



Development and Validation of UV-Spectrophotometric Method for Estimation of Doxofylline in Bulk and Tablets

SUBRATA PAUL¹, MD. ABDUL KARIM MIA², SABARNI SARKER³, SHETA BISWAS⁴,
PUJA BAL⁵, TRISHA RANI DEY³ and TARUN KUMAR PAL^{6*}

¹Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh.

²Faculty of Medicine, University of Dhaka, Dhaka-1000, Bangladesh.

³Department of Pharmacy, Faculty of Life and Earth Science, Jagannath University, Dhaka-1100, Bangladesh.

⁴Department of Biochemistry and Molecular Biology, Noakhali Science and Technology University, Noakhali-3814, Bangladesh.

⁵Department of Pharmacy, Faculty of Allied Health Science, Daffodil International University, Dhaka-1207, Bangladesh.

⁶Department of Chemistry, Rajshahi University of Engineering & Technology, Rajshahi-6204, Bangladesh.

*Corresponding author E-mail: (tkpchem@gmail.com)

<http://dx.doi.org/10.13005/ojc/360516>

(Received: August 05, 2020; Accepted: September 10, 2020)

ABSTRACT

Doxofylline is a xanthine derivative and it has its application as a bronchodilator in different pulmonary conditions like asthma and COPD (chronic obstructive pulmonary disease). The research was conducted with the objective to develop and validate a simple, easy, rapid and cost-effective UV-Spectrophotometric method to estimate the amount of doxofylline in bulk and tablets. The validating parameters and methodology were selected and performed according to ICH and USP guidelines. In the accuracy study, the mean % recovery was within the limit (100.20%) with %RSD 0.77. The mean of % assay and %RSD of intra-assay precision study was found 99.81 and 0.79 respectively with only 0.57% variation between two analysts in intermediate precision study. The linearity study demonstrated the value of the correlation coefficient is 0.9999 whereas the robustness studies showed a slight but negligible variation of absorbance while changing different operating parameters. In system suitability study, the %RSD was found less than 2.00%. According to the specificity study, there were no placebo and diluent effect on the absorbance measurements.

Keywords: Method Validation, Doxofylline, UV-Spectrophotometer, Accuracy, Linearity.

INTRODUCTION

The number of patients with respiratory

diseases such as COPD and asthma is increasing day by day. These two diseases are considered as major risk factors for other respiratory diseases



like severe acute respiratory syndrome and novel coronavirus diseases^{1,2}. In COPD, consistent respiratory problems result in shortness of breath and other problems limiting the airflow in the lungs are observed^{3,4}. Persistent symptoms do not require hospitalizations but an exacerbation of the disease signatures often occurs affecting patients severely leading to poor health and high expenses^{5,2}. At the same time, asthma is very much prevalent worldwide and symptoms may vary from people to people, ranging from wheezing, shortness of breath to severe life-threatening asthma attacks^{6,7}.

Both COPD and asthma can be treated by specific strategies with multiple modes of drugs, including a bronchodilator or an anti-inflammatory drug along the way^{2,8}. Theophylline, an anti-inflammatory and immunomodulatory drug with small bronchodilatory action, had been used since the 1930s against pulmonary diseases^{9,10}. In the recent guidelines of treating asthma and COPD, theophylline still has a place although having a narrow therapeutic window, lack of selective actions and incompatibility with multiple drugs^{11,12,4}. Due to those pharmacodynamic disadvantages, new derivatives of theophylline with more selectivity and fewer side-effects had been discovered such as bamifylline, acebrofylline, doxofylline^{13,14}.

Doxofylline or 7-(1,3-dioxolan-2-ylmethyl)-3,7-dihydro-1,3-dimethyl-1H-purine-2,6-dione (Fig. 1) is a xanthine derivative in which 1 and 3 position of the purine base is replaced by two methyl groups similar to theophylline. However, it shows less side-effect profile than theophylline due to its less affinity towards adenosine A₁ and A₂ receptors. It does not interact with calcium channel blockers as it does not obstruct Ca²⁺ entry into cells¹⁵⁻¹⁷. The capability of the drug of being both bronchodilator and anti-inflammatory agent bearing the larger therapeutic window with having lesser side-effects infers the drugs more randomized clinical trials in the future¹⁷. Doxofylline is used and produced in different countries in the world, such as India and China both single as well as in combination with another drug¹⁸.

