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Risk assessment and uncertainty appraisal for quantification of Aripiprazole and lamotrigine in combination: A neoteric validation approach

Krishna Jadav and Rajashree Mashru

Abstract
Risk profile and uncertainty approximation are two prime and important parameters that need to be accepted for the evolution of the pharmaceutical process to obtain reliable results. The ordinary method validation process needs to be extempore so, as to provide to a high level of assurance of method reliability to quantify the quality element of a drug product. Risk contour, combined standard uncertainty and expanded uncertainty in the analysis of Aripiprazole and Lamotrigine in the synthetic mixture were analysed by absorption correction method in this present research work. The cause-effect diagram was used to determine uncertainty parameters and to make it more accurate and precise method was validated in our laboratory as per the ICH guidelines. Whilst evaluate the results of validation, a calibration model was established by Levene's test. In uncertainty, the major contribution is owing to sample concentration and mass of the sample. This present research work distinctly illustrates the application of the theoretical concept of calibration model tests, relative bias, risk analysis and uncertainty in the method used for analysis in drug invention activity.

Keywords: Aripiprazole, lamotrigine, risk profile, combined standard uncertainty, expanded uncertainty

1. Introduction
Measurements are made and utilized to create decisions. When we utilize the numbers to create decisions, each time we run with chances of making mistake since all numbers are more or less unreliable. On the off chance that we measure something, we can be sure of just a single thing: estimation is uncertain. Since each and every measurement uncertain, we should essential to know how and why it is so. Consequently, a new approach known as a measurement of uncertainties in sample analysis was recently introduced to keep away from doubts. As a result of the measurement, the measurement value (μ) is only estimated. The measured value is only accepted when accompanied by a quantitative statement of its uncertainty (U) and it is expressed as (μ+U). Managing uncertainty is a fundamental effort in analytical method development and validation. The quality of the method is expressed as its uncertainty and uncertainty assessment has become an important parameter for a validated method to obtain accreditation. Identification of uncertain components and reasonable estimation are essential for a rigorous and statistically valid calculation of the measurement uncertainty [1, 2].
The third edition of guidelines for quantifying uncertainty in analytical measurement dependent on developments in uncertainty estimations is introduced by Eurachem in 2012 [3]. For the uncertainty estimation, the steps included begin with measurand specification and end with expanded uncertainty calculation. The Analytical method for assessments of drug component in the pharmaceutical formulation has been presented and accessible around the world. However, validation by total error approach and quantification of causes of uncertainties in this developed and published method were missing. A few articles also suggest ordinary estimation of analytical measurement and uncertainty. In the present study, simple method for quantification of uncertainty components and combined standard uncertainty (CSU) is presented by evaluating this computation for spectrophotometric measurement of aripiprazole and lamotrigine in synthetic mixture. For a precise and complete study of uncertainty components, a new UV method for Aripiprazole and Lamotrigine in a synthetic mixture form have been developed and validated as per the ICH guideline [4-5]. β-expectation tolerance interval, relative bias, accuracy profiles, calibration model and risk profile had been studied.

This approach also consists of a detailed analysis of the factors impacting analytical results using cause-effect diagram and risk profile of future assessment.

Aripiprazole (ARP) is chemically called 7-[4-[4-(2,3-dichlorophenyl) piperazin-1-yl] butoxy]-3,4-dihydro-1H-quinolin-2-one -quinolone and piperazine derivative that is used as an antipsychotic agent. Aripiprazole has partial agonistic activity on dopamine D2 receptors and serotonin 5-HT1A receptors, further as potent antagonistic activity on serotonin 5-HT2A receptors. it is used in acute manic and mixed episodes associated with bipolar I disorder. Lamotrigine (LMG) is chemically called as 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine -phenyltriazine compound, is considered sodium and calcium channel blocker that is used for the treatment of seizures and bipolar disorder. It can also improve depression in patients with epilepsy and reduces the risk of relapse in bipolar disorder [6-9]. It has been demonstrated to aripiprazole has low in solubility and low in permeability and to be considered as a typical Biopharmaceutics Classification System (BCS) Class IV drug from the United States of Food and Drug Administration (USFDA). And lamotrigine has low solubility and high permeability and to be considered as BCS Class II from USFDA [10-11]. In combination of aripiprazole and lamotrigine demonstrated a delay in time to depressive relapses and tended to prolong the time to manic/mixed relapses. So, this combination may provide a synergistic treatment effect in preventing depressive relapses for this subpopulation in mixed-episode patients. The combination of aripiprazole and lamotrigine demonstrated a safe and adequate tolerability profile [12-13].

Few methods for determination of Aripiprazole and Lamotrigine individual and in combination with other drug has been reported previously, alike spectrophotometric [14-16], high-performance liquid chromatography (HPLC) [15-21], capillary electrophoresis [18]. be that as it may, all these methods are onerous, time-consuming and require derivatization of the drug. Spectrophotometric analysis is considered more convenient alternative techniques since of their inherent simplicity and high sensitivity. Even though the individual UV spectrophotometric method for simultaneous estimation of Aripiprazole and Lamotrigine has already been developed however this technique consist of the uncertainty measurement and risk profiling using total error approach and developed method is more efficient than the previously posted method.

Uncertainty estimation and relative bias, β-expectation tolerance interval, accuracy profiles, risk profile and calibration model, which have not been studied either, are critical parameters in present method validation procedures. Some articles also propose the conventional estimation of analytical measurements and uncertainty [22-26]. Be that as it may, most of these methods are applied to food samples and a very few methods have been found for pharmaceutical formulations [27]. Hence, we developed a new simple spectrophotometric method that defeats these drawbacks by applying a wide estimation of uncertainty and total error estimation approach.

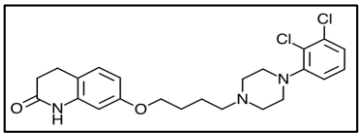

Fig 1: Structure of Aripiprazole

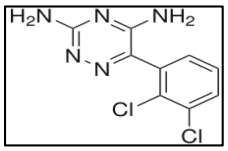

Fig 2: Structure of Lamotrigine

The current research paper represents a validated Absorption correction method for quantification of Aripiprazole and Lamotrigine in synthetic mixture with total error approach and uncertainty measurement.

2. Experiment Part

2.1 Instrumentation

Spectrophotometric measurements were performed on a Shimadzu 1700 double beam UV-VIS Spectrophotometer with a fix slit width of 1nm coupled with Shimadzu UV Probe PC software version 2.10. Electronic Weighing Balance of Shimadzu AX120, Borosil Glassware, and Ultra Sonicator Bath were used for experimental purpose.

