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Spectrofluorimetric determination of Trimethoprim in pharmaceutical preparations

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Abstract: The development of a spectrofluorimetric method for the determination of trimethoprim according to the reaction between O-phthalaldehyde (OPA) in highly alkaline media, and 2-mercaptoethanol (2ME) and this reaction gives fluorescent product measured at (458) nm when excited at (342) nm. The optimization of the analytical parameters that influence intensity was investigated. The intensity of fluorescence of the formed product was linearly related to the concentration of trimethoprim in the (100-1200) ng mL^{-1} range. The limit of detection and limit of quantification were estimated to be (22.54) ng mL^{-1} and (75.15) ng mL^{-1} respectively. The utility of the proposed methods was successfully verified by analysis of trimethoprim in pure and real pharmaceutical preparations with high accuracy, the recovery percentages Re%, were found to be (100.5) % and (99.76) % for pure drug and pharmaceutical preparations respectively.

Key words: trimethoprim, O-phthalaldehyde reagent and spectrofluorimetric

1. Introduction

Trimethoprim (TMP)[1] (2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine is an antibiotic used to treat and prevent urinary tract infections and treat ear infections caused by certain bacteria.[2] It is also used in combination with sulfamethoxazole to treat several infections, including those of the gastrointestinal tract, respiratory tract, and urinary tract. The chemical formula of TMP is $C_{14}H_{18}N_4O_3$ molar mass 290.32 g mol^{-1}.[3,4] (*Fig.* 1) shows the structural formula of TMP.

The determination of TMP in pharmaceutical preparations including spectrophotometric methods,[5,6] spectrofluorometric methods,[7] liquid chromatography,[8] HPLC,[9,10] Micellar electrokinetic capillary chromatography,[11] voltammetry,[12] Continuous wavelet transforms,[13] Electrochemical[14] and ion-selective electrode (ISE).[15] This study used the spectrofluorometric method for the determination of trimethoprim according to the reaction with O-phthalaldehyde and 2-mercaptoethanol in alkaline media to form a labeling fluorescent product at λ_{exc} = 342 nm and $\lambda_{em.}$= 458 nm. In comparison to several other methods, which require costly hazardous solvents and sample pretreatment, this method is characterized by simplicity, rapidity, and reproducibility, allowing them to be used in routine analysis of trimethoprim in pharmaceutical formulations.

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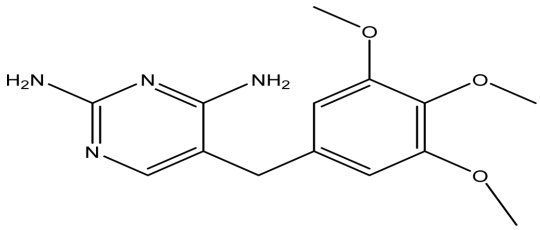

Fig. 1. Structural formula of TMP.

The limitations of this study are the inability to estimate trimethoprim in the presence of other primary amino compounds since those compounds may produce fluorescent chemical compounds that interfere with the fluorescence spectrum of OPA-2ME-TMP fluorophore. In this case, a method must be followed to resolve this overlap in order to the determination of trimethoprim, such as the H-point standard addition method, derivative spectrophotometric method, etc. Also, this reaction cannot be carried out in acidic or neutral media because OPA reacts with 2ME and TMP to form a stable isoindole ring only in alkaline media (pH 8-12).

2. Experimental

2.1. Apparatus

Fluorescence spectra were collected by using a Shimadzu (RF-5301 PC, Kyoto, Japan) equipped with a 150 W xenon lamp and a photomultiplier tube. Slit width set at 5 nm for both excitation and emission monochromatic. All pH measurements were made with a digital pH meter (in-lab pH 720, Germany). Sensitive digital balance (BP 3015, Sartorius Germany).