The repeatability of analytical techniques can be guaranteed by the analytical method validation technique. For orphan and investigational drugs, more and more method validation studies optimize the information regarding accuracy,

precision, linearity, robustness etc¹⁹. Literature survey indicated that several studies had been done regarding the RP-HPLC analytical method validation of doxofylline^{20,21,18}. On the other hand, few studies were done to perform the UV-spectrophotometric analysis of the drug. Among them, studies were successfully done previously to quantitatively analyze and test the photo-stability of doxofylline from bulk, pharmaceutical formulations and tablets^{22,23,24}. From the literature, it was clear that spectrophotometric methods can offer a suitable and cost-effective method for routine analysis of doxofylline. In the current study, UV-spectrophotometric method for the determination of doxofylline from bulk and marketed tablets was validated according to the ICH and USP guidelines^{25,26}.

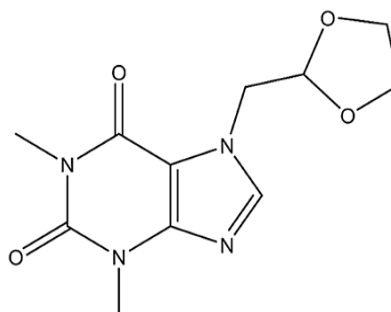


Fig. 1. Chemical Structure of doxofylline

MATERIALS AND METHODS

Instrumentation and reagents

A calibrated UV-Visible spectrophotometer (UV-3600i Plus, Shimadzu, Japan) run with Lab Solutions software was used for the development and validation of the method. Doxofylline 200 mg tablet and doxofylline standard (Potency 99.40%) were gifted by Drug International Limited, Bangladesh. Sodium hydroxide, potassium dihydrogen phosphate, orthophosphoric acid were purchased from Sigma Aldrich (Germany). Double distilled and deionized water was prepared in the laboratory.

Preparation of diluent

Approximately 2.446 g of sodium hydroxide and 1.575 g of potassium dihydrogen phosphate were taken in a 1000 mL volumetric flask and then water was added to fill the volume. The pH was adjusted to 7.6 with dilute orthophosphoric acid or sodium hydroxide as per requirement.

Preparation of standard 1 and standard 2

20 mg of doxofylline standard was weighed

and poured into a 100 mL volumetric flask and diluent was added to make the volume about 70 mL. It was sonicated for 15 min and cooled down the solution afterwards. Further, the entire volume was filled up with the same solvent. 5 mL of this solution was poured into another volumetric flask to make the final volume 50 mL by adding the same solvent. So, the concentration of the solution became 0.02 mg/mL. For the preparation of standard 2, the process was repeated and the similarity factor was calculated using the following equation:

$$\frac{\text{Absorbance of Standard 1} \times \text{Weight of Standard 2}}{\text{Weight of Standard 1} \times \text{Absorbance of Standard 2}}$$

Sample preparation

20 tablets were weighed and milled into fine powders. After that, accurately weighed 350 mg powder (equivalent to 200 mg doxofylline) was taken into a 100 mL volumetric flask. Then 70 mL diluent was mixed and sonicated for 15 minutes. Subsequently, the solution was cooled down and diluent was added up to the mark. After that, the resulting solution was centrifuged at 3000 RPM for 20 minutes. From this, 1 mL was poured into a 100 mL volumetric flask and the required volume was filled by adding the same solvent to attain a final concentration of 0.02 mg/mL. Then the absorbance of standard and sample solution was taken with UV-Spectrophotometer at 272 nm. Then the amount of doxofylline/unit dose was determined with the following equation:

$$\frac{\text{Abs. of sample} \times \text{Std. wt (mg)} \times 5 \times 100 \times 100 \times \text{Std. potency (\%)} \text{ as it is} \times \text{Avg. wt (mg)}}{\text{Abs. of Std} \times 100 \times 50 \times 1 \times \text{Sample wt. (mg)} \times 100}$$