2.2 Chemicals and Reagents

Aripiprazole and Lamotrigine were provided as a gift sample from Zydus Pharmaceutical Pvt. Ltd., India. All the analytical grade reagents used for the study was supplied from Research-Lab fine chem industries, Mumbai.

2.3 Preparation of standard solution of Aripiprazole and Lamotrigine

For standard stock solution (1000 µg/ml) of Aripiprazole and Lamotrigine were prepared by accurately weighed 10mg of individual both drug and transferred in 10 ml calibrated volumetric flask and dissolved in a 6 ml of methanol and sonicated for 5 min then volume was made up to the mark with methanol. Different volumes of this stock solution were then further diluted with methanol to get the working standard solutions.

2.4 Preparation of calibration curves

From the working standard solution of Aripiprazole (100 µg/ml) aliquots ranging from 0.1 ml to 0.35 ml were taken, in 10 ml volumetric flask and diluted to volume with methanol to obtained final concentrations of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 µg /ml of ARP. And aliquots ranging from 1.0 ml to 3.5 ml were taken, from the working standard Lamotrigine (100 µg/ml) in 10 ml volumetric flask and diluted to volume with methanol to obtained final concentrations of 10, 15, 20, 25, 30, 35 µg/ml of LMG. The Calibration curve was constructed by plotting absorbance v/s concentration of each drug and the regression equation was calculated.

2.5 Method for spectrophotometric determination

All reagents were tested for solution stability during the actual analysis. The behavior of analyte stayed unchanged for around 24 hours from their preparation at room temp. the drugs were found to be stable throughout each type of experimental measurements. Each and every measurement was done at room temperature. The absorption spectrum of the standard solutions was recorded between 200-400nm against blank methanol the usage of a 1.0 cm quartz cell cuvette. The zero-order overlain spectrum of Aripiprazole and Lamotrigine and synthetic mixture was obtained and the maximum absorption was found to be at 255nm and 307nm, respectively. Absorption correction method is modification of

simultaneous equation method. This method utilizes the absorbances at two selected wavelengths, one at λmax of one drug where other drug also shows considerable absorbance (λ2) and other being the wavelength at which the first drug has practically negligible absorbance (λ1).

2.6 Validation parameters studied utilizing Total Error Approach

The total error approach dependant on the use of two-sided β-content tolerance intervals was developed. The total error approach provides a formal statistical system for analytical method performance assessment. The approach is reliable with the concept of method suitability and controls the risk of incorrect acceptance of unsuitable analytical methods. Risk profiling is a procedure for finding the ideal level of risk for a developed method and risk related to the method. The proposed method was validated as per ICH guideline [3, 4] and the ISO-IEC applying accuracy profiles, which depend on the total error approach [1]. In this approach "total error" was evaluated by combining the systemic error and random error to recognize the difference between observed value and true value. In the present method sensitivity of the method and robustness were also studied.

2.6.1 Calibration curves (response function)

In the present method six sets of calibration curves were plotted between absorbance and different concentrations of Aripiprazole and Lamotrigine which follows Beer's-Lambert law and on these six different regression analyses was performed and the series with the best coefficient of assurance was selected and the selected linearity has been analyzed by Levene's test and standard residual plot.

2.6.2 Trueness

As indicated by ISO, trueness of an analytical procedure expresses the closeness of agreement between the average value obtained from repeated measurements and a conventional true value [35]. In this method trueness of calibration curves is calculated to justify the calibration line by back-calculating concentrations and results are expressed in terms of absolute and relative bias and additionally, linear relationship between nominal and back-calculated concentrations has been plotted to illustrate linearity of the method.

2.6.3 Precision

The precision of an analytical method expresses the closeness of agreement between the values obtained from repeated measurements. In this method precision was studied at two levels: the first was repeatability under the same operating conditions over a short time interval and second was the intermediate precision assessed on different days. The results of precision are expressed using relative standard deviation (RSD). Relative precision and absolute precision were calculated at these two levels and additionally 95% upper confidence limit was determined for both levels.

2.6.4 Accuracy

Accuracy is the most critical parameter in method validation. So, it requires an additional consideration during the study. Subsequently, the results of accuracy studies had been represented in the β- expectation tolerance limits. Further to this, risk profile was also studied to recognize the future application of the method in synthetic mixture of both drugs. Accuracy profiles for the synthetic mixtures were plotted with β- expectation tolerance limits. The Linearity profile was also studied to illustrate the relationship between introduced and observed concentrations in synthetic mixture and besides, a residual plot was created to know outliers in the determination of aripiprazole and lamotrigine in a synthetic mixture.

2.6.5 The Detection and Quantification Limit

Limit of detection (LOD) and limit of quantification (LOQ) are two important parameters which show the application of methods in detection and quantification of synthetic mixture. There were calculated according to ICH guidelines [4, 5].

2.6.6 Robustness

Robustness was examined by evaluating the influence of small variation of method variables including detection wavelengths and solvent grades. In the present study, one parameter was changed while the others were kept unchanged and recovery percentage was calculated each and every time for both drugs. The robustness studies were performed with three different grades of solvents and three different wavelengths. The study was carried out by keeping one standard parameter constant and varying the second parameter respectively.

2.7 Estimation of Measurement Uncertainty

To avoid a risk of making mistake with every measurement and to clear worries associated with measurement recently a new approach has been familiarized known as a measurement of uncertainties in the analysis of sample. The quality of method is expressed in terms of its uncertainty and assessment of uncertainty becomes a key parameter for method validation to get certification. Developed method was successfully validated, still measurements always create some doubts, so the uncertainty of measurements was performed.

2.7.1 Cause-effect diagram

Despite the fact that method was validated as per guidelines yet some doubts were there in results as during the validation of method small impact which can influence the results has not been studied, such as an error during mass of sample taken, discharge of volumetric flask etc. Consequently, to overcome such doubt during result comparison were clarified by estimation of uncertainty in result obtained from validation. The uncertainty estimation protocol starts with identification of sources of uncertainty. The good way to listing the sources of uncertainty is to use the cause-effect diagram because it traces the sources connected to each other illustrating their effect on the result. Hence, a cause-effect diagram was constructed as appeared in Figure 3, which points out the different sources which may influence the sample analysis measurements. The parameters taken in consideration was volume of volumetric flask V_{10}, concentration of analyte C, precision of method P, recovery of method R_m and mass of sample m_{sample}. All these parameters make a contribution to uncertainty in the interpreted results.

