-5}$ , $0.024 \pm 2.627 \times 10^{-3}$ $r^2= 0.9989, 0.9992$	[7]
Spectrophotometric	Sample: Sulfamethoxazole , trimethoprim $\lambda_{\max} = 237, 257 , 288 \text{ nm}$ Concentration Range=2.0-11.0 , 3.0-18.0 $\mu\text{g/ml}$	[8]
First-Order Derivative Spectroscopy	Sample : trimethoprim, sulphamethoxazole $\lambda_{\max} = 247.8 , 257.9 \text{ nm}$ Concentration Range= 10-50 ,250-350 $\mu\text{g/ml}$	[2]
UV-VIS Spectrophotometric-Colorimetric	Sample : trimethoprim Concentration Range= 1–35 ,20–90 $\mu\text{g/ml}$ $r^2= 0.9976, 0.9981$ LOD= 2.72 4.90 $\mu\text{g/ml}$ % Average recovery= $99.07 \pm 1.46 , 99.86 \pm 2.35$	[9]
Spectrophotometric	Sample sulphamethoxazole $\lambda_{\max} = 460 \text{ nm}$ Concentration Range= 5-50 $\mu\text{g/ml}$ Molar absorptivity= $6.7878 \times 10^4 - 7.0918 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$ Limit of detection= 0.3755– 0.3594 $\mu\text{g/ml}$	[10]
UV Spectrophotometry	Sample : trimethoprim–sulphamethoxazole $\lambda_{\max} = 265 , 289 \text{ nm}$ Concentration Range=2-9 , 9.08-41 $\mu\text{g/ml}$ %Recovery= 98.20-99.25 %RSD=0.440 , 0.569	[11]
First-order derivative spectrophotometric	Sample : trimethoprim–sulphamethoxazole $\lambda_{\max} = 247.8 , 257.9 \text{ nm}$ %RSD= 2.08 ,1.79 R=0.998	[12]
Spectrofluorometry	Sample : trimethoprim–sulphamethoxazole $\lambda_{\max} = 295 , 330 \text{ nm}$ Concentration Range= 0.5 to 40,1 -400 $\mu\text{g/ml}$ R=>0.99	[13]
Derivative Spectrophotometry	Sample : trimethoprim–sulphamethoxazole $\lambda_{\max} = 257.8, 251.5 \text{ nm}$ R=0.9992,0.9995 Concentration Range=2.00 to 25.00 $\text{mg/ml}$ LOD=0.36 ,0.382 $\text{mg/ml}$ %Re= 97.23, 102.13	[14]