Validation parameters

Accuracy

Accuracy was determined by calculating the % recovery according to ICH guidelines²⁵. It was done by adding a known amount of standard above and below the label claim i.e. 80%, 100% & 120%. Accurately weighed 160 mg of doxofylline and 190 mg of placebo for 80%, 200 mg of the drug and 150 mg of placebo for 100% and 240 mg of the drug and 110 mg of placebo for 120% in a 100 mL volumetric flask. After that, 70 mL of diluent was mixed and placed in an ultrasonic bath for 15 min the solution was cooled down, the required volume was filled with diluent and it was centrifuged at 3000 RPM for 20 minutes. Furthermore, 1 mL of the solution was poured into another 100 mL volumetric flask and the volume was filled with the required amount of diluent.

The percent of recovery was calculated using the following equation. For each level, the experiment was carried out three times.

$$\frac{\text{Absorbance of recovery sample} \times \text{Weight of standard (mg)} \times \text{Sample dilution}}{\text{Absorbance of standard} \times \text{Standard dilution}}$$

Precision

Precision studies were carried out through intra-assay and intermediate precision according to ICH guidelines^{25,27}. Intra-assay precision, also termed as repeatability, demonstrates how precise a process is after a small interval involving similar operating conditions in a specific laboratory. In this precision study, same operator, same analytical machines in a same day was involved. On the other hand, in the intermediate precision study, same sample within different days, different personnel and equipment's were involved and the contrasting results were checked. For intra-assay and intermediate precision, six experiments were done and %RSD was calculated to assess the precision of the method.

Linearity

Linearity studies were performed and linearity graph was plotted according to USP guidelines²⁶. At first, incremental concentrations of samples were prepared in the working range. Linearity samples were prepared at concentrations 50%, 80%, 100%, 120% and 150% of the theoretical claim of a single unit. To prepare the mother stock solution, accurately weighed 20 mg of doxofylline was taken in a 100 mL volumetric flask and required amount of diluent was added. The concentration of this solution was 0.2 mg/mL. For 50%, 80%, 100%, 120% and 150% sample, 2.5 mL, 4 mL, 5 mL, 6 mL, 7.5 mL of the stock solution were taken respectively into a 50 mL volumetric flask and required diluent was added. The concentrations of the sample solutions became 0.010 mg/mL, 0.016 mg/mL, 0.020 mg/mL, 0.024 mg/mL, 0.030 mg/mL respectively. The absorbance of samples was taken as per assay method and a graph of doxofylline concentration versus the corresponding absorbance was plotted.

Robustness

Robustness indicates the analytical method's capacity to produce similar results if some intentional and experimental changes are made in the parameters²⁹. The study was carried out by checking result variations in the wavelength range of 272 nm \pm 2 nm, carrying out filtration step with filter

and without filter paper, running samples with and without centrifuge and a pH variation of 7.6 ± 0.1 .

System suitability study

System suitability is an integral and initial stage of method validation and used to verify reproducibility of the method. It is generally performed to ensure the performance of the instrument and to make sure that the system is ready for starting the analysis³⁰. In this study five replicates of the standard at 100% level were prepared to check the system suitability.

Specificity

The capacity of the method to determine the exact concentration of active ingredients in the presence of excipients and impurities is termed as the specificity of an analytical method²⁸. For the preparation of the placebo solution, 150 mg of placebo was used. The procedures mentioned in sample preparation were followed before it was transferred into a 100 mL volumetric flask. The specificity of the method was determined by checking and comparing the absorbance pattern of standard solution, sample

solution and placebo solution.

Stability

Analytical solutions stability was evaluated by taking and comparing the absorbance of standard and sample solution at 0 h (freshly prepared) and at 24 hours. The sample and standard solution were prepared according to the method described in sample and standard preparation section. After taking the spectrum, solutions were kept at room temperature for another 24 h and the spectrum was taken again.