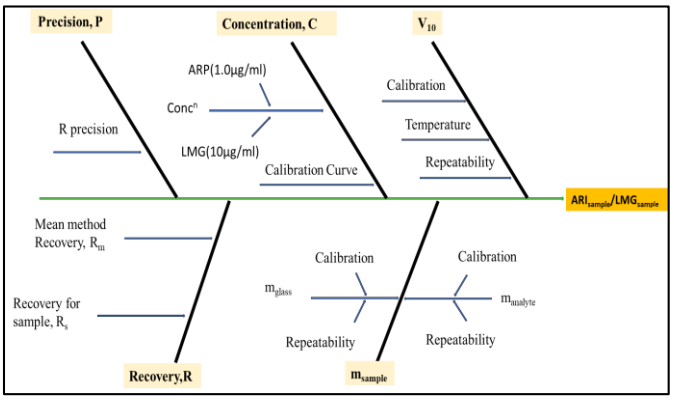

Fig 3: Cause and effect diagram to identify the sources of uncertainty

This diagram also facilitates in resolving any repeatability of component in uncertainty. These parameters come into consideration after constructing a cause-effect diagram was outlined in Eq. (1).

$$ARP/LMG_{sample}=C*V_{10}10^{-3}/m_{sample}R_m \quad (1)$$

Where, ARP_{sample} and LMG_{sample} is ARP and LMG quantity in (mol/kg); C is ARP and LMG concentration in 10 mL volumetric flask (M); V_{10} is volume of 10 mL volumetric flask (mL); m_{sample} is ARP and LMG sample mass taken (kg); Rm is Recovery of method.

After identification of parameters affecting the uncertainty of results, these sources were individually quantified and their effect on overall uncertainty was studied as Combined Standard Uncertainty (CSU) and Expanded Uncertainty (EU).

2.8 Effect of individual parameters on overall uncertainty [28-29].

2.8.1 Discharge of solution from volumetric flask

The uncertainty due to discharge of solution from volumetric flask was assessed by performing experiment including filling up and weighing of 10 mL volumetric flask with standard solution for 10 times.

2.8.2 Concentration (C)

The uncertainty in concentration of drugs obtained from calibration curve is expressed as uncertainty due to concentration (C). This is evaluated using Equation (2).

$$U(c)=\frac{Sr}{b}\sqrt{\frac{1}{n}+\frac{1}{p}+\frac{(C-\bar{C})2}{sxx}} \quad (2)$$

Where

$$S_r=\sqrt{\sum_{j=1}^{n}\frac{[Yj-(bxi+a)]2}{n-2}} \quad (3)$$

$$S_{xx}=\sqrt{\sum(Ci-\bar{C})\,2} \quad (4)$$

Where

S_r: residual standard deviation;
b: slope of calibration curve(L/mol);
n: number of solution measurements used for calibration curve;
p: number of measurements used to obtain concentration of unknown sample;
C: concentration in sample(M);
$\bar{C}$: average of standard concentration(M);
Y_j: analytical signal response obtained from measurement;
j: index for number of measurements made in order to obtain calibration standard;
i: index for number measurements for calibration;
a: calibration curve intercept.

The sample solution was measured 10 times so p=10 and concentration has been obtained from the calibration curve regression equation. Eq. (5)

$$Y = mx + C \quad (5)$$

Where, Y is absorbance of sample solution; m is calibration curve slope; C is intercept and x is concentration of ARP and LMG.

2.8.3 Precision (P)

During the validation of method precision studies were carried out. In this study repeatability and variability related with the measurement were included in overall precision uncertainty estimation.

2.8.4 Recovery of method (R_m)

Uncertainty related to a recovery of a method was evaluated using Equation (7) Simple recovery was calculated by Eq. (6) and it depends upon spiked and recovered standard concentration in the sample.

$$R(m)=\frac{Cobs}{Cspike} \quad (6)$$

$$U(R_m)=Rm\times\sqrt{\left(\frac{Sobs2}{n\times Cobs2}\right)+\left(\frac{U(Cspike)}{Cspike}\right)^2} \quad (7)$$

Where,
C_{obs}: replicate analysis of spiked sample;
C_{spike}: nominal concentration of drug in spiked sample;
S_{obs}: standard deviation of results from the replicate analyses of spiked sample;
n: number of replicates;
U (C_{spike}): standard uncertainty in concentration of spiked sample.

2.8.5 Mass of sample (m_{sample})

The sample mass was obtained through calculating the difference between weighing glass with and without the sample.

3. Results and Discussions

The absorption spectra of Aripiprazole, Lamotrigine and mixture were recorded and these are overlapped in the region of their absorption maxima at 255nm and 307nm, respectively as shown in Figure 4. Thus, all the studies were performed at these wavelengths respectively. Direct Ultraviolet Spectrophotometry cannot be used to determine the two compounds individually in their mixtures but Absorption Correction Method seemed to offer great potential.

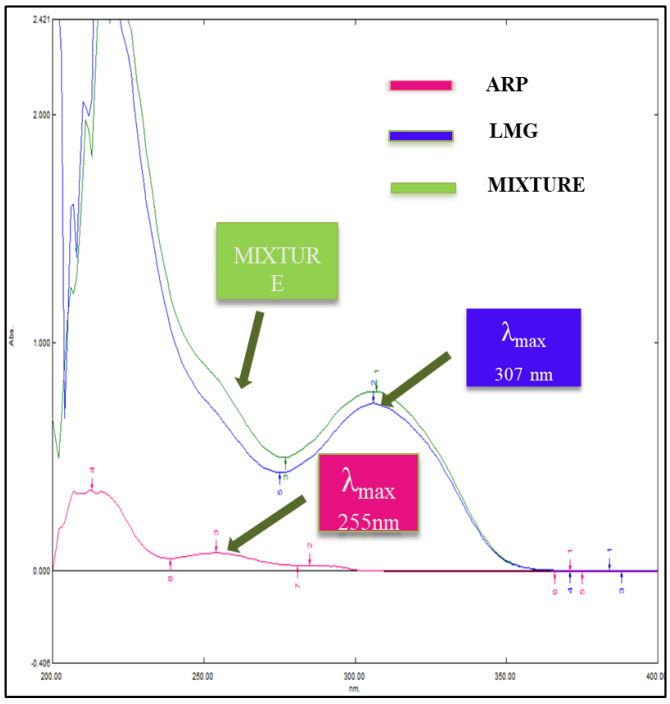

Fig 4: overlain spectrum of Aripiprazole, Lamotrigine and mixture

3.1 Validation parameters

3.1.1 Calibration curves

Calibration curves from the response of different concentratio ns were prepared using linear regression model in this present method. The six different sets were prepared for response function studies with range of 1.0-3.5 µg/ml for ARP and 10-35 µg/ml for LMG, from their regression analysis studies series all are shows the best results with coefficient of determination r^2 0.9997 and 0.9999 with regression equation (y= 0.0293x - 0.0073) and (y = 0.0258x - 0.0275) for ARP and LMG respectively, which was selected for further validation and sample analysis studies. Besides, the selected series and regression model was analysed and confirmed using Levene's test. The p-value were calculated and found to be greater than 0.05 represented in Table 1 and further to demonstrate that no outliers were found in calibration curve and standard residual plots were also plotted as shown in Figures 5 and 6. Presently to confirm the chosen regression equation, back-calculation was done and linear plot using absolute β-expectation limit was constructed between nominal and back-calculated concentration which showing r^2 the 0.9996 and 0.9998 for ARP and LMG respectively and it turned out that authenticity of regression equation was evident.

