Available online on 15.03.2020 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

© 2011-18, publisher and licensee JDDT, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited

Open Access Research Article

A Highly Specific Colorimetric Method for On-Spot Determination of Lidocaine Using Color Kit and Application of Uncertainty Principles

Margi Gandhi, Rajashree Mashru*

Faculty of Pharmacy, G.H. Patel Building, Donors Plaza, The M.S. University Of Baroda, Vadodara, 390002, INDIA

ABSTRACT

A simple, accurate, precise, selective and detectable Colorimetric method was developed for estimation of Lidocaine in five different Pharmaceutical Formulations. The method is an azo C-coupling reaction where Lidocaine undergoes series of reaction and finally couples with resorcinol to form yellow color azo compound. The colored complex was measured at 430 nm. Beers law was obeyed in concentration range of 0.05-0.8 ug/ml. The method was validated and was found to be accurate, precise and robust with limit of detection and quantification to be 0.014 and 0.045 ug/ml. The Color kit was also developed for On-Spot detection of Lidocaine. Measurement uncertainty principles were also adopted to obtain reliable results where the process of uncertainty started from specifying measurand, then identifying uncertainty sources by cause-effect diagram, quantification of these sources of uncertainty and then finally calculating combined standard uncertainty and expanded uncertainty. In the present experiment, Concentration of sample and mass of sample was the major contributor towards uncertainty for all five Formulations. From the five formulations combined standard uncertainty of Transdermal patch was high, followed by Aerosol, Ointment, Gel and Injection.

Keywords: Lidocaine, Uncertainty, Transdermal patch, Aerosol, Injection, Ointment, Gel

Article Info: Received 03 Jan 2019; Review Completed 12 Feb 2020; Accepted 24 Feb 2020; Available online 15 March 2020

Cite this article as:

Gandhi M, Mashru R, A Highly Specific Colorimetric Method for On-Spot Determination of Lidocaine Using Color Kit and Application of Uncertainty Principles, Journal of Drug Delivery and Therapeutics. 2020; 10(2):86-96
http://dx.doi.org/10.22270/jddt.v10i2.3970

***Address for Correspondence:**

Rajashree Mashru, Faculty of Pharmacy, G.H. Patel Building, Donors Plaza, The M.S. University Of Baroda, Vadodara,

INTRODUCTION

Lidocaine (LID) is chemically acetamide, 2-(diethylamino)-N-(2,6-dimethylphenyl)-;2-(Diethylamino)-2,6-acetoxylidide. It acts as local anesthetics by interfering with the propagation of peripheral nerve impulses by blocking the sensation of pain. LID is, also employed in spinal anesthesia and as an antiarrhythmic drug. [1]

Figure 1: Structure of Lidocaine

Depending on the type of aromatic chain, local anesthetic can be divided into two main classes: amide (e.g., articaine, bupivacaine, lidocaine, & ropivacaine) and ester (e.g., benzocaine, cocaine, proparacaine, & tetracaine). Compared to ester anesthetics, amide local anesthetics are more commonly used in clinics because of relatively lower allergic reactions of human associated to amide local anesthetics. Apart from that, amide local anesthetics have better lipid solubility, higher potency and longer duration of action than the ester type.[1] The structure of Lidocaine is given in Figure 1

Various analytical methods are available in the literature for estimation of LID in biological and pharmaceutical samples which includes GC[2,3], Spectrophotometric determination of Lidocaine in pharmaceuticals [4], with bromocresol purple[5], bromocresol green[6] , sodium nitroprusside[7], Methylene Blue[8].

No method is reported in the literature for estimation of LID by using resorcinol as the coupling reagent. Hence, it is proposed to use resorcinol as coupling reagent for the estimation of the lidocaine by Spectrophotometry. The method is simple, rapid, reproducible, precise, and needs no extraction or heating, colour development is instantaneous, and the color is stable for more than 24 hours. Further, the controlling of experimental conditions is minimum [9]. The

method was also validated according to current ICH guidelines[10]

Also application of uncertainty principles to this developed Colorimetry was performed.