2.2. Chemicals and reagents

A stock solution of Trimethoprim (TMP) (100 μg·mL^{-1}) was prepared by dissolving 10 mg of TMP in 100 mL of distilled water. The working standard solutions were prepared by suitable dilution with distilled water to the appropriate concentration. O-phthalaldehyde (OPA) (0.1 w/v%) was prepared by dissolving (100) mg of (OPA) in 100 mL of methanol. 2-mercaptoethanol (2ME) (1 v/v%) was prepared by pipetting 1 mL of 2-mercaptoethanol into 100 mL volumetric flask and completing the volume with methanol. Boric acid (0.2 mol·L^{-1}) was prepared by accurately weighed of 1.237 g, and dissolved in 100 mL of distilled water. Borate buffer solution was prepared from 0.2 mol·L^{-1} boric acid and 1.00 mol·L^{-1} sodium hydroxide. The sodium hydroxide solution was added drop by drop to the boric acid solution, with stirring, until a pH of (9.4) was reached.

2.3. Recommended procedure

Aliquots of TMP working standard solutions were quantitatively transferred using a micropipette into sets of 10 mL volumetric flasks to prepare a TMP concentration within the range of (100-1200 ng mL^{-1}). Then, 2 mL of 0.2 molq·L^{-1} borate buffer solution at (pH = 9.8) together with 1 mL of 1 %v/v 2-ME were added for each flask and mixed well. The reaction mixture was allowed to stand for 4 min. Thereafter, 2.5 mL of 0.1 % w/v OPA was added and mixed gently. The reaction mixture was left again for 40 min at room temperature. The solution in each flask was made up to the mark by methanol solvent. The intensity of the resulting solution is measured at λ_{em} 458 nm when excited at λ_{exc} 342 nm.

3. Results and Discussion

The O-Phthalaldehyde (OPA) is a most popular fluorogenic agent that is widely used for the detection of a nanogram of compounds with primary amine moiety. Primary amines such as TMP form a highly fluorescent compound when they react with OPA and a thiol compound (like 2-mercaptoethanol) through condensation reaction under basic condition. The reaction is essentially complete within 5.0-40.0 minutes. The reaction product intensity is attributed to the formation of the isoindole group which triggers the compound to give fluorescence intensity as shown in (*Fig.* 2). The fluorescence spectrum of OPA-2ME-TMP fluorophores using drugs concentration 300 ng mL^{-1} was shown in (*Fig.* 3). The formed fluorophores exhibit an excitation wavelength of 342nm with emission wavelengths of 458 nm.

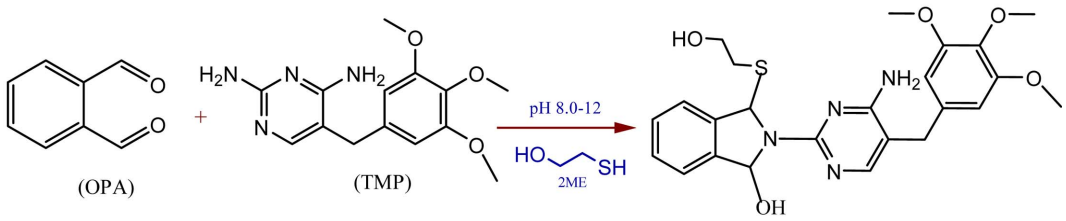

Fig. 2. The suggested reaction between TMP and OPA reagent.