Table 1: Levene's test for regression model.

Drug	Source	SS	df	MS	Fcalc	Fcrit,95%	p-value
ARP	Model	0.000000369	1	0.000000369	0.000374	5.3176	0.9790
	Error	0.004020000	8	0.000502000			
LMG	Model	0.00002424	1	0.00002424	0.000489	5.3176	0.9828
	Error	0.39652374	8	0.04956546			

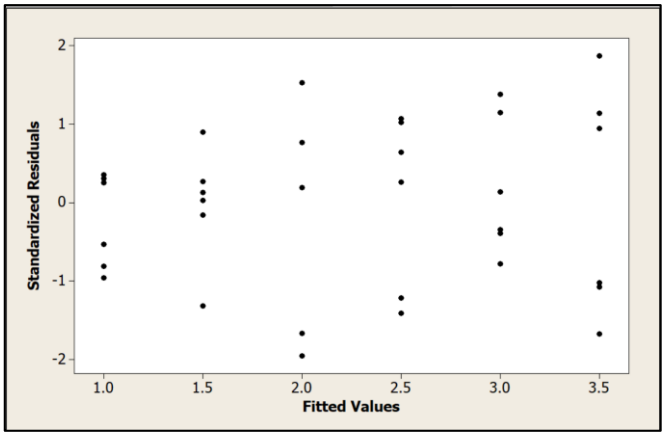

Fig 5: Standard residual plot of six different series of representing absence of outliers in all different concentration levels of Aripiprazole

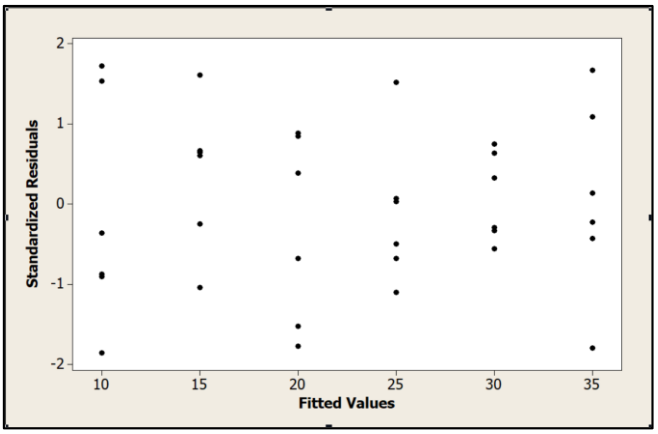

Fig 6: Standard residual plot of six different series of representing absence of outliers in all different concentration levels of Lamotrigine

3.1.2 Trueness

Trueness of method justified by calculating the percentage relative bias which was found to be limited between [-1.5602 to 0.9774] for ARP and [-0.6590 to 0.9528] for LMG as shown in Tables 2 and 3 from which it has been concluded that the trueness of method is adequate.

Table 2: Result of Trueness in terms of relative bias (%) for ARI

Mean introduced concentration (µg/ml)	Mean back calculated concentration (µg/ml)	Absolute Bias (µg/ml)	Relative Bias (%)	Recover y (%)	95% Confidence Interval of Recovery (%)
1.000	1.006	0.0064	0.6396	100.64	[100.61, 100.67]
1.500	1.516	0.0162	1.0785	101.08	[101.05, 101.10]
2.000	1.971	-0.0293	-1.4673	98.533	[98.507, 98.559]
2.500	2.485	-0.0150	-0.602	99.398	[99.372, 99.424]
3.000	3.023	0.0226	0.7524	100.75	[100.73, 100.78]
3.500	3.492	-0.0075	-0.215	99.785	[99.759, 99.811]

Table 3: Result of Trueness in terms of relative bias (%) for LMG

Mean introduced concentration (µg/ml)	Mean back calculated concentration (µg/ml)	Absolute Bias (µg/ml)	Relative Bias (%)	Recover y (%)	95% Confidence Interval of Recovery (%)
10.00	1.005	0.0953	0.9528	100.95	[100.47, 101.20]
15.00	1.515	-0.0990	-0.6590	99.341	[98.86, 99.58]
20.00	1.969	0.0724	0.3622	100.36	[99.88, 100.61]
25.00	2.483	0.0512	0.2047	100.20	[99.72, 100.45]
30.00	3.020	-0.1720	-0.5720	99.428	[98.94, 99.67]
35.00	3.497	0.1776	0.5073	100.51	[100.03, 100.76]

3.1.3 Precision

The method precision and reproducibility were confirmed by the result obtained from precision studies which were found to be <2% in terms of RSD for both level repeatability and intermediate as illustrated with 95% confidence upper limit in Tables 4 and 5. These results of precision suggest that the developed analytical method was precise and reproducible.

Table 4: Results of relative and absolute intermediate precision and repeatability in terms of %RSD(ARP).

Nominal concentration (µg/ml)	Relative intermediate precision and repeatability				Absolute intermediate precision and repeatability	
	Repeatability (%RSD)	Inter-mediate precision (%RSD)	95% Upper confidence limit		Repeatability (SD) (µg/ml)	Inter-mediate precision (SD) (µg/ml)
			Repeatability (SD) (µg/ml)	Inter-mediate precision (SD) (µg/ml)		
1.0	0.6444	0.8589	0.2893	0.2842	0.00185	0.00242
1.5	0.8782	0.9431	0.4131	0.4331	0.00359	0.00404
2.0	0.8142	0.5887	0.5671	0.5625	0.00458	0.00329
2.5	0.9173	0.7297	0.6927	0.6924	0.00629	0.00501
3.0	0.9735	0.8116	0.8324	0.8352	0.00802	0.00672
3.5	0.7139	0.6278	0.9966	0.9940	0.00706	0.00620

Table 5: Results of relative and absolute intermediate precision and repeatability in terms of %RSD(LMG).