Measurement uncertainty [11,12,13,14]

Analysis results are affected by random errors whose magnitudes depend on the measurement conditions. In order to determine these error sizes, measurement uncertainty of the analysis must be expressed together with the measured value, thus providing the result as a range of values with an accepted level of confidence. Measurement uncertainty is defined as a non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used.[11] for the uncertainty estimation, the steps involved start with measurand Specification and end with expanded uncertainty (EU) calculation.[12] Thus ,the current study aims to adopt a simple methodology for quantification of uncertainty components and Combined Standard Uncertainty is Presented by assessing these computations for colorimetric measurement of Lidocaine in its different pharmaceutical formulation.[12,13]

MATERIALS AND METHODS

Gift samples of standard Active Pharmaceutical Ingredient-Lidocaine were provided by SIDMAK LABORATORIES (INDIA) PVT.LIMITED. All chemicals used in the present study were of analytical grade. The pharmaceutical samples used in the present study include Lidocaine 5% ointment, lignocaine hydrochloride injection 2%, Lidocaine spray 10%w/w, Lidocaine hydrochloride gel 2%, Lidocaine patch 5%.

Instrumentation

Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with Shimadzu UV-Probe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-800 nm. The samples were weighed on electronic analytical balance (A×120, Shimadzu).

Reagents

LID stock solution (500ug/ml)

5 mg of drug was dissolved in 10 ml methanol as LID is practically insoluble in water. A series of standard solutions from stock were prepared by a suitable dilution of stock standard solution with distilled water to get final concentration of 10 ug/ml.

HCl (0.4%w/v): 0.4 ml of concentrated hydrochloric acid was measured out and made upto 100 ml with distill water.

$NaNO_3$ (0.1%w/v): 0.1g of sodium nitrate was dissolved in 100ml of distill water.

NaOH (2.5%w/v): 2.5 g of sodium hydroxide was dissolved in 100 ml of distill water.

Resorcinol (0.5%w/v): 0.5g of resorcinol was dissolved in 100 ml distill water.

Preliminary Tests

10 ug/ml was prepared by serial dilution of standard stock solution. Aliquots ranging from 0.05-0.8ug/ml were transferred into series of 10 ml volumetric flask. To each flask 0.5ml HCl(0.5%w/v),1ml of NaNO3 (0.1%w/v), NaOH(2%W/V) and 0.5 ml Resorcinol (0.5%w/v) was added. The volumes was made up to mark with distil water. The absorbance of yellow chromogen was measured at 430nm against reagent blank which was prepared in similar manner without LID.

Optimum Conditions

To achieve the optimum conditions for this method, the following parameters were studied.

1) Effect of Concentration of HCl

Various concentration of HCl was optimized. It was observed that the maximum color intensity and highest absorbance values were recorded in 0.4%w/v of HCl shown in Figure 2

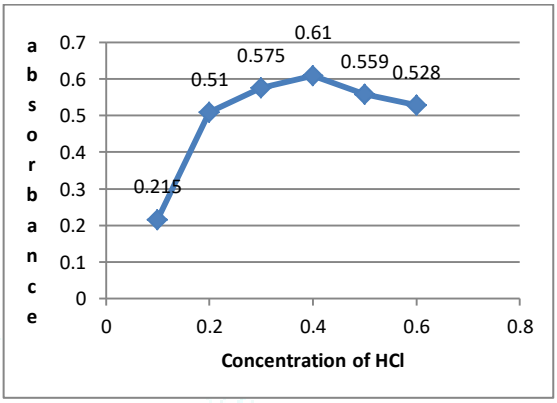

Figure 2: Optimization of Concentration of HCl

2) Effect of HCl volume(ml)

