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AN INDIRECT ANALYTICAL METHOD FOR DEVELOPMENT AND VALIDATION OF α-β ARTEETHER IN PURE BULK PHARMACEUTICAL FORMULATION BY UV-SPECTROPHOTOMETRY

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ABSTRACT

The exploration work depends on the turn of events and approval for assessment of α-β arteether. UV spectroscopy's simple, accurate, precise, sensitive, and economical method has been developed, validated for the estimation of α-β arteether in bulk pharmaceutical raw material form as per ICH guidelines Q2(R1). The method employed 5M HCl as a solvent and was used to derivatize a drug. The proposed method obeyed Beer's law in the concentration range of 10-80µg/ml. The linear regression showed a good linear relationship with R^2= 0.997, slope and intercept were 0.012 and 0.009 respectively. The method was validated statistically where SD and % RSD were found to be satisfactorily low. The average recovery of the drug for the projected method was found in the range of 99.96% indicating no interference of the excipients. The results of the pure drug analysis were validated concerning the accuracy, precision, and recovery studies which were found to be satisfactory. LOD and LOQ for α-β arteether were found to be 0.0138µg/ml and 0.0433µg/ml respectively. The utility of the created strategy has been exhibited by examination of the business definsition containing this medication. The UV spectroscopy method showed good accuracy and precision which shows no significant difference between these methods. In this way, the proposed techniques were found to have equivalent appropriateness for assessment and routine investigation of arteether in drug crude material.

Key-words:α-β Arteether, UV spectroscopy, intercept, linearity, calibration curve

INTRODUCTION

α, β-Arteether is an ethyl ether derivative of artemisinin which is an effective schizontocidal drug in mild falciparum malaria. Chemically, ART also called dihydroartemisinin methyl ether, is a synthetic derivative of artemisinin, widely used in malaria treatment in endemic areas **[1]**. This drug can be administered as an oily solution by intramuscular injection or in capsules orally. ART is active against the plasmodium genera that cause malaria. The key structure characteristic appears to be a "trioxane" consisting of endo paroxide and doxepin oxygen's which are a simpler class of 3-aryl trioxane **[2]**. It is regulated by i.m. infusion for the treatment of serious intestinal sickness contaminations in kids and youths. It acts quickly against Plasmodium during the early blood phase of its turn of events. It like wise shows gametocytocidal movement against *Plasmodium falciparium*. The underlying endorsement was distinctly for treatment of youngsters; however, clinical preliminaries demonstrate that the medication acts quickly and effectively against Plasmodium in grown-ups **[3]**. Endoperoxide moiety is required for antimalarial activity whereas substitution on lactone carbonyl group markedly increases potency. It's in vivo potency is 10 – 100folds greater than other antimalarial drugs.

The proposed UV spectrophotometric method is modest, precise, swift, and exact for quantitative estimation of ART. The results obtained have been statistically validated following the ICH guidelines **[4]** and can be effectively used for the determination of ART in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

A standard gift sample of (Arteether-API) (ART) was provided by Thimas pharmaceuticals Haridwar. Spectroscopic Grade HCl was purchase from CDH Darya Ganj New Delhi. Double Distilled water was used throughout the study and 5M HCl was used for derivatization of a drug.

Instrumentation:

UV-Visible double-beam spectrophoto-meter, Shimadzu model 1800 with spectral bandwidth of1 nm, wavelength accuracy ± 0.2 nm, and a pair of 10-mm matched quartz cells was used. All the weighing was done on an electronic balance.

Methods-

Preparation of standard stock solution of Arteether:

Accurately weighed 10 mg of ART was transferred to a 100 ml volumetric flask. To it add 25 mlof 5M HCl and this solution was heated on the water bath for 20 minutes at temperature 80 ±2ºC. The solution was permitted to cool at room temperature and volume was then made up

to the mark with distilled water to get the concentration of 100µg/ml and used as a stock solution.

Preparation of calibration curve:

Appropriate aliquots of the stock solution of ART (1 – 8 ml) were taken in 10 ml volumetric flasks. Flasks were shaken for a few minutes and volume was then made up to the mark with distilled water to prepare a sequence of standard solutions containing 10-80µg/ml in the concentration range. The absorbance of the complex was measured at 258 nm against blank. Blank was prepared by heating 2.5 ml 5M HCl in the same condition and diluting up to 10 ml with distilled water. Then calibration curve was plotted for ART in the concentration range of 10-80µg/ml at 258 nm as shown in **Figure 1.3.**

Determination of Absorbance maxima:

The stock solution was further diluted with distilled water to get the concentration of 20µg/ml. This solution was then scanned in the range of 200 – 400 nm where distilled water was used as a blank. The wavelength of maximum absorbance of ART was found at 258 nm as shown in **Figure 1**.