Nominal concentration (µg/ml)	Relative intermediate precision and repeatability				Absolute intermediate precision and repeatability	
	Repeatability (%RSD)	Inter-mediate precision (%RSD)	95% Upper confidence limit		Repeatability (SD) (µg/ml)	Inter-mediate precision (SD) (µg/ml)
			Repeatability (SD) (µg/ml)	Inter-mediate precision (SD) (µg/ml)		
10	0.9578	0.91001	0.2801	0.2811	0.00266	0.00253
15	0.97148	0.91322	0.4188	0.4187	0.00403	0.00379
20	0.73242	0.6076	0.5615	0.5686	0.00408	0.00343
25	0.87153	0.64382	0.7032	0.7089	0.00607	0.00453
30	0.03335	0.94485	0.8486	0.8567	0.00028	0.00802
35	0.65939	0.55733	0.982	0.9822	0.00643	0.00544

3.1.4 Accuracy

After the confirmation of accuracy of all the parameters associated with the system and developed method, sample matrix was incorporated in validation process which includes recovery studies. Recovery studies were conducted using standard addition method in sample matrix. These recovery studies receipts into account total error of test result and is represented through the β-expectation tolerance limit. The result of accuracy studies has been shown in Table 6. The β-expectation tolerance limit was also found to be in the acceptance as accuracy profile showed in Figures 7 and 8. Furthermore, Risk profile keeping maximum risk level at 5.0% from which it was concluded that risk of outliers is within limits and in future analysis of the sample using this developed and validated method will fall inside range.

Table 6: Results of method accuracy and risk analysis of ARP and LMG by considering linear regression model

Drug	Concentration level (%)	Concentration (µg/ml)	β- expectation tolerance limit (µg/ml)	Relative β- expectation tolerance limit (%)	Risk (%)
Aripiprazole	80	0.80	[0.774, 0.803]	[-3.250, 0.375]	0.013936
	100	1.00	[0.972, 0.988]	[-2.781, -1.132]	0.019568
	120	1.20	[1.192, 1.207]	[-0.666, 0.583]	0.0000948
Lamotrigine	80	8.00	[8.152, 8.187]	[1.901, 2.337]	0.021221
	100	10.0	[10.180, 10.307]	[1.803, 3.071]	0.024367
	120	12.0	[12.351, 12.479]	[2.925, 3.991]	0.034615

3.1.5 The detection and Quantification Limit

The results of LOD and LOQ show that this method is sufficiently sensitive to analyze synthetic mixture; LOD was found to be 0.37 µg/ml and 0.96 µg/ml for ARP and LMG respectively. LOQ was found to be 1.12 µg/ml and 2.91 µg/ml for ARP and LMG respectively.

3.1.6 Robustness

Robustness of the developed method was determined in the form of % RSD by small yet sdeliberate changes in the detection wavelengths and solvent grades for synthetic mixture. The results of robustness studies are illustrated in table 7 and 8 showing the impact of variation on amount found in sample matrix and from these results it is concluded that the method has sufficient capacity to bear up to some extent human or system errors.

Table 7: results of robustness studied of aripiprazole in terms of mean back concentration and % RSD (n=6)

Parameter studies		Nominal concentration (µg/ml)	Mean Back concentration (µg/ml) ± %RSD
λmax(nm)	254	1.0	1.01 ± 0.85
	255	1.0	0.997 ± 0.58
	256	1.0	0.999 ± 0.67
Solvent grade	AR grade	1.0	0.993 ± 0.92
	HPLC grade	1.0	1.020 ± 0.42
	LR grade	1.0	0.997 ± 0.59

Table 8: results of robustness studied of Lamotrigine in terms of mean back concentration and % RSD (n=6)

Parameter studies		Nominal concentration (µg/ml)	Mean Back concentration (µg/ml) ± %RSD
λmax(nm)	306	10	9.99 ± 0.89
	307	10	10.02 ± 0.61
	308	10	9.96 ± 0.77
Solvent grade	AR grade	10	9.94 ± 0.42
	HPLC grade	10	9.91 ± 0.74
	LR grade	10	10.03 ± 0.65

3.2 Application of proposed method

3.2.1 Analysis of synthetic mixture

It is evident from the previously mentioned results that proposed method gave palatable results with the ARP and LMG in bulk drug. The synthetic mixture was subjected to analysis for their substance of active drug material by the proposed method. The percentage purity for synthetic mixture was found to be 99.61% for ARP and 100.10% for LMG. It is obvious from the above-mentioned results that proposed method is relevant to the analysis of drug in its bulk drug as well as synthetic forms with comparable analytical performance.

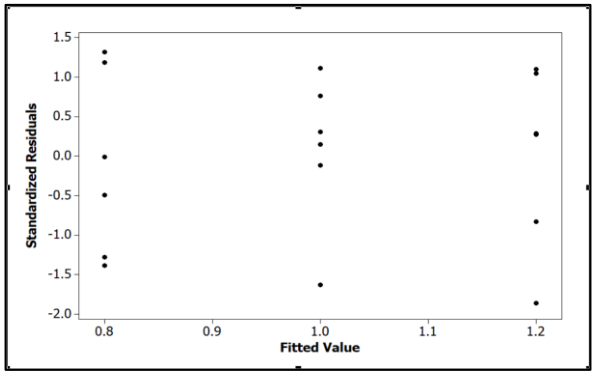

Fig 7: Standard residuals plot confirming absence of outliers in determination of accuracy of Aripiprazole

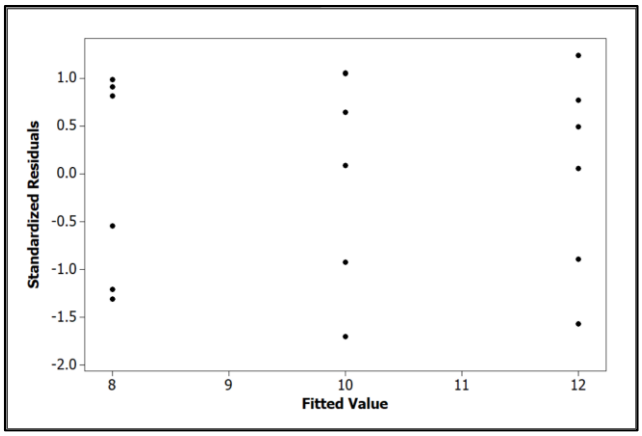

Fig 8: Standard residuals plot confirming absence of outliers in determination of accuracy of Lamotrigine

3.3 Uncertainty measurements

When uncertainty sources have been identified, they were evaluated and their greatness was determined. In order to assure the detectability for uncertainty results, all the calculation have been done in the International System of Unit as a concentration in M and weight in kg.