The effect of different volumes of HCl to obtain maximum sensitivity was investigated. The volume of the HCl required to obtain maximum absorption was 0.5 mL, as shown in Figure 3

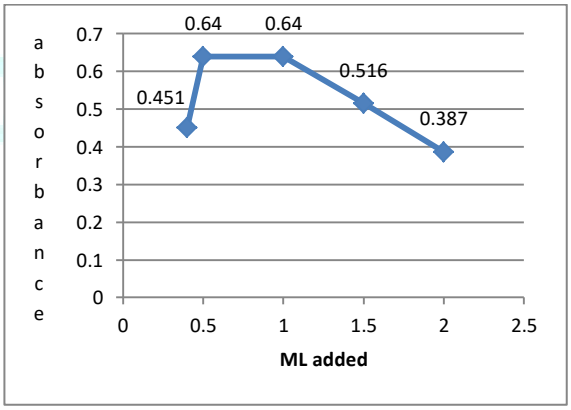

Figure 3: Optimization of ML of HCl added

3) Effect of Concentration of sodium nitrite

Various concentration of sodium nitrite was optimized. It was observed that the maximum color intensity and highest absorbance values were recorded in 0.1%w/v of sodium nitrite. The results are shown in Figure 4

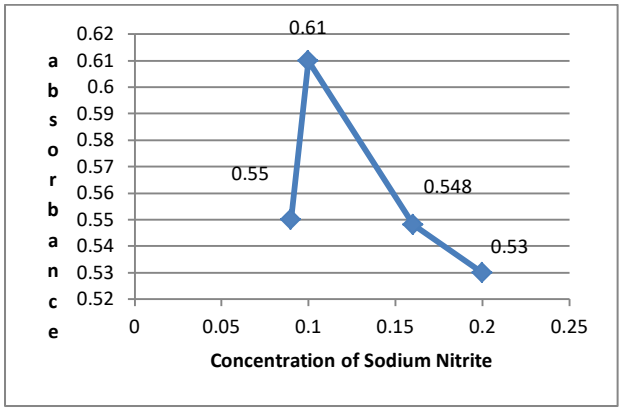

Figure 4: Optimization of Concentration of Sodium Nitrite

4) Effect of sodium nitrite Volume (ml)

The effect of different volumes of $NaNO_3$ to obtain maximum sensitivity was investigated. The volume of the $NaNO_3$ required to obtain maximum absorption was 1 mL, as shown in Figure 5

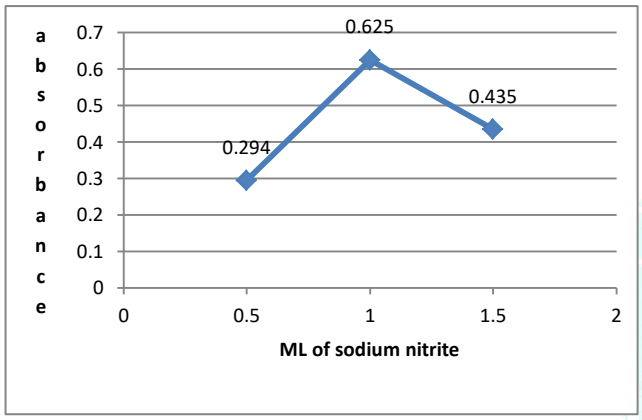

Figure 5: Optimization of ML of Sodium Nitrite Added

5) Effect of Concentration of sodium hydroxide

Various concentration of NaOH was optimized and it was found out that maximum absorbance was found at 2.5%w/v, as shown in Figure 6