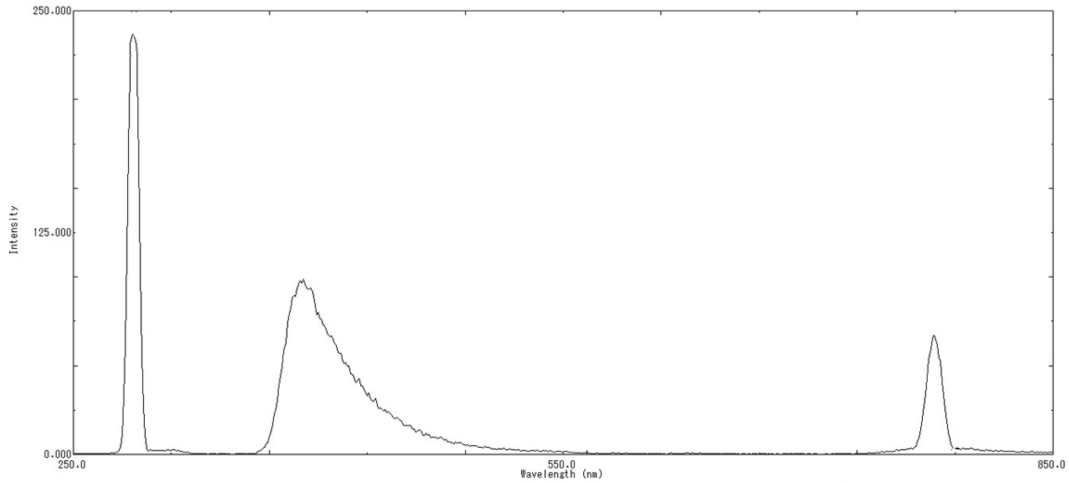

Fig. 3. Fluorescence spectrum of OPA-2ME-TMP fluorophores.

3.1. Optimization of conditions

The experimental parameters of the proposed method that effect on fluorescence intensity of OPA-2ME-TMP fluorophore were estimated and optimized.

3.1.1. Effect of pH and buffer's volume

To investigate the impact of PH value on the formation of the fluorescent compound, a series of buffer solutions were used in the pH range (6.5-11.5). The fluorescent intensity of the formed OPA-2ME-TMP fluorophore increases as a function of solution pH until it reaches a maximum value at pH= 10.0 and no longer increases. Otherwise, it decreased gradually due to may be of background compound formation. The influence of borate buffer volume in the range of (0.5-4.0 mL) on the fluorescent intensity of the formed fluorophore was studied at (pH = 9.8), the maximum fluorescent intensity was reached at 2 mL which was selected as the optimal buffer volume. As observed in (*Fig.* 4(a)).

3.1.2. Effect of OPA volume

The effect of different volumes of OPA reagent on the formation of the fluorescent complex has been studied. *Fig.* 4(b) shows that (2.5) mL of 0.1 % w/v OPA was optimized and selected for subsequent experiments.

3.1.3. Effect of 2-mercaptoethanol volume

Different volumes of 2-ME 0.5 % w/v within the range (0.1-2.0 mL) were used. Hence, 1 mL of 2-mercaptoethanol was selected as the optimal volume as shown in (*Fig.* 4(c)).

3.1.4. Effect of time after addition of 2-mercaptoethanol

The effect of time after the addition 2-mercaptoethanol on the fluorescence intensity of the fluorescent compound was tested. As shown in (*Fig.* 4(c)). Five minutes was selected as the optimal time to formation of thiolate ion.