RESULTS AND DISCUSSION

Accuracy

The spiked-placebo (product matrix) recovery of the analytical method was applied to determine the recovery of a known amount of standard. In this study, the accuracy determination of doxofylline was checked at three concentration levels each in triplicate. The obtained percentage of recovery was within the range of 99.10–101.11% and % RDS of 0.77, which supports the suitability of the method for routine drug analysis Table 1.

Table 1: % Recovery study for doxofylline

Level	Weight of Placebo (mg)	Amount of Std. added (mg) X	Amount Recovered (mg) Y	%Recovery (Y/X*100)	Mean	%RSD
80%	190.3	160.1	160.71	100.38	100.6	0.36
	190.5	161.6	163.19	100.98		
	190.6	160.2	160.92	100.45		
100%	150.1	200	198.3	99.15	99.16	0.029
	150.2	200.1	198.3	99.1		
	150.1	200.1	198.52	99.21		
120%	110.2	240.3	242.97	101.11	100.84	0.347
	110.4	240.4	241.94	100.64		
	110.3	240.4	242.26	100.77		
Mean (%recovery)					100.2	
%RSD					0.77	

Acceptance limit: Mean (% recovery) 98.00 % -102.00 % and %RSD must be $\leq 2.00\%$

Precision

In this experiment, both intra-assay precision (repeatability) and intermediate precision were performed. Repeatability was performed by same analyst with same instrument on same day. Intermediate precision was performed by two analysts

on a different day with different instrument, and it was found that the two results have a disparity of 0.57% (acceptance Limit: $\pm 2.00\%$). On the other hand, %RSD for Intra and intermediate precision were 0.79% and 0.27%, respectively which is well below the permissible range, $\leq 2.00\%$ Table 2 and Table 3.

Table 2: Repeatability study for doxofylline

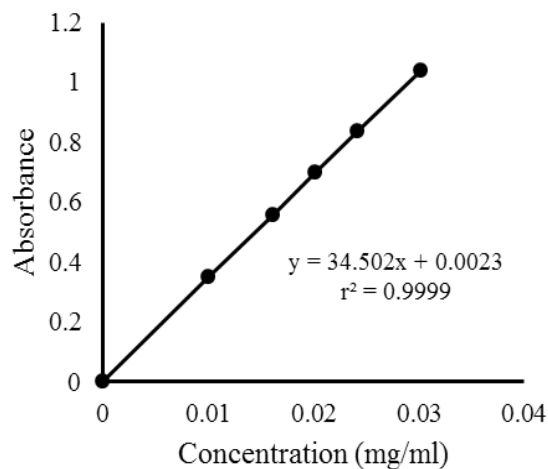
Determination No.	Weight of Sample (mg)	Weight of Std. (mg)	Result (mg/Unit Dose)	Assay (%)
1	352.2	20.01	199.43	99.71
2	352.9		200.64	100.32
3	352.5		199.93	99.96
4	352.1		196.65	98.33
5	352.1		199.87	99.93
6	352.6		201.16	100.58
			Mean of % Assay	99.81
			%RSD	0.79

Table 3: Intermediate precision study for doxofylline

Analyst 1 (Day 1)			Analyst 2 (Day 2)		
Determination No.	Weight of sample (mg)	Assay (%)	Determination No.	Weight of sample (mg)	Assay (%)
1	352.2	99.71	1	349.6	99.09
2	352.9	100.32	2	349.9	99.45
3	352.5	99.96	3	349.5	99.51
4	352.1	98.33	4	350.1	99.21
5	352.1	99.93	5	349.5	99.38
6	352.6	100.58	6	352.1	98.79
Mean		99.81			99.24
%RSD		0.79			0.27

Linearity

The linearity was evaluated in the drug concentration range of 0.010 mg/mL to 0.030 mg/mL. The line must be linear over the experimental range and the correlation coefficient of 0.9999 is generally considered as acceptable³¹. From the regression analysis, a linear equation was obtained: $y = 34.502x + 0.0023$, and r^2 was found to be 0.9999, which assures linearity between absorbance and the concentration of the analytes Fig. 2 and Table 4.