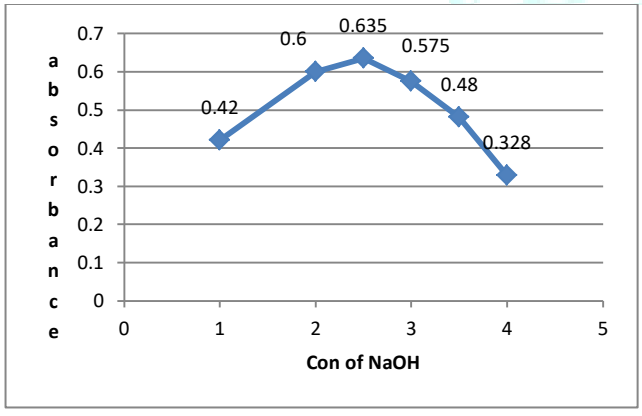

Figure 6: Optimization of Concentration of NaOH

6) Effect of sodium hydroxide Volume (ml)

The effect of different volumes of NaOH to obtain maximum sensitivity was investigated. The volume of the NaOH required to obtain maximum absorption was 1 ml, as shown in Figure 7

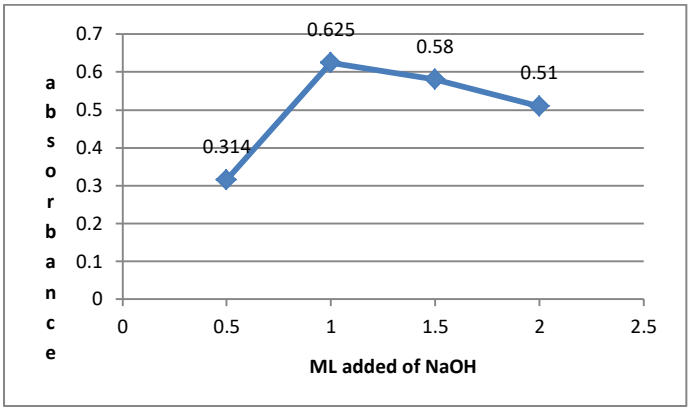

Figure 7: Optimization of ML of NaOH added

7) Effect of Concentration of Resorcinol

Various concentration of resorcinol was investigated and it was found out that maximum color intensity was found at 0.5%w/v of resorcinol, as shown in Figure 8

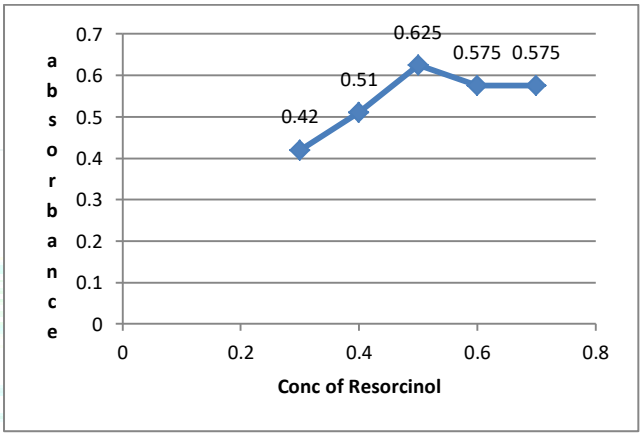

Figure 8: Optimization of Concentration of Resorcinol

8) Effect of Resorcinol Volume (ml)

The effect of different volumes of Resorcinol to obtain maximum sensitivity was investigated. The volume of the Resorcinol required to obtain maximum absorption was 1 ml, as shown in Figure 9