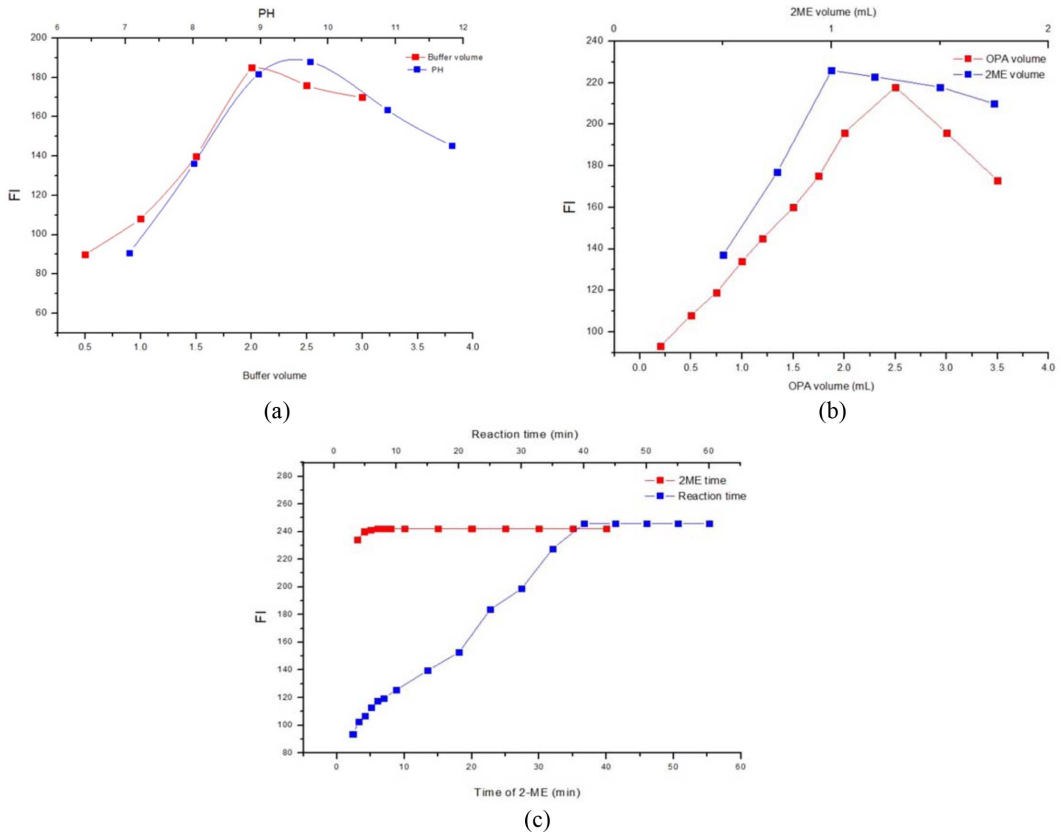

Fig. 4. Effect of various experimental parameters on the fluorescence intensity of the OPA-2ME-TMP fluorophore.

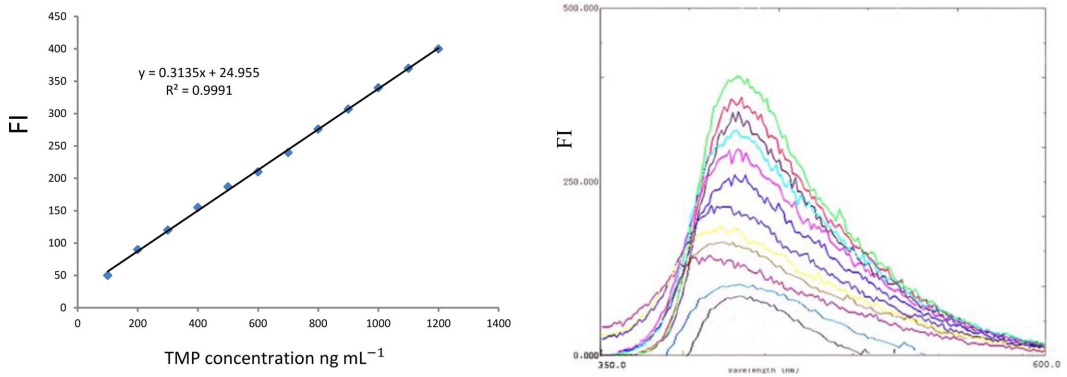

Fig. 5. Calibration curve of TMP drug.

3.1.5. Effect of reaction time

The time required for the reaction completion was investigated. The results indicated that (40) min is sufficient to complete the reaction as observed in (*Fig.* 4(c)).

3.1.6. Solvents effect

To select a more suitable solvent that obtains the highest fluorescence intensity of the formed fluorophore different solvents were tested, such as acetone, acetonitrile, methanol, butanol, ethyl acetate, DMSO, ethanol, DMF and distilled water. It was found that

the dilution of the reaction mixture with methanol gives maximum fluorescent intensity.

3.2. Method validation

3.2.1. Calibration curve

The values of the fluorescence intensity at 458 nm were plotted vs TMP concentration to obtain a calibration curve whose linear In concentration range (100-1200) ng·mL^{-1} as shown in (*Fig.* 5). The regression parameters were illustrated in *Table* 1.

3.2.2. Accuracy

The suggested approach accuracy was verified by measuring the fluorescence intensity of TMP-2ME-OPA by selecting three concentration levels within the calibration curve (500, 800, and 1000) ng·mL^{-1}) by repeating the experiment five times for each concentration. As shown in (*Table* 2). The percent recovery (Re%) was obtained by analyzing the results. The Re% values indicate that the proposed method is highly accurate.