**Fig. 2. Linearity of doxofylline****Table 4: Linearity study for doxofylline**

Determination No.	Concentration (mg/ml)	Absorbance
	(X Axis)	(Y Axis)
1	0.010065	0.35046
2	0.016104	0.55805
3	0.02013	0.70016
4	0.024156	0.83775
5	0.030195	1.03971

Correlation of coefficient (r^2) = 0.9999**Robustness**

Different parameters of robustness studies and their effects on %recovery are tabulated in Table 5. Changing of pH by ± 0.1 showed no significant effect on the absorbance of the drug. The results showed that a minor change of other parameters such as change of wavelength, filtration effect and centrifugation effect did not affect much on the final results. As the changes are not significant and %RSD is $\leq 2.00\%$ so we can say that the method is robust.

Table 5: Robustness study

Parameters	% Recovery	%Target	% RSD
With filtration	99.26	100	1.64
Without filtration	101.59	100	
With Centrifuge	99.41	100	0.64
Without Centrifuge	100.32	100	
Wavelength 270 nm	97.78	100	
Wavelength 272 nm	99.26	100	0.95
Wavelength 274 nm	99.52	100	
pH 7.5	99.44	100	
pH 7.6	100.56	100	0.59
pH 7.7	99.64	100	

Table 6: System suitability study for doxofylline

Day-1			Day-2		
Determination No.	Absorbance	Similarity Factor	Determination No.	Absorbance	Similarity Factor
1	0.70011	1.00	1	0.70329	0.99
2	0.70025		2	0.70027	
3	0.69937		3	0.69967	
4	0.69966		4	0.69895	
5	0.70134		5	0.70145	
	% RSD 0.10		% RSD 0.24		

Acceptance limit: % RSD $\leq 2.00\%$, Similarity Factor: 0.98-1.02

System suitability

The results of the system suitability of the method are shown in Table 6. The %RSD found for five replicates from both day 1 and day 2 were 0.10 and 0.24 respectively, which were within the acceptance limit. Besides, for day 1 and day 2, the similarity factors of the standard solution 1 and 2 were also within the permissible range. These results suggest the suitability of the proposed validated method.

Specificity

The UV-spectrum of the drug was not changed due to the presence of excipients used in the formulation. The spectrum of standard, sample and placebo solution were taken and showed in Fig. 3. The spectrum showed that there was no effect of diluent and placebo solution during analysis. So the proposed method is selective and specific.

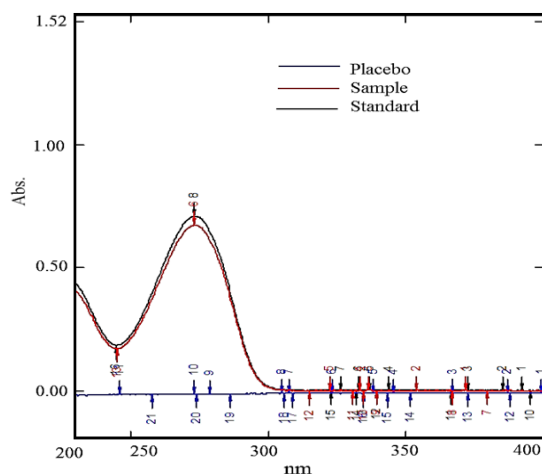


Fig. 3. UV-absorption spectra of doxofylline standard, sample and placebo

Stability study

Doxofylline standard and sample solution in diluent showed no spectrophotometric changes during 24 hours. After 24 h the spectrum of sample and standard was almost same and overlapped with the freshly prepared solutions which indicate good stability of analytical solutions in diluent Figure 4.

Assay of market formulation

Five brands of doxofylline tablets were collected and analyzed for the determination of drug content by the developed validated method. Each experiment was performed 3 times and the results are summarized in Table 7.