3.3.1 Uncertainty due to discharge of solution from volumetric flask

The uncertainty effect on a discharge of solution from the

volumetric flask is mainly affected by the three components i.e. calibration of the volumetric flask (during manufacturing time), repeatability and temperature.

3.3.1.1 Calibration of volumetric flask

Deviation of esteem from nominal volume for 10ml volumetric flask was ± 0.005ml (at 27˚C) as provided by assuming that standard deviation isn't claimed by manufacturer with confidence interval limit. The standard value of uncertainty was calculated with triangular distribution. Hence, uncertainty related to discharge of solution from volumetric flask due to calibration u (V_{10cal}) is showed in Eq. (8)

$$u(V_{10cal})=\frac{0.005}{\sqrt{6}}=2.04\times10^{-3} \quad (8)$$

3.3.1.2 Repeatability

In experiment repeatedly filling and weighing volumetric flask, standard uncertainty of volumetric flask was established as 0.0014ml.

3.3.1.3 Temperature

The manufacturer has calibrated volumetric flask at the time of manufacturing at a temperature of 27˚C, while temperature in the laboratory varied in a range of Δt = ±4˚C. This distinction was overwhelmed by computing uncertainty value with estimation of temperature range and volume dilation coefficient. Volume expansion of liquid was taken into consideration, as it is very higher than expansion of volumetric flask. The volume expansion coefficient, γ, of Methanol is 1.49×10^{-3}/˚C. so, uncertainty for 10ml volumetric flask ΔV_{10} was calculated by Eq. (9)

$$\Delta V_{10}= V_{10}\times \gamma\times \Delta t \quad (9)$$

Where, ΔV_{10}, uncertainty of the 10 ml volumetric flask; V_{10} volume of the 10 ml volumetric flask; γ, volume dilation coefficient; Δt, temperature variation in the laboratory.

Hence, we get that uncertainty for volumetric flask of 10 ml is 0.0596 ml, also assuming temperature variation is rectangular distribution, standard uncertainty for 10ml volumetric flask due to the temperature effect will be u (V_{10temp}) as shown in Eq. (10).

$$u\,(V_{10temp}) = \frac{4\times 1.49\times 10-3\times 10}{\sqrt{3}} = 3.44\times10^{-2}\,\text{ml} \quad (10)$$

Thus, standard uncertainty due to discharge of solution from 10 ml volumetric flask was calculated according to Eq. (11) and was found to be 0.034ml. Standard relative uncertainty was calculated and shown in Eq. (12).

$$u(V_{10})=\sqrt{(u(V10-cal))2 + (u(V10-rep))2 + (u(V10-temp))2} \quad (11)$$

$$u\,(V10) = 0.0348\ \text{ml}$$

The Relative Standard Uncertainty will be:

$$\frac{u(V10)}{V10}=3.48\times10^{-3} \quad (12)$$

3.3.2 Uncertainty owing to concentration

After the scanning of standard solutions, values in terms of absorbance have been obtained and calibration curve was plotted as depicted by Equation (5). Regression equation of calibration curve was identified as, slope 0.0293 and intercept 0.0073 for ARP and slope 0.0258 and intercept 0.0275 for LMG. For the determination of calibration curve, six solutions have been measured three times (total no. of measurements n=18). The sample solution was measured ten times, and analytes concentration was obtained in synthetic mixture and results are represented. S_r and S_{xx} value were calculated as shown in Equation (3) and (4), which were additionally used to calculate standard relative uncertainty, due to concentration.

For ARP,

$S_{xx} = 1.18585\times10^{-11}$

$Sr = 7.982\times10^{-2}$

For LMG,

$S_{xx} = 1.18585\times10^{-9}$

$Sr = 2.506\times10^{-2}$

3.3.3 Uncertainty due to repeatability (precision)

Method validation results show the repeatability for determination of Aripiprazole and Lamotrigine in terms of percentage RSD. This equation can be used directly for calculation of Combined standard uncertainty.

U (Rep) = RSD

U (Rep) $_{ARP}$ = 0.004558

U (Rep) $_{LMG}$ = 0.005389

3.3.4 Uncertainty due to sample mass(m_{sample})

sample mass estimation has three types of uncertainty sources such as sensitivity, linearity, and repeatability. Mass of the sample was becoming expressed in kg for convenient detectability of results.

3.3.4.1 Sensitivity

The range of difference in weighed mass was very less and the same weighing balance was used each time. Hence, uncertainty due to sensitivity of balance can be neglected.

3.3.4.2 Linearity

As the manufacturer data indicated a linearity value is 0.00015 g for both drugs. Thus, to determine overall uncertainty value, standard uncertainty due to linearity was considered. A rectangular distribution was accepted to convert contribution of linearity. It was calculated and is expressed in Eq. (13)

$$u=\frac{0.00015\times10^{-3}}{\sqrt{3}}=8.66\times10^{-8}\ \text{Kg.} \quad (13)$$

3.3.4.3 Repeatability

Uncertainty associated with repeatability is found to be 0.00025 g for both ARP and LMG.

3.3.4.4 Calculation of relative standard uncertainty due to sample mass

Uncertainty due to sample mass u (m_{sample}) was calculated using linearity and repeatability as shown in Equation (14).

$$u(m_{sample})=\sqrt{2\times U(L)^2 + U(R)^2}=2.78\times10^{-7}\,\text{kg}$$

for ARP and LMG (14)

From the values of Eq. (14) the relative uncertainty due to sample mass in synthetic mixture was found to be 0.0279 for Aripiprazole and 0.0277 for Lamotrigine.

3.3.5 Uncertainty due to recovery of method

Results of recovery are assessed as percentage recovery from sample matrix of representative spiking. The value of recovery has been obtained from validation of method as examined earlier. When a 'spike' is utilized to estimate recovery, the recovery of analyte from the sample may differ from recovery of spike so that uncertainty must to be evaluated. It became evaluated as equation (6) and $u(C_{spike})$ is determined using equation (15) and results of uncertainty due to spiking concentration as standard are illustrated in Table 9.

$$U(C_{spike})=C_{spike}\times\sqrt{\frac{U(Cbal)^2}{(Cbal)^2}+\frac{U(v)^2}{(v)^2}} \quad (15)$$

Therefore, the standard relative uncertainty of method recovery was calculated using uncertainty due to mass of Aripiprazole and Lamotrigine (from balance), calibration of pipette, calibration of flask and temperature effect, which was found to be 3.19×10^{-6}, 0.0054, 0.0023 and 0.0348 respectively. Combined uncertainty due to these factors was found to be U(v)= 0.009124 (ARP) and 0.006291 (LMG) ml. Now using the equation (3) the relative standard uncertainty due to recovery of method has been calculated and results are illustrated in Table 9 for both drugs.