Table 1. Analytical parameters of the suggested approach

Parameter	Suggested method
λ_{exc} (nm)	342 nm
λ_{em} (nm)	458 nm
Concentration range (ng mL^{-1})	100-1200 ng·mL^{-1}
Slope	0.3135
Determination coefficient (r^2)	0.9982
Correlation coefficient (r)	0.9991
Intercept	24.995
SD of slope	0.0122
SD of intercept	2.0570
LOD[a] (ng mL^{-1})	22.54
LOQ[b](ng mL^{-1})	75.15

LOD[a]: Limit of detection. LOQ[b]: Limit of quantitation

Table 2. Accuracy data of the suggested spectrofluorimetric method

Sample	Concentrations ng·mL^{-1}	Rec%*± SD
1	500	101.2+2.65
2	800	101.31+2.54
3	1000	98.97+3.05

*Mean of five determinations

3.2.3. Precision

Three concentrations of TMP (300, 600 and 900 ng·mL^{-1}) were prepared and analyzed to verify the precision of the suggested method. The R.S.D% of the results was less than 2 indicating acceptable precision as shown in *Table* 3.

3.2.4. Robustness

To verify the robustness of the proposed method, three experimental variables (OPA volume, pH, and 2-ME volume) were slightly altered in this study. The slight alterations in conditions of the experiment did not effect on the determination of TMP by suggested method. As shown in *Table* 4.

3.3. Application

Trimethoprim has been successfully estimated by the proposed method in its pharmaceutical tablets as shown in *Table* 5. The results of the suggested method have been statistically compared with those obtained

Table 3. Precision of the proposed method

Concentrations ng·mL^{-1}	Intra-day precision		Inter-day precision	
	Rec%*	R.S.D %	Rec%*	R.S.D %
300	101.76	1.89	101.43	1.79
600	101.03	1.78	100.95	1.34
900	100.95	1.69	99.30	1.22

*Mean of five determinations

Table 4. Robustness evaluation for the proposed approach

Experimental parameters	Rec%* ± R.S.D.%
OPA volume (mL)	
1.8	101.67±1.750
2	101.58±1.648
2.2	101.49±1.653
Buffer PH	
9.6	99.42±1.482
9.8	101.24±1.341
10	101.75±1.527
2-ME volume (mL)	
0.9	98.97±1.78
1	101.65±0.983
1.1	101.77±1.418

*Mean of five determinations.

Table 5. Determination of TMP in different pharmaceutical preparations

Drug company	Concentration of TMP ($ng.mL^{-1}$)		R_e*%	SD	R.S.D%
	Present	Found			
Crescent	200	202.6	101.3	0.6999	0.778
	500	497.8	99.56	0.6925	0.379
	800	802.5	100.31	1.2650	0.461
Activas	200	198.2	99.1	0.5160	0.585
	500	503.5	100.7	0.8490	0.460
	800	797.5	99.68	1.2650	0.455

*Mean of five determinations

Table 6. Statistical comparison between the proposed and official method

	Proposed method	Official method[16]
Re% mean	100.39	99.8
N	5	5
t_{test}	0.452 ((2.447)	
F_{test}	0.972 (9.277)	

by the official methods. *Table* 6 shows that the t_{test} and F_{test} values of the proposed method were less than theoretical ones, which indicates that the suggested approach can be effectively used to determination of TMP in a real pharmaceutical preparation (Tablets).

The results reported by many published methods for the determination of TMP are given in *Table* 7. As can be seen, the lowest detection limit was obtained for the determination of TMP by the proposed method.

4. Conclusions

The spectrofluorimetric method has been proposed for the estimation of TMP in pure and pharmaceutical dosage forms according to the reaction of TMP with OPA in highly alkaline media in the presence of 2-mercaptoethanol and this reaction gives fluorescent product measured at (458) nm when excited at (342) nm