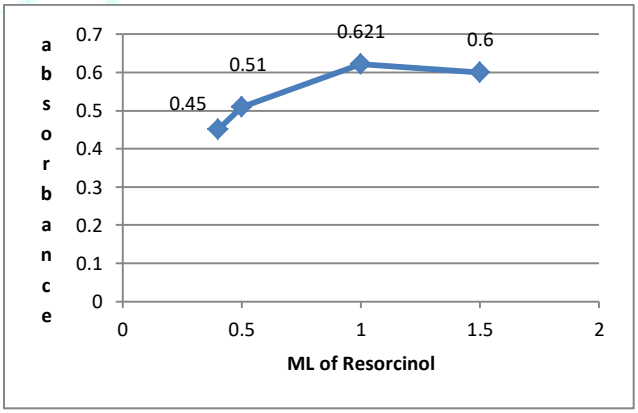

Figure 9: Optimization of ML of Resorcinol Added

Recommended Procedure

From standard stock solution (500ug/ml) of LID, series of dilutions was done to obtain 10ug/ml of LID. Aliquots ranging from 0.05-0.8ug/ml were transferred into series of 10 ml volumetric flask. To each flask 0.5ml HCl (0.4%w/v), 1ml of $NaNO_3$ (0.1%w/v), 1 ml of NaOH (2.5%w/v) and 1ml of Resorcinol (0.5%w/v) was added. The volumes were

made up to mark with distill water. The absorbance of the yellow colored chromogen was measured at 430 nm against reagent blank which was prepared in the similar manner without LID.

Reaction mechanism

LID is an amide local anesthetic where it is hypothesized that it is an azo C-coupling reaction were Lidocaine reacts with water to form 2,6 Dimethyl Xylidine which reacts with nitrous acid formed insitu by reaction of sodium nitrite and hydrochloric acid to form benzene diazonium chloride. This undergoes coupling reaction with resorcinol using sodium hydroxide as catalyst to form yellow color azo compound. Thus the intensity of color formed is directly proportional to amount of LID present.

RESULT AND DISCUSSION

Validation Parameters for the Proposed Method[10]

1) Linearity

For preparing calibration graph of LID, spectra of calibration standards (0.05-0.8ug/ml) were recorded in the range of 200 to 800, under the optimum experimental conditions and the absorbance vs. concentration plot was found to be a linear plot as shown in Fig 10

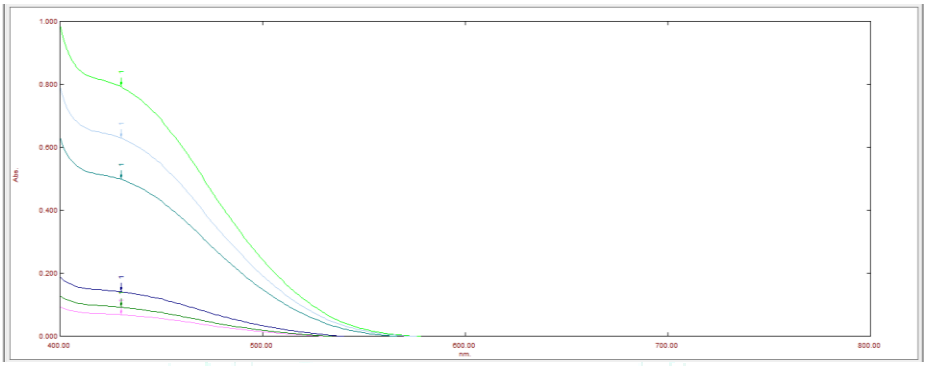

Figure 10: UV Spectrum Showing Linearity of the Proposed Colorimetric Method

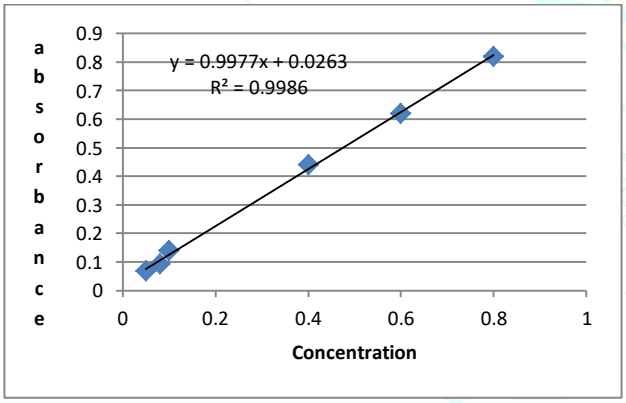

Figure 11: Linearity Graph of Lidocaine

Table 1: Statistical data for the regression equation of the proposed method

PARAMETERS	VALUE
Analytical wavelength(nm)	430
Linearity range (ug/ml)	0.05-0.8
Regression equation	Y=0.997x+0.026
Correlation co-efficient(R^2)	0.998
Slope	0.997
Intercept	0.026
Detection limit (ug/ml)	0.014
Quantification limit (ug/ml)	0.045