Analytical Method Validation of the proposed method:

The analytical method validation includes linearity, precision, accuracy, robustness, the limit of detection (LOD), and the limit of quantification (LOQ) as per ICH guidelines.

Linearity and range:

The linearity of the analytical method is the ability to produce test results that are directly proportional to analyte concentration in samples within a given range.Accurately weighed 10 mg of ART was transferred to a 100 ml volumetric flask with 5ml of methanol. To it add 25 ml of 5M HCl and this solution was heated on the water bath for 20 minutes at temperature 80 ± 2ºC. The solution was allowed to cool at room temperature and volume was then made up to the mark with distilled water to get the concentration of(100µg/ml) and used as a stock solution. The various aliquots were prepared by suitable dilution of the standard stock solution (100µg/ml) ranging from (10-80µg/ml) and the samples were scanned in UV-Vis Spectrophotometer against 5ml methanol, 5M HCL (25ml), and 70ml of double distilled water as blank. The absorbances of respective concentrations were then calculated for the coefficient of correlation using Microsoft excel **[5]**.

Specificity:

Solutions of the pure drug as well as the drug with common formulation ingredients were prepared having known concentrations of the drug (e.g. 40µg/ml, 60µg/ml, and 80µg/ml) were prepared separately in the respective solvent system. All solutions were scanned between 400 to 200 nm and checked for any difference in

corrected absorbance of the pure and impure solution of the drug at wavelengths of study. The spectra of the pure and impure solutions of the drug were also experimental for any alteration in wavelength of maximum absorbance.

Accuracy:

The accuracy studies were performed at three levels i.e. 80%, 100%, and 120% by adding known amounts of the drug to a known concentration of the standard and analyzing the percent drug content (standard addition method) **[6]**.

Precision:

Repeatability was determined by analyzing different levels of drug concentrations from independent stock solutions (n=6). Intraday and interdayvariations in estimation were determined to assess the intermediate precision of the proposed method **[7]**. Different levels of 80%, 100%, and 120% of drug concentrations in 6 replicates were analyzed three times a day for intraday variation. The same method was followed for three different days to study interday variation. The precision was determined as percent relative standard deviation.

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) of the drugs by the proposed method were determined using calibration standards **[8]**. The LOD and LOQ were calculated as per equations 1 and 2 respectively, (ICH Guidelines Q2 (R1).

$$LOD = 3.3\left(\frac{SD_{Intercept}}{Slope}\right) \quad \text{----------1}$$

$$LOD = 10\left(\frac{SD_{Intercept}}{Slope}\right) \quad \text{----------2}$$

where "$SD_{intercept}$" is the standard deviation of the intercept of the regression line and "Slope" is the slope of the calibration curve.

Robustness:

The robustness of the projected method was determined by carrying out analysis under different wavelengths (256 nm, 258 nm, 260 nm) and by making 5mlMethanol, 5M HCl(25ml), and double-distilled waterused for UV spectrometer.

RESULTS:

Pure drug (Arteether)of Absorbance maxima:

The UV spectra of the prepared pure, as well as impure solutions of the drug, were recorded between 400 nm and 200 nm and analyzed.

The spectra of the pure drug are shown in **Figure 1**. It was clear from **Figure 1.2** that pure drug exhibited a prominent peak at the same characteristic wavelength i.e. 258 nm and this was selected for further studies as λ max for the drug. The calibration curves were prepared by plotting absorbance on Y-axis against the concentration on X-axis.

Linearity and Range:

The calibration curve was obtained by its correlation coefficient. The curve of

Arteether was linear in the concentration range of 10-80µg/ml with a correlation of 0.998567 for UV spectroscopy **(Table 1)**.

Beer's law was obeyed and validated from 10 µg/ml-80 µg/ml for Arteether The linear regression equations were found to beY=0.012X+0.009 Arteether, where A is the absorbance and C is the concentration, with a correlation coefficient of 0.998 for each **(Table 2)**.