Table 2: HPLC for determination of Trimethoprim

Method	Column	Mobil Phase	Elution	Flow Rate	Results	t <sub>R</sub>	λ(nm)	Ref.
Simultaneous HPLC	C18 column (5m, 150, 4.6 mm)	acetonitrile, 0.5% triethylamine in 1% acetic acid, pH 3	(18:82, v/v)	1.5 ml/min	Conc. Range=35-101, 102-306 µg/ml, R= 0.9980 , 0.9998 %Re= 99.7±0.92 %RSD=0.05-8.94	3.2, 16 min	271	[15]
HPLC-DAD	RP-18	32 ml methanol-dichloromethane	70:30, v/v)	1 ml/min	Conc. Range= 22.0-54.0 , 21.3 - 32.7 ng/L	-	-	[16]
Liquid Chromatography	C18	0.1% formic acid in water and acetonitrile	isocratic elution mode		Conc. Range= 5–250 ng·g <sup>-1</sup> LOD= 1 ng·g <sup>-1</sup> %R = 0.9984			[17]
RP-LC	C18 (150 x 4.6 mm, 5 mm).	water, pH 3.5, and methanol	(60:40, v/v)	1.0 ml/ min	Conc. Range= 5 – 70, 1 -30 mg mL <sup>-1</sup> µg/ml, R≥ 0.99	-	213 and 230	[18]
HPLC	-	Phosphate buffer 0.1 M, acetonitrile and methanol	(65:20:15)	1.0 ml/ min	Conc. Range= 0.25 – 5, 5-100 µg/ml R= 0.9998, 0.9996 %Re= 92.23, 96.85 DOL=0.2, 3 µg/ml	-	225	[19]
RP-HPLC	C18 (250mm x 4.6 mm, 5 µm).	Triethylamine:Acetonitrile	(30:70)	1.0 ml/ min	Re=100.23, 99.30 %RSD=0.6, 0.4	2.688, 4.388	260	[20]
High Performance Liquid Chromatography	C18 analytical column (stainless steel, 25 cm, 4.6 mm)	0.025 M sodium phosphate as aqueous phase , acetonitrile , 0.4% triethylamine as organic phase	(80/20)	1.2 ml/ min	R= 0.986, 0.949	-	260	[21]
HPLC	C18 (250 mm X 4.6 mm X 5µm particle size)	20% 0.05 M potassium dihydrogen orthophosphate, pH 3.5–80% acetonitrile	-	1.5 ml/ min	Conc. Range= 1-18, 1-16 µg/ml R= 0.9998, 0.9996 %Re=100.02±0.869, 100.51 ± 0.978	-	225	[22]
RP-HPLC	C18 Phenomenex	acetonitrile: potassium dihydrogen orthophosphate buffer, pH adjusted to 3.8 using orthophosphoric acid	(20: 80)	1 ml/ min	Conc. Range= 2-10, 1-5 µg/ml R= 0.999, LOD= 0.0024, 0.0009 µg/ml and 0.0072 µg/ml , 0.0028 µg/ml	5.2 , 2.4	248	[23]
RP-HPLC	C18 (4.6, 150 mm, 4 m)	30 mM sodium phosphate (pH 5.8), acetonitrile, and 0.05% triethylamine	(83:17:0.05 ,) v/v	1.2 ml/ min	Conc. Range= 0.10-6.0 , 1.0-70 µg/ml, R= 0.999, LOD= 0.0024, 0.0009 µg/ml and 0.0072 µg/ml , 0.0028 µg/ml		235	[24]

Table 3: Ion – Selective Electrodes for Determination of Trimethoprim

Type of Ion –pair for Electrodes	Slope mV/decade	Conc. Range	Detection Limit	R	Response Time	PH	Life Time	Ref.
Trimethoprim- Molybdophosphoric acid	58.61, 58.41, 15.30	1.2×10 <sup>-5</sup> -1.0×10 <sup>-2</sup> and 6.1×10 <sup>-5</sup> -1.0×10 <sup>-2</sup> and 2.5×10 <sup>-5</sup> -1.0×10 <sup>-2</sup> mole/L	2.5×10 <sup>-6</sup> , 6.3×10 <sup>-6</sup> , 5.9×10 <sup>-6</sup> mole/L	0.9996, 0.9996, 0.9997	-	3.0-8.0, 3.0-6.5 7.0-8.5	45, 1, 2 days	[25]
Trimethoprimium-phosphomolybdata	56.5	2.3 × 10 <sup>-6</sup> –10 <sup>-2</sup> mole/L	-	0.00091	-	1.6–6.2	24 hours	[26]
Trimethoprim- methyl orange	57.31, 42.00	6.0×10 <sup>-6</sup> –1.0×10 <sup>-2</sup>	3.0×10 <sup>-6</sup>	0.9903, 0.9990	-	2.0-5.5, 1.5-4.5, 3.0-4.5 and, 4.5-5.2, 3.0-4.0, 1.0-3.5	27, 3 days	[27]

## 2. Conclusion

In this review, a theoretical study of more analytical technique used for evaluation trimethoprim were listed in Table 1, 2 and 3. From these tables founded the best method was high performance liquid chromatography, also according to the United States Pharmacopeia (USP) [3], the official method for the simultaneous analysis of sulfamethoxazole and trimethoprim in pharmaceutical formulations implicates to use High Performance Liquid Chromatography (HPLC). This method was gave a wide concentration range determined in ng/ml and µg/ml, with low detection limit and the most solvent used were acetonitrile and methanol. In the other hand, spectrophotometric and ion selective electrodes were successfully applied to the determination of trimethoprim and sulfamethoxazole in tablet dosage forms with accuracies comparable to the official BP method. These methods were very easy to use and easy to apply and besides very cheap to quality control and routine analysis of two active veterinary compounds in commercial samples.

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