Precision

Reproducibility of methods was checked by performing intra-day precision (three times a day) and inter-day precision (repeated triplicates for three consecutive days). Results are expressed in terms of standard deviation and %Relative standard Deviation (%RSD) as shown in Table 2. It can be observed that the %RSD was less than 2 for the proposed methods.

Table 2: Precision Data of Lidocaine

	SD	%RSD
Intraday precision	0.002082	1.469
Interday precision	0.001528	1.088

Accuracy

To check the Accuracy of the proposed methods, Recovery studies were carried out at three deferent level of standard addition 80%, 100% and 120%. Results of Recovery studies are shown in Table 3. %Recovery was the average of three determinations at each standard addition level. %Recovery for the proposed methods was found to be between 95-105% which proves that the method was accurate.

Table 3: Accuracy Data of Lidocaine

% spiking	Conc actual(ug/ml)	Conc added(ug/ml)	Conc recover(ug/ml)	% Recovery ± SD*
80	0.1	0.08	0.083	103.7±0.00577
100	0.1	0.1	0.099	99±0.0022
120	0.1	0.12	0.119	99.16±0.0152

* Average ± SD of (n= 3) experiment

Robustness

Robustness was performed by deliberately changing method parameters (wavelength ±2nm) to find out indication of its reliability during normal usage. [10]

Table no 4: Robustness Data of Lidocaine

S.N.	FACTOR Wavelength (±2nm)	SD	%RSD
1	428 430 432	0.0015	1.08

Ruggedness

Ruggedness studies were performed by altering analyst and instrument to find out ability of analytical method to remain unaffected by small variations in method parameters. [10]

Table 5: Ruggedness Data of Lidocaine

Sr.no	Parameters		SD	%RSD
1	Analyst	1	0.0005	0.41
		2		
2	Instrument	UV1700	0.002	1.49
		UV1800		

Applicability of proposed method

The proposed method was applied for quantification of five different marketed formulations. The formulation extraction process is mentioned below. Base concentration selected was 0.1ug/ml

1) Aerosol, Ointment, Gel

An amount equivalent to 5mg (Two sprays for Aerosol) was taken and dissolved in 10 ml of methanol to get 500ug/ml of stock concentration.

The solution was sonicated for 5-10 min and then filtered through 0.22um syringe filter. Further it was diluted according to dilution scheme followed in the proposed method and colorimetric estimation was performed.

2) Lidocaine patch 5%

An amount equivalent to 5 mg(0.1g) was taken in 10 ml of Dipotassium monohydrogen phosphate buffer 10 mM of ph 7.2 and was magnetically stirred for 2 hours. The solution was then sonicated for 15 minutes and was then filtered through whatman filter paper. Further it was diluted according to dilution scheme followed in the proposed method and colorimetric estimation was performed. The results of the assay are given in table 6

Table 6: Assay Results of Formulations

Sr.no	Marketed formulations of LID	Taken (mg)	Recovered(mg)	% Recovery
1	Ointment (5%)	5	5.15	103
2	Aerosol (10%)	5	5.065	101.3
3	Gel (2%)	5	5.1	102
4	Injection	5	4.96	99.29
5	Patch (5%)	5	4.75	95

A Cost Effective Color Kit For On Spot Determination Of Lidocaine From Formulation Was Designed.

The kit contains 4 bottle of reagents with the brochure mentioning the procedure to be followed along with color card which shows quantification relationship between intensity of color formed and amount of LID present. Thus using this color kit u doesn't need to have UV spectrophotometer every time to quantify Lidocaine.