Specificity-

Specificity studies for the developed analytical method

The method was found to be specific as indicated by less than 0.5% difference in absorbance at different concentration levels of pure and impure solutions for Arteether (40µg/ml, 60 µg/ml, and 80 µg/ml) **(Table 3)**.

Accuracy:

Accuracy was determined by calculating the recovery and the mean was determined. The assay values concerning the label pure claim raw formulation of arteether in both methods ensure the accuracy of the proposed methods. The results of accuracy for UV are mentioned in **Table 4**.

Accuracy studies for the developed analytical method

The percent recovery of the added known amounts of the drug to a known concentration of the sample was found to be 99.95%±0.05%-99.98%±0.02%, pure solution of.

Precision-

Precision was calculated as intraday and interday variation (%RSD) for the drug. The results confirmed adequate sample stability and method reliability. The results of repeatability,interday and intraday,and precision for UV analysis are mentioned results are summarized in **Table 5**, **Table 6**, and **Table 7**.

The low values of RSD (<0.25%, Tables) indicated that the developed method was precisely repeatable.

Limit of detection and limit of quantification

The limit of detection and limit of quantification for the UV method was found to be 0.0138µg/ml, 0.0433µg/ml.

Robustness:

Robustness was calculated by varying wavelengths and results are shown in **Table 8** for UV analysis.