Table 7. The most important methods used for the determination of TMP

Method	Linear range	R.S.D %	Rec %	LOD	LOQ	Ref.
Spectrophotometric	10-60 μg mL^{-1}	1.40	99.22	-	-	5
	3-18 μg mL^{-1}	2.75	102.2	-	-	6
	10-75 μg mL^{-1}	1.70	100.76	-	-	17
	10-100 μg mL^{-1}	0.75	100	0.028 μg mL^{-1}	-	18
	1.25-10.71 μg mL^{-1}	0.902	99.96	0.154 μg mL^{-1}	0.513 μg mL^{-1}	19
HPLC	0.5-10 μg mL^{-1}	-	70.8	15 $ng.mL^{-1}$	-	20
	0.11-690 μmol. L^{-1}	-	99.8	-	-	21
	0.05-10 μg mL^{-1}	-	97.9	25 $ng.mL^{-1}$	-	22
LC	0.1-92.4 ng. mL^{-1}	2.3	98.3	5 $ng.mL^{-1}$	13.8 μg mL^{-1}	23
	0.5-20 μg mL^{-1}	1.56	79.6	0.1 μg mL^{-1}	0.5 μg mL^{-1}	24
Electrochemical	0.4-1.1 μmol L^{-1}	0.970	97.4	0.6 $\mu mol.L^{-1}$	-	25
Ion-selective electrodes	6.0×10^{-6}-1.0×10^{-2} mol L^{-1}	0.98	96.56	3.0×10^{-6} mol L^{-1}	-	26
	2.3×10^{-6}-10^{-2} mol L^{-1}	-	99.8	-	-	27
Charge-Transfer	0.2-0.4 μg mL^{-1}	0.7407	-	0.0104 μg mL^{-1}	0.0316 μg mL^{-1}	28
Flow injection	10-70 μg mL^{-1}	1.40	99.7	0.6 μg mL^{-1}	2.0 μg mL^{-1}	29
	0.5-5 μg mL^{-1}	-	104	0.008 μg mL^{-1}	-	30
	25-150 μg mL^{-1}	1.1	101.4	-	-	31
Spectrofluorimetric	100-1200 $ng.mL^{-1}$	1.45	100.5	22.54 $ng.mL^{-1}$	75.15 $ng.mL^{-1}$	This work

within a concentration range (100-1200) ng.mL^{-1} and low detection limit (22.54) ng·mL^{-1}.

The proposed method can determine the unknown concentration of analyte giving a heightened precision and accuracy, where the value of R.S.D% is less than 2 % and the recovery percentage is equal to 100.5 % and 99.76 % for the determination of TMP in pure and real pharmaceutical formulations respectively.