Recommended Procedure for Color Kit

This color kit can detect Lidocaine present in very minute amount (0.05-0.8ug/ml) which makes this colorimetric technique highly specific method for On-Spot determination.

The sample containing Lidocaine was transferred into 10 ml volumetric flask. To each flask 10 drops of Hcl (bottle no 1), 15 drops of sodium nitrite (bottle no 2), 15 drops of sodium hydroxide(bottle no 3) and 10 drops of resorcinol (bottle no 4) was added. The volumes were made up to mark with distill water (bottle no 5). The intensity of yellow color was used for quantification of Lidocaine using color card.

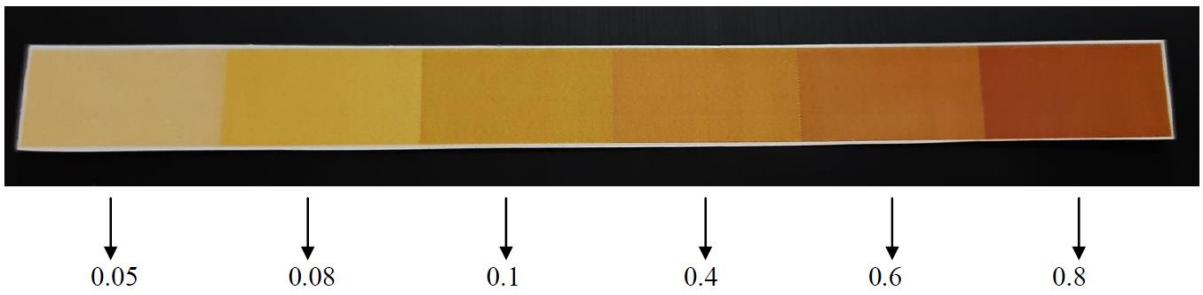

Figure 12: Color Card to Quantify Lidocaine from Range (0.05-0.8 ug/ml)

Estimation of measurement uncertainty [12,13,14]

Identification and quantification of different uncertainty parameters

Although the method was validated, still there were some doubts in the results, as few factors were not included in the validation such as errors during mass of sample taken etc. So

Uncertainty estimation was carried out starting with the identification of sources of uncertainty and compiled up with the Combined Standard Uncertainty and Expanded Uncertainty results.[12,13]

Identification of sources of uncertainty

Construction of the cause and effect diagram

In order to list uncertainty sources, it is very convenient to use the cause and effect diagram because it shows how the sources link to each other and indicate their influence on the result.

So a cause and effect diagram was constructed as shown in Fig. 13, which points out the different sources which may affect the sample analysis measurement. These parameters are:

Uncertainty associated with standard and test solution preparation V, uncertainty due to Concentration of analyte C, uncertainty associated with sample mass measurement Msample, uncertainty due to Recovery of method R and Precision of method P. These all parameters contribute to the overall uncertainty in final analytical results in marketed formulations. This diagram will also help in resolving any repeatability of components in uncertainty. These parameters are shown in Eq.(1)

$$\text{Lidocaine}_{\text{sample}} = C^* V^* 10^{-3} / M_{\text{sample}} R \quad (1)$$

Where, Lidocaine sample, Lidocaine quantity (mol/kg); C, Lidocaine concentration in 10 mL

Volumetric flask (M); V, volume of 10 mL volumetric flask (mL); Msample, Lidocaine sample

Mass taken (kg); R, Recovery of method.