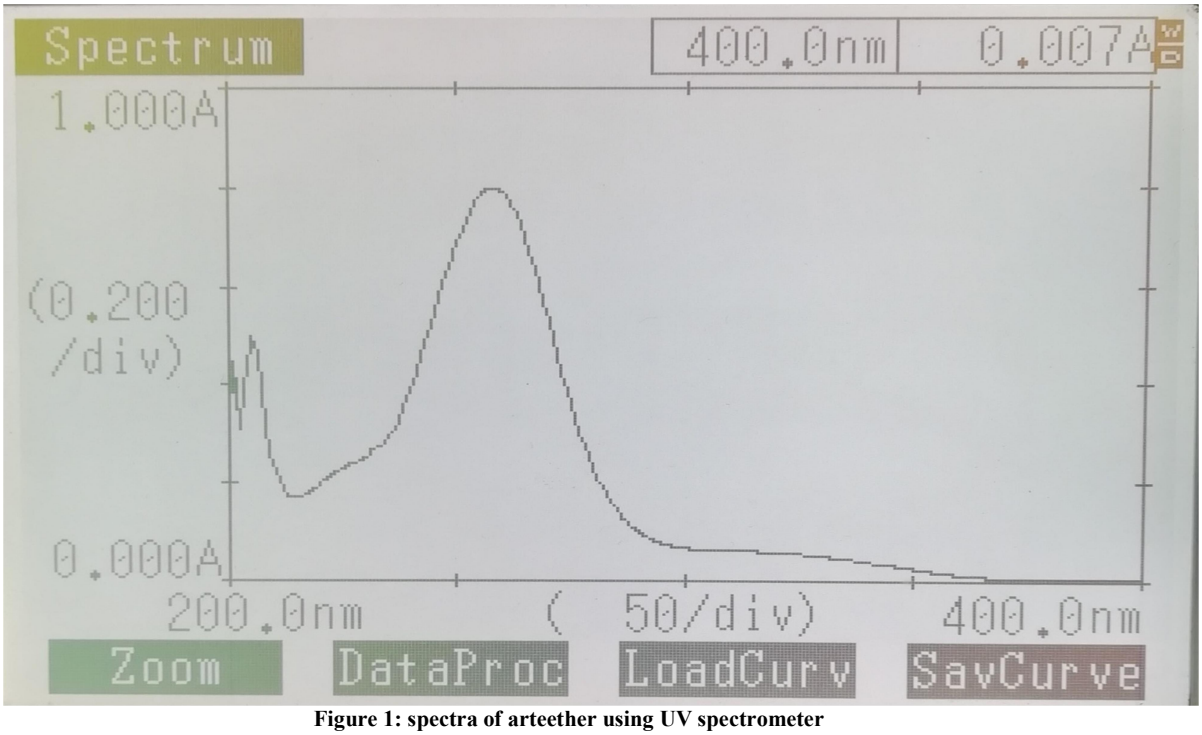

Figure 1: spectra of arteether using UV spectrometer

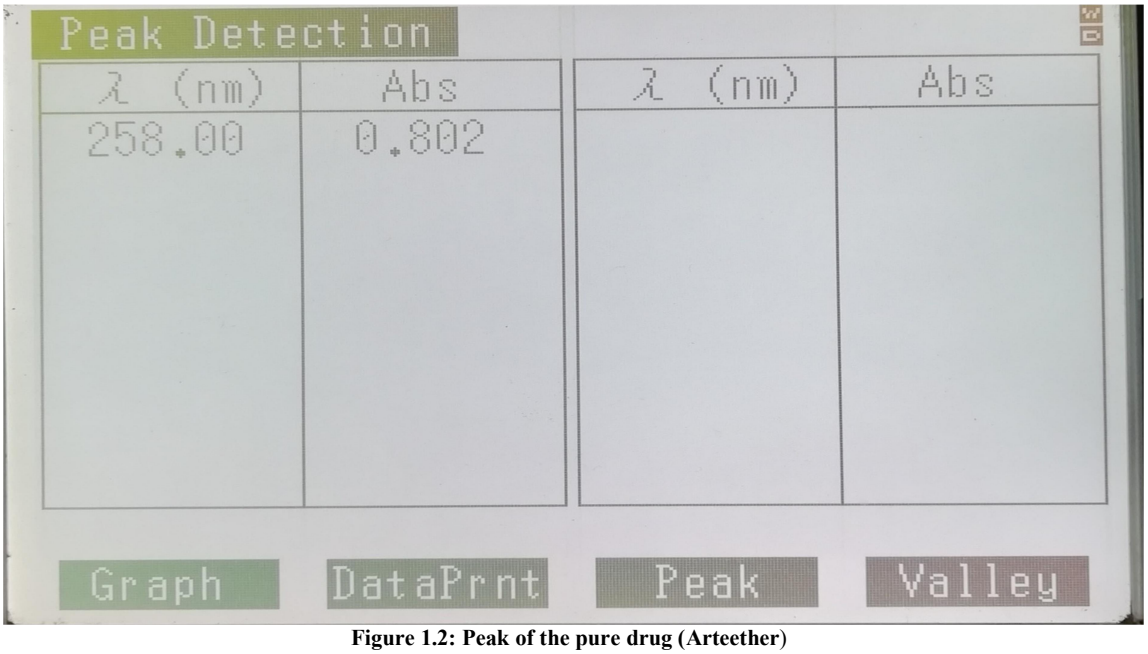

Figure 1.2: Peak of the pure drug (Arteether)

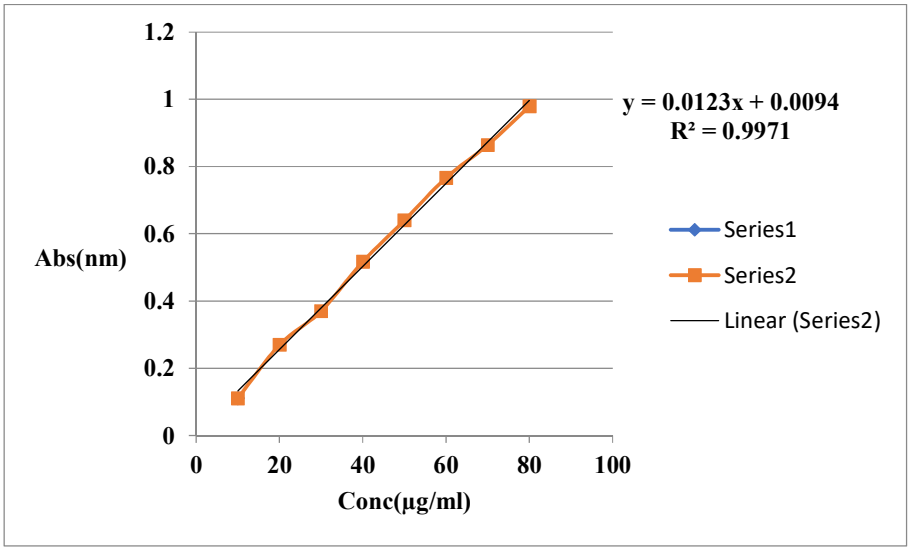

Figure 1.3: Graph of the Calibration curve

Table 1: Linearity and Range

Parameter	Value
Analytical wavelengths (nm)	λmax=258 nm
Linearity range (µg/ml)	10-80
Regression equation (A=aC+b)[a]	a= value of slope b= value of intercept
$SD_{Intercept}$ (n=6)	0.008565
Correlation coefficient (r)	0.998567