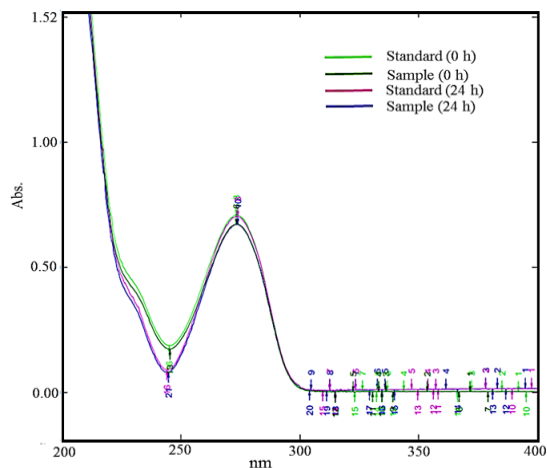


Fig. 4. Stability study of analytical solutions

Table 7: Estimation of doxofylline in commercial formulations

Brand	Labeled amount (mg)	Obtained amount (mg)	% Drug content
B-1	200	199.6	99.8
B-2	200	200.8	100.4
B-3	200	197.5	98.75
B-4	200	198.5	99.25
B-5	200	200.2	100.1

CONCLUSION

In this current study, an accurate, precise, linear, robust, specific and cost-effective UV-visible spectrophotometric method for analysis of doxofylline has been developed. The main traits of the developed method were an accuracy with acceptable range and both repeatability and intermediate precision has %RSD below 2.00%. Besides, the linearity of the method was obtained and the process was found to be robust. The system was similar on different days and it has no placebo or diluent effect. Moreover, the analytical solution was found to be stable over a period of 24 h at room temperature. This validated method of doxofylline is simple and this method validation procedure is replicable in any laboratory conditions and can be used for quantitative analysis of doxofylline in bulk and tablets.

ACKNOWLEDGEMENT

The first author, Subrata Paul is grateful to Drug International Limited, Bangladesh for supplying the doxofylline (sample) and doxofylline (standard).

Conflict of interest

No conflict of interest to declare.