Now after identification, these sources were quantified and their individual effect on overall

Uncertainty was studied and compiled up in the form of CSU and EU by carefully choosing Coverage factor.[12,13]

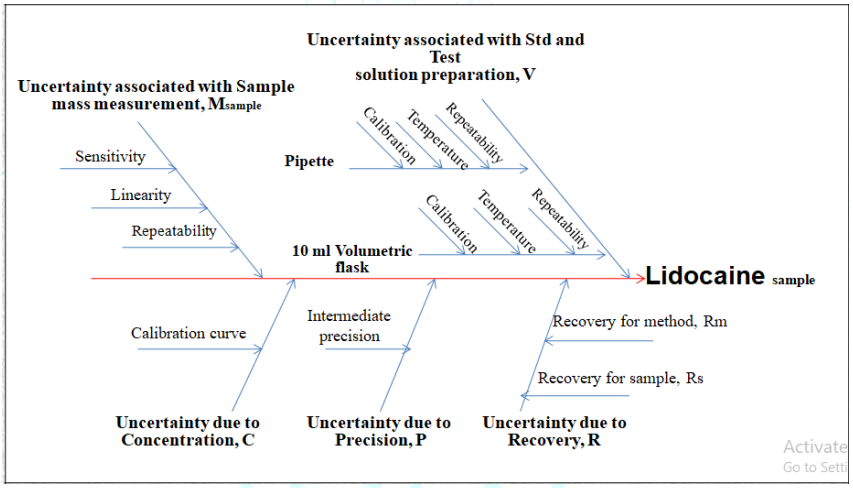

Figure 13: Cause-Effect Diagram

Effect of individual parameters on measurement uncertainty i.e Quantify uncertainty components

1) Uncertainty associated with standard and test solution preparation, (V)

The effect on volume of 1 mL pipette and 10 mL volumetric flask is mainly influenced by the three parameters i.e. calibration of the volumetric flask and pipette (at the time of manufacturing), repeatability and temperature.

1.1) Effect of volume of 10 mL volumetric flask - calibration of 10 ml volumetric flask

u (v_{cal})

Deviation of value from nominal volume for 10 mL volumetric flask was ± 0.009 mL (at 27°C)

by assuming that standard deviation is not claimed by Manufacturer with confidence interval limit, standard value of uncertainty can be calculated with triangular distribution. Thus, uncertainty associated with liberation of 10 mL volume of 10 mL volumetric flask due to calibration u (Vcal) is shown in Eq.(2)

$$u(V_{10\text{-cal}}) = \frac{SD}{\sqrt{n}} = \frac{0.009}{\sqrt{6}} = 0.0036 \text{ mL} \quad (2)$$

1.2) Repeatability u (V_{rep})

After filling and weighing of 10 mL volumetric flask, standard uncertainty of volumetric flask

was established at 0.0014 mL.

1.3) Temperature u (V_{temp})

The manufacturer has calibrated volumetric flask at the time of manufacturing at a temperature

of 27°C, while temperature in the laboratory varied within a range of Δt = ± 4°C. This

difference was overcome by calculating uncertainty value with estimation of temperature range

and volume dilatation coefficient. Volume expansion of liquid was taken into consideration, as it

is quite higher than expansion of volumetric flask. The volume expansion coefficient, γ, of water

is 2.1×10^{-4} /°C. Thus uncertainty for 10 mL volumetric flask ΔV10 was calculated by Eq. (3)

$$\Delta V_{10} = V_{10} \times \gamma \times \Delta t \quad (3)$$

Where Δ V10, uncertainty of the 10 mL volumetric flask; V10, volume of the 10 mL volumetric

flask; γ, volume dilatation coefficient; Δt, temperature variation in the laboratory. Thus, we obtain that uncertainty for volumetric flask of 10 mL is 0.0084 mL, also assuming

temperature variation is rectangular distribution, standard uncertainty for 10 mL volumetric flask

due to the temperature effect will be u ($V_{10\text{-temp}}$) as shown in Eq. (4).

$$u(V_{10\text{-temp}}) = \frac{4 \times 2.1 \times 10^{-4} \times 10}{\sqrt{3}} = 0.0048 \text{ mL} \quad (4)$$

Thus, standard uncertainty due to liberation of 10 mL volume of 