Table 9: Summary of contribution to the measurement uncertainty for determination of ARP and LMG

Drug	Parameter	Volume, V_{10} (ml)	Sample conc^n, C_{10} (M)	Mass sample m_{sample} (kg)	Recovery method	Repeatability
ARP	Value	10	1.063×10^{-9}	9.98×10^{-6}	98.56×10^{-2}	--
	Standard uncertainty, u(x)	3.48×10^{-2}	3.12×10^{-11}	2.78×10^{-7}	5.13×10^{-3}	4.55×10^{-3}
	RSU*, u(x)/x	3.48×10^{-3}	2.94×10^{-2}	2.79×10^{-2}	5.21×10^{-3}	4.55×10^{-3}
LMG	Value	10	1.049×10^{-8}	1.005×10^{-5}	102.12×10^{-2}	--
	Standard uncertainty, u(x)	3.48×10^{-2}	3.79×10^{-10}	2.78×10^{-7}	5.89×10^{-4}	5.38×10^{-3}
	RSU*, u(x)/x	3.48×10^{-3}	3.61×10^{-2}	2.77×10^{-2}	5.77×10^{-4}	5.38×10^{-3}

3.3.6 Combined Standard Uncertainty (CSU)

Determine the combined standard uncertainty of the measurement result by combining the individual standard uncertainties using the usual "root-sum-of-squares" method.

The values of all the parameters having impact on Aripiprazole and Lamotrigine determination are compiled up in table 9 for Synthetic mixture.

These values were further used to calculate Aripiprazole and Lamotrigine quantity by using Eq. (1) and thus, we obtained a quantity of 1.05×10^{-5} mol/kg and 9.74×10^{-6} mol/kg for Aripiprazole and Lamotrigine, respectively.

The CSU is calculated as shown in Equation (16),

$$\frac{u(Q_{sample})}{Q_{sample}}=\sqrt{\left(\frac{u(V_{10})}{V_{10}}\right)^2+\left(\frac{u(C_{10})}{C_{10}}\right)^2+\left(\frac{u(m_{sample})}{m_{sample}}\right)^2+\left(\frac{u(R_m)}{R_m}\right)^2+\left(\frac{u(Rep)}{Rep}\right)^2} \quad (16)$$

$u(ARP_{sample}) = 4.18\times10^{-4}$ mol/kg

$u(LMG_{sample}) = 4.59\times10^{-4}$ mol/kg

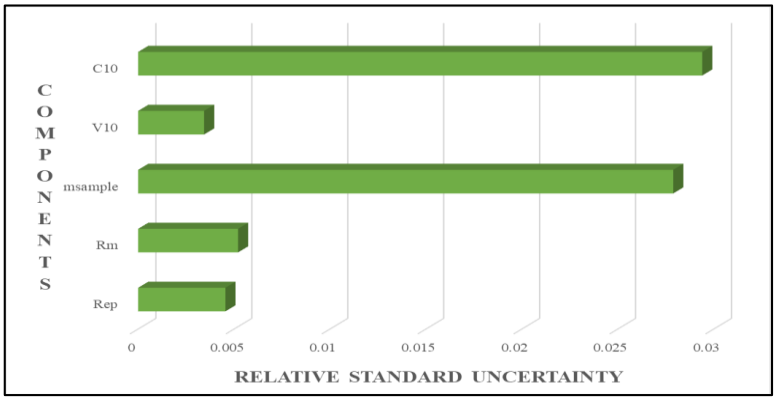

Fig 9: Uncertainty profile for Aripiprazole determination

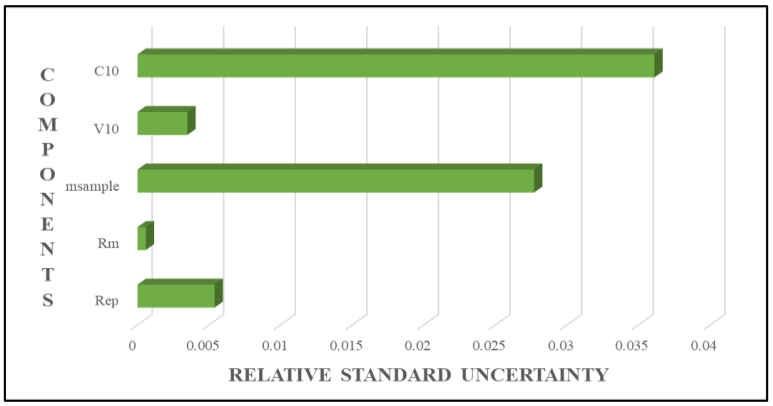

Fig 10: Uncertainty profile for Lamotrigine determination

3.3.7 Expanded Standard Uncertainty (EU)

Expanded uncertainty of Aripiprazole and Lamotrigine in synthetic mixture was obtained by multiplying the combined standard uncertainty by coverage factor, k=2, at confidence level of 95%, EU = $k \times U_c$ and the EU (ARP_{sample}) and EU (LMG_{sample}) are as shown,

EU (ARP_{sample}) = 8.37×10^{-4} mol/kg
EU (LMG_{sample}) = 9.19×10^{-4} mol/kg

The contribution of different components in uncertainty is shown individually for both drugs in Figure 9 and 10.

4. Conclusion

All analytical enterprise generates measurement data and hence, should necessarily utilize relevant statistical techniques and methods of inference, to present and interpret the data. The precise estimation of variability is challenging. Bayesian approaches offer a distinct way to the evaluation of variability by means of combining probabilities estimated from a detailed study of subprocesses. Developing a novel pharmaceutical product requires the designing and testing of manufacturing and measurement processes. The resulting process produces quality products when estimations demonstrative of product quality is on target with the least variance. In the current study, error propagation breaks up statistical methods that are efficiently applied. In this validation was depended on the "total error" approach, and it can be seen that the method is appropriate for a routine analysis of Aripiprazole and Lamotrigine in synthetic mixture with minimal errors. Furthermore, it also outlines the utilization of cause-effect analysis in order to estimate the uncertainty in the measuring of Aripiprazole and Lamotrigine from the synthetic mixture through UV-VIS Spectrophotometry. The estimation of uncertainty parameters proved to be a great way for the experimental model to obtain contribution of the uncsertainty in the analytical results. In the current study, concentration of the sample and mass of the sample are the primary contribution closer to uncertainty.