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References

1. J. H. Darrell, L. P. Garrod, and P. M. Waterworth, *J. Clin. Pathol.,* **21**, 202-209 (1968). http://dx.doi.org/10.1136/jcp.21.2.202
2. S. G. B. Amyes and J. T. Smith., *Biochem. Biophy. Res. Commun.*, **58**(2), 412-418 (1978). https://doi.org/10.1016/0006-291X(74)90380-5
3. M. A. Perazella, *Drug Safety,* **22**, 227-236 (2000). https://doi.org/10.2165/00002018-200022030-00006
4. R. Gleckman, M. S. Alvarez, and D. W. Joubert, *Am. J. Hosp. Pharm.*, **36**(7), 893-906 (1979). https://doi.org/10.1093/ajhp/36.7.893
5. S. Z. Qureshi, M. I. H. Helaleh, N. Rahman, and R. M. A. Q. Jamhour, *Fresenius J. Anal. Chem.,* **357**, 1005-1007 (1997). https://doi.org/10.1007/s002160050293
6. Ş. E. Hişmioğulları and E. Yarsan, *Hacettepe University Journal of the Faculty of Pharmacy*, **Year 2009**(2), 95-104 (2009).
7. D. M. Lichtenwalner, B. Suh, B. Lorber, and A. M. Sugar, *Antimicrob. Agents Chemother.*, **16**(5), 579-83 (1979). https://doi.org/10.1128/aac.16.5.579
8. C. Akay and S. A. Ozkan, *J. Pharm. Biomed. Anal.*, **30**(4), 1207-1213 (2002). https://doi.org/10.1016/s0731-7085(02)00460-0
9. E. Sayar, S. Sahin, S. Cevheroglu, and A. A. Hincal, *Eur. J. Drug Metab. Pharmacokinet*, **35**, 41-46 (2010). https://doi.org/10.1007/s13318-010-0006-9
10. R. W. Bury and M. L. Mashford, *J. Chromatogr.*, **163**, 114-117 (1979). https://doi.org/10.1016/S0378-4347(00)81178-0
11. J. J. Berzas, G. C. Peñalvo, and F. J. Guzmán Bernardo, *J. Sep. Sci.*, **28**(6), 543-548 (2005). https://doi.org/10.1002/jssc.200400098
12. G. N. Calaca, C. A. Pessoa, K. Wohnrath, and N. Nagata, *Int. J. Pharm. Pharm. Sci.*, **6**(9), 438-442 (2014).
13. E. Dinç, Y. Kadioğlu, F. Demirkaya, and D. Baleanu, *J. Iran. Chem. Soc.*, **8**, 90-99 (2011). https://doi.org/10.1007/BF03246205
14. L. G. Chatten, B. S. Pons, and P. Mcleod, *Analyst*, **107**, 1026-1031 (1982). https://doi.org/10.1039/an9820701026
15. M Aboudan, Y M. Issa, and A F. Shoukry, *J. Chem. Tech. Biotechnol.*, **61**(1), 31-35 (1994). https://doi.org/10.1002/JCTB.280610105
16. The British Pharmacopoeia, Stationary Office, Appendix 1D, Buckingham Palace Road, London, UK, 2016.
17. T. Al-Sabha and I. A. Hamody, *Journal of Education and Science*, **24**(2), 1-11 (1999). https://doi.org/10.33899/edusj.1999.58728
18. M. R. Raauf, *Tikrit J. Pharm. Sci.*, **8**(2), 217-228 (2023). https://doi.org/10.25130/tjphs.2012.8.2.10.217.228
19. P. Nagaraja,; A. Shrestha, A. Shivakumar, and A. K. Gowda, *J. Food Drug Anal.*, **18**(4), 239-248 (2010). https://doi.org/10.38212/2224-6614.2244
20. O. Spreux-Varoquaux, J. P. Chapalain, P. Cordonnier, C. Advenier , M. Pays, and L. Lamine, *J. Chromatogr. B Biomed. Sci. Appl.*, **274**, 187-199 (1983) https://doi.org/10.1016/s0378-4347(00)84422-9
21. K. Hoppu and P. Arjomaa, *Clin. Chim. Acta*, **163**(1), 81-86 (1987). https://doi.org/10.1016/0009-8981(87)90036-2
22. M. J. B. Mengelers, M. B. M. Oorsprong, H. A. Kuiper, M. M. Aerts, E. R. van Gogh, and A. S. J. P. A. M. van Miert, *J. Pharm. Biomed. Anal.*, **7**(12), 1765-1776 (1989). https://doi.org/10.1016/0731-7085(89)80192-X
23. G. C. Batzias, N. A. Botsoglou, V. P. Kotsaki-Kovatsi, and G. Kounenis, *J. Chromatogr. B*, **769**, 253-259 (2002). https://doi.org/10.1016/S1570-0232(01)00625-0
24. M. W. Hruska and R. F. Frye, *J. Chromatogr. B*, **807**(2), 301-305 (2004). https://doi.org/10.1016/j.jchromb.2004.04.021
25. T. T. Guaraldo, L. A. Goulart, F. C. Moraes, and M. R. V. Lanza, *Appl. Surf. Sci.*, **470**, 555-564 (2019). https://doi.org/10.1016/j.apsusc.2018.09.226
26. S. A. Abrahem, A. M. Abass, and A. Ahmed, *Asian J.*

Pharm. Clin. Res., **12**(6), 83-87 (2019). https://doi.org/10.22159/ajpcr.2019.v12i6.32959
27. A. Mahmoud, M. I. Yousry, and F. S. Adel, *J. Chem. Tech. Biotechnol.*, **61**, 31-35 (1994), https://doi.org/10.1002/jctb.280610105
28. A. Bagheri and S. R. Khomami, *J. Appl. Chem. Res.*, **7**(3), 25-33 (2013). https://dorl.net/dor/20.1001.1.20083815.2013.7.3.3.0
29. M. L. Fernández, P. O. Barrales, G. R. Torné and A. M. Díaz, *J. Pharm. Biomed. Anal.*, **31**(4), 669-677 (2003). https://doi.org/10.1016/S0731-7085(02)00685-4
30. A. Waseem, M. Yaqoob, and A. Nabi, *J. Chem. Soc. Pakistan*, **30**, 703-707 (2008). https://doi.org/10.4172/pharmaceutical-sciences.1000243
31. W. Z. Liu and J. Q. Gao, *Acta Pharmacol. Sin.*, **26**(9), 710-713 (1991).

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