[a]A= Absorbance, C= Concentration, a= Slope and b= Intercept
$SD_{Intercept}$= Standard deviation of intercept,
λmax= wavelength of maximum absorbance

Table 2:Vaule of Pure drug ART [absorbance(nm) and concentration(nm)]

S.No.	Concentration(µg/ml)	Absorbance(nm)mean±SD*(n=6)
1	10	0.110±0.0053
2	20	0.269±0.0368
3	30	0.372±0.0139
4	40	0.517±0.0150
5	50	0.641±0.0214
6	60	0.766±0.0195
7	70	0.864±0.0254
8	80	0.978±0.0083

Table 3: Specificity studies for the developed analytical method

Concentration taken (µg/ml)	Absorbance* for		The difference in absorbance (A_c-A_t)	The difference in absorbance (%) (w. r. t. A_c)
	Pure solution (A_c)	Impure solution (A_t)		
Arteether				
40	0.334±0.001	0.333±0.00208	0.001	0.29
60	0.520±0.001528	0.520±0.001528	0.000	0.00
80	0.975±0.000577	0.972±0.001528	0.003	0.30

*Mean±SD of 6 replicate determinations

Reference range- Less than 0.5%

Table 4: Accuracy studies for the developed analytical method

C_s (µg/ml)	Level of addition(%)	C_a (µg/ml)	Amount recovered	% Recovery	Average recovery (%)	S.D.
20	80	16	35.97±0.01	99.95±0.05	99.9633	0.0152
	100	20	39.99±0.01	99.96±0.04		
	120	24	43.99±0.01	99.98±0.02		

Reference range SD- Less than 2.0%

C_s= Concentration of standard solution, C_a= Concentration of sample solution added and C_t= Total concentration found, #% Recovery= $[(C_t-C_s)/C_a]x100$, *Mean±SD of 6 replicate determinations.

Table 5: Repeatability studies for the developed analytical method

Solution	The concentration of drug solution (µg/ml)								%RSD
	Prepared	Found							
		1	2	3	4	5	6	Mean*	
Arteether									
Pure Solution	40	40.07	40.16	40.08	40.03	40.06	40.01	40.068 ± 0.051	0.129

Reference range RSD- Less than 2.0%

*Mean±SD

RSD = (SD / Mean) × 100

Table 6: Intraday precision of the developed analytical method

Solution	The concentration of drug solution (µg/ml)					%RSD
	Taken	Found				
		t1**	t2**	t3**	Mean*	
Arteether						
Pure Solution	30	29.92±0.01	29.94±0.01	29.97±0.02	29.94±0.015	0.084
	40	40.01±0.015	39.97±0.01	39.97±0.015	39.98±0.013	0.057
	50	50.01±0.01	49.96±0.015	50±0.015	49.99±0.013	0.052

Reference range RSD- Less than 2.0%

*Mean±SD of 18 determinations (6 replicate determinations every time for 3 points of time in a day), **Mean±SD of 6 replicate determinations

Table 7: Interday precision of the developed analytical method

Solution	The concentration of drug solution (µg/ml)					%RSD
	Taken	Found				
		Day 1**	Day 2**	Day 3**	Mean*	
Arteether						
Pure Solution	30	29.93±0.01	29.92±0.01	29.93±0.005	29.92±0.008	0.019
	40	40.02±0.01	40.04±0.01	39.96±0.01	40±0.041	0.104
	50	50.01±0.01	49.96±0.01	50.01±0.015	49.99±0.011	0.057

Reference range RSD- Less than 2.0%

*Mean±SD of 18 determinations (6 replicate determinations every day for 3 days), **Mean±SD of 6 replicate determinations

Table 8: Robustness was a calculation on different absorbance

Wavelength (nm)	Concentration (µg/ml)	Absorbance (nm)			Mean	S.D.	%R.S.D.
256	40	0.101	0.101	0.102	0.1013	0.0005	0.5697
	60	0.361	0.364	0.364	0.3626	0.0015	0.4211
	80	0.423	0.424	0.425	0.424	0.001	0.2358
258	40	0.106	0.105	0.107	0.106	0.001	0.9433
	60	0.412	0.415	0.416	0.414	0.0020	0.5052
	80	0.492	0.494	0.496	0.494	0.002	0.4048
260	40	0.123	0.123	0.124	0.1233	0.0055	0.4464
	60	0.372	0.374	0.375	0.373	0.0015	0.4087
	80	0.397	0.398	0.399	0.398	0.001	0.2512