5. References

1. International organization for standardization, ISO-IEC, General Requirements for the competence of Testing and Calibrating Laboratories, 17025, Geneva, 1999.
2. Amanatidou Elisavet, Trikoilidou Eleni, Tsikriktsis Lazors, Foteini Katsiouli; Uncertainty in spectrophotometric analysis-error propagation break up, a novel statistical method for uncertainty management. A. Elsevier *et al*. 2011.
3. Eurachem guide: Quantifying Uncertainty in Analytical Measurement, Third Edition, S L R Ellison (LGC, UK), A Williams (UK); Laboratory of government Chemist, London, 2012, (isbn: 978-0-948926-30-3)
4. International Conference on Harmonization; Q2B Validation of Analytical Procedure: Methodology, Consensus Guideline, ICH Harmonized Tripartite Guidelines, 1997.
5. International Conference on Harmonization; Q2A Text on Analytical validation Procedures, Consensus Guideline, ICH Harmonized Tripartite Guidelines, 1995.
6. Martindale, The complete drug reference, 36th edition, pharmaceutical press, London, 2009, 1241.
7. Roger J. Porter, Antiseizures agents; Charles DeBattista, Antipsychotic Agents; Bertram G. Katzung MD, & Michael A. Rogawski, MD, Ph.D., Basic & Clinical Pharmacology, 14thedition, librarie du liban, Beirut, Lebanon, 2001, 422.
8. Bhattacharjee J, El-Sayeh HG: Aripiprazole typical antipsychotic drugs for schizophrenia. Cochrane Database Syst Rev. 2008; 16(3):CD006617.
9. National Center for Biotechnology Information, U.S. National Library of Medicine; 8600, Rockville Pike, Bethesda, MD20894, USA, https://pubchem.ncbi.nlm.nih.gov/compound/lamotrigine#section=Top & https://pubchem.ncbi.nlm.nih.gov/compound/Aripiprazole#section=Top
10. International council for harmonisation of technical requirements for pharmaceuticals for human use, ich harmonised guideline; bio pharmaceutics classification system-based; bio waivers; m9; draft version endorsed on, 2018.
11. Poli JE, Abrahamson BS, Lawrence XY. Summary workshop report: bioequivalence, bio pharmaceutics classification system and beyond, AAPS 10, 2008.
12. Bristol-Myers Squibb. A Phase IV Study of the Safety and Efficacy of Aripiprazole in Combination with Lamotrigine in the Long-Term Maintenance Treatment of Patients with Bipolar I Disorder with A Recent Manic or Mixed Episode; Clinical Trials. gov Identifier: NCT00277212, 2013.
13. Carlson BX, Ketter TA, Sun W, Timko K, McQuade RD, Sanchez R *et al*. Aripiprazole in combination with lamotrigine for the long-term treatment of patients with bipolar I disorder (manic or mixed): a randomized, multicentre, double-blind study (CN138-392), 2012.
14. abdel-monem E. ahmed bhagat, Micro determination of carvedilol and aripiprazole using haematoxylin dye in pure form and pharmaceutical formulations using spectrophotometry, Egypt; 10.7897/2230-8407.0911250, 2018.
15. Kalaichelvi R, Thangabalan B, Srinivasa Rao D, Jayachandran E. UV Spectrophotometric Determination of Aripiprazole in Bulk and Pharmaceutical Formulation; august, 2009, dx.doi.org/10.1155/2009/542919.
16. Alizadeh N, Khakinahad R, Jabbari A. Spectrophotometric determination of lamotrigine in pharmaceutical preparations and urine by charge-transfer complexation Pharmazie, 63, 2008.
17. Akamine Y, Yasui-Furukori N, Kojima M, Inoue Y, Uno T. A sensitive column-switching HPLC method for aripiprazole and dehydroaripiprazole and its application to human pharmacokinetic studies, japan, 10.1002/jssc.201000457, 2010.
18. Musenga M, Saracino D, Spinelli E, Rizzato G, Boncompagni E, Kenndler M. Raggi. Analysis of the recent antipsychotic aripiprazole in human plasma by capillary electrophoresis and high-performance liquid chromatography with diode array detection. 18358867, 2008.
19. Nadia Rezende B, Antonio Flavio M. Validated high-performance liquid chromatographic method for the determination of lamotrigine in human plasma, Journal of Chromatography B. 2000; 741(2):289-293.
20. Merce T, Miquel R, Santiago A, Jacint C. Optimized procedure for lamotrigine analysis in serum by high-performance liquid chromatography without interferences from other frequently coadministered anticonvulsants

Therapeutic Drug Monitoring. 2000; 22(5):621-625.
21. Jaber E, Neda G, Fatemeh A. Development and validation of a new HPLC method for determination of lamotrigine and related compounds in tablet formulations, Journal of Pharmaceutical and Biomedical Analysis. 2006; 40(4):999-1005.
22. Taylor BN, Kuyatt CE. guidelines for evaluating and expressing the uncertainty of NIST measurements results, physics laboratory national institute of standard and technology, NIST technical note 1297, USA, 1994.
23. Ulrich JC, Shihomastu HM, Hortellani MA, Sarkis J. Estimate of uncertainty of measurement in the determination of methyl mercury in fish sample by FIA-CV-AAS, XVIII Imeko World Congress metrology for a sustainable development, 2006.
24. Karan M, Kaushal R, Mashru RC. Estimation of the galanthamine using derivative spectroscopy in bulk drug and formulation. J Biomed I Sci. Eng. 2010; 3:439-441.
25. Kaur N, Mittal K, Nagar R, Nepali K, Thakkar A. A sensitive spectrophotometric method for the determination of pregabalin in pure and pharmaceutical formulation through benzoylation. IJRP. 2010; 1:175-180.
26. Mittal K, Dhingra T, Upadhyay A. Estimation of uncertainty for measuring galanthamine hydro bromide in pharmaceutical formulation using ultraviolet spectroscopy. J Pharm Res. 2013; 12:34-38.
27. Diaconu I, Aboul-Enein HY, Bunaciu AA *et al*., Estimation of uncertainty for measuring codeine phosphate tablets formulation using UV-VIS spectrophotometry, anal. Let, 2010, 43.
28. Mittal K, Patadiya R, Vora C, Mashru RC. A newfangled study using risk silhouette and uncertainty approximation for quantification of acyclovir in diverse formulation Elsevier, jpa-2011, 2014.
29. Dharmendra D, Karan M, Bhoomi P, Rajshree CM. Quantification of Cilostazol and Telmisartan in Combination Using Risk Profile and Uncertainty Contour: A Contemporary Validation Approach. 2015; 6(5):1000278. http://dx.doi.org/10.4172/2157-7064.1000278,