REFERENCES

1. Lippi, G; Henry, B.M. *Int. Urol. Nephrol.*, **2020**.
2. Singh, D.; Agusti, A.; Anzueto, A.; Barnes, P.J.; Bourbeau, J.; Celli, B. R.; Criner, G. J.; Frith, P.; Halpin, D.M.G.; Han, M.; López Varela, M. V.; Martinez, F.; Montes de Oca, M.; Papi, A.; Pavord, I.D.; Roche, N.; Sin, D.D.; Stockley, R.; Vestbo, J.; Wedzicha, J.A.; Vogelmeier, C. *Eur. Respir. J.*, **2019**, *53*(5).
3. Criner, G.J.; Celli, B.R.; Brightling, C.E.; Agusti, A.; Papi, A.; Singh, D.; Sin, D.D.; Vogelmeier, C.F.; Sciurba, F.C.; Bafadhel, M.; Backer, V.; Kato, M.; Ramírez-Venegas, A.; Wei, Y.F.; Bjerner, L.; Shih, V.H.; Jison, M.; O'Quinn, S.; Makulova, N.; Newbold, P.; Goldman, M.; Martin, U. J. N. *Engl. J. Med.*, **2019**, *381*, 1023–1034.
4. Vogelmeier, C.F.; Criner, G.J.; Martinez, F.J.; Anzueto, A.; Barnes, P.J.; Bourbeau, J.; Celli, B.R.; Chen, R.; Decramer, M.; Fabbri, L.M.; Frith, P.; Halpin, D.M.G.; Varela, M.V.L.; Nishimura, M.; Roche, N.; Rodriguez-Roisin, R.; Sin, D.D.; Singh, D.; Stockley, R.; Vestbo, J.; Wedzicha, J.; Agustí, A. *Am. J. Respir. Crit. Care. Med.*, **2017**, *195*(5), 557-582.
5. MacIntyre N.; Yuh, C.H. *Proc. Am. Thorac. Soc.*, **2008**, *5*(4), 530-535.
6. Asher, M.I.; Montefort, S.; Bjorksten, B.; Lai, C.K.; Strachan, D.P.; Weiland, S.K.; Williams, H. *Lancet.*, **2006**, *368*(9537), 733-743.
7. Eder, W.; Ege, M.J.; Von Mutius, E. N. *Engl. J. Med.*, **2007**, *355*(21), 2226-35.
8. Kalayci, O.; Abdelateef, H.; Pozo Beltrán, C.F.; El-Sayed, Z.A.; Gómez, R.M.; Hossny, E.; Morais-Almeida, M.; Nieto, A.; Phipatanakul, W.; Pitrez, P.; Wong, G.W.; Xepapadaki, P.; Papadopoulos, N.G. *World Allergy Organ. J.*, **2019**, *12*(9), 100054.
9. Nyhan, W.L.; Shirkey, H.C.; Weinberger, M.M.; Bronsky, E.A. *J. Pediatr.*, **1974**, *84*(3) 421-427.
10. Weinberger, M.; Hendeles, L. N. *Engl. J. Med.*, **1996**, *334*(21), 1380-1388.
11. Horak, F.; Doberer, D.; Eber, E.; Horak, E.; Pohl, W.; Riedler, J.; Szépfalusi, Z.; Wantke, F.; Zacharasiewicz, A.; Studnicka, M. *Wien. Klin. Wochenschr.*, **2016**, *128*(15-16), 541-54.
12. Spina D.; Page, C.P. *Handb. Exp. Pharmacol.*, **2016**, *237*, 63-91.
13. Calzetta, L.; Page, C.P.; Spina, D.; Cazzola, M.; Rogliani, P.; Facciolo, F.; Matera, M.G. *J. Pharmacol. Exp. Ther.*, **2013**, *346*(3), 414-423.
14. Abbracchio, M.P.; Cattabeni, F. *Pharmacol. Res. Commun.*, **1987**, *19*(8), 537-545.
15. Franzone, J.S.; Cirillo, R.; Reboani M.C. *Int. J. Tissue React.*, **1991**, *13*(3), 131-138.
16. Matera, M.G.; Page, C.; Cazzola, M. *Int. J. Chron. Obstruct. Pulmon. Dis.*, **2017**, *12*, 3487-3493.
17. Page, C. P. *Pulm. Pharmacol. Ther.*, **2010**, *23*(4), 231-234.
18. Samanthula, G.; Yadiki, K.; Saladi, S.; Gutala, S.; Surendranath, K.V. *Sci. Pharm.*, **2013**, *81*(4), 969-982.
19. Ozkan, S.A. *Pharm. Sci.*, **2018**, *24*(1), 1-2.
20. Revathi, R.; Ethiraj, T.; Thenmozhi, P.; Saravanan, V.S.; Ganesan, V. *Pharm. Methods.*, **2011**, *2*(4), 223-8.
21. Patil, P.S.; Hamrapurkar, P.D.; Phale, M.D.; Desai, M.I.; Pawar, S.B. *J. AOAC Int.*, **2013**, *96*(4), 765-770.
22. Rekha, P.S.; Gunasekaran, S. *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.*, **2018**, *190*, 140-149.
23. Kamila, M.M.; Mondal, N.; Ghosh, L.K. *Indian J Chem Techn.*, **2007**, *14*, 523–525.
24. Patel, A. H.; Captain, A. D.; Joshi H. R. *J Young Pharm.*, **2010**, *2*(3), 289-296.
25. ICH Guideline, Validation of Analytical Procedures: Text and Methodology. Topic Q 2 (R1), Geneva, Switzerland., **2005**.
26. United States Pharmacopoeia United States Pharmacopoeial Convention, 26th Edition, Rockville, MD., **2003**.
27. Haque, M.A.; Naznin, A.; Kabir, A.H.; Hossain, M.K.; Islam, S.A. *Dhaka Univ. J. Pharm.*, **2010**, *9*(2), 131-138.
28. Batrawi, N.; Naseef, H.; Al-Rimawi, F. *J. Anal. Methods. Chem.*, **2017**, 1529280.
29. Naseef, H.; Moqadi, R.; Qurt, M. *J Anal Methods Chem.*, **2018**, 1902510.
30. Sharma, S.; Goyal, S.; Chauhan, K. *Int. J. App. Pharm.*, **2018**, *10*(6), 8-15.
31. Miller, J.N. *Analyst.*, **1991**, *116*(1), 3-14.