DISCUSSION

The proposed methods provide sensitive, precise, economical, and accurate UV spectrophotometric methods for the estimation of arteether in the bulk pharmaceutical dosage form. In the UV spectrometric method, methanol was used as a solvent, and HCl was used for acid decomposition, which induces the formation of UV detectable degradation products. The maximum absorption was found to be 258 nm for UV spectrometric analysis. The linearity range was found to be 10-80µg/ml with a correlation coefficient of 0.998 for the UV method. The method was found to be precise as % RSD values for intraday, interday, and repeatability were within the limits of less than 2%. The accuracy of the proposed methods was determined by the recovery studies and the mean recoveries (% RSD) for the three concentrations were found to be average recovery 99.963 for UV analysis. The good % recovery of the drug obtained indicates that the methods are accurate. The proposed method was found to be robust as the % RSD values were found to be less than 2%. The limit of detection and limit of quantification for the UV method was found to be 0.0138µg/ml and 0.0433µg/ml representativeof the methods developed are sensitive.

CONCLUSION

The developed spectroscopic methods are not only rapid but also simple, sensitive, accurate, and precise and hence used for the routine analysis of arteether in bulk and pharmaceutical formulation. This method supports us in estimating that in contrast to UV spectrophotometric technique, outcomes of the analysis were more sensitive and accurate as the accuracy Based on the results obtained, it can be concluded that the proposed UV spectrophotometric method for determination of ART is quick, cost-effective, accurate and precise. The utility of the developed method has been established by analysis of raw formulation. Hence, the proposed method can be used for the quantitative determination of pharmaceutical formulation containing this ingredient.

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CONFLICTS OF INTEREST: The authors report no conflict of interest.

Abbreviations:

UV: Ultra-Violet Spectroscopy.

API: Active Pharmaceutical Ingredient

ART: α-β ARTEETHER

%RSD: Percent Relative standard deviation

SD: standard deviation

µg/ml: microgram per ml

REFERENCES

[1] Tiwari MK, Chaudhary S. Artemisinin-derived antimalarial endoperoxides from bench-side to bed-side: Chronological advancements and future challenges. Medicinal research reviews. 2020; 40(4): 1220-75.

[2] Chandra R, Puri SK. Arteether resistance reversal by ketoconazole/ fluconazole in rodent malaria parasite *Plasmodium vinckei*. Parasitology research. 2015; 114(3): 1239-43.

[3] Cheong DHJ, Tan DWS, Wong FWS, Tran T. Anti-malarial drug, artemisinin and its derivatives for the treatment of respiratory diseases. Pharmacological research. 2020; 158: 104901.

[4] Singh RP, Sabarinath S, Gautam N, Gupta RC, Singh SK. Liquid chromatographic tandem mass spectrometric assay for quantification of 97/78 and its metabolite 97/63: a promising trioxane antimalarial in monkey plasma. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2009; 877(22): 2074-80.

[5] Ding J, Zhang Y, Cui X, Zhao C, Zhang S. Development of a novel analytical method for determining trazodone in human plasma by LC-MS coupled with automatic 2-dimensional liquid chromatograph-mass spectrometer coupler 9500 and its application to therapeutic drug monitoring. Therapeutic drug monitoring. 2021.

[6] Baldi A, Sanadya J, Patidar A, Method development and validation for estimation of quetiapine-fumerate by RP-HPLC, Asian Journal of Research in Chemistry, 2010; 3(3): 604-607.

[7] Bui VT, Doan CS, Tong TTV, Le DC. Development and Validation of a Simple, Green Infrared Spectroscopic Method for Quantitation of Sildenafil Citrate in Siloflam Tablets of Unknown Manufacturing Formula.

Journal of analytical methods in chemistry. 2021; 2021: 6616728.

[8] Hasan N, Chaiharn M, Toor UA, Mirani ZA, Sajjad G, Sher N, et al. Development, Validation and Application of RP-HPLC Method: Simultaneous Determination of Antihistamine and Preservatives with Paracetamol in Liquid Formulations and Human Serum. The open medicinal chemistry journal. 2016;10: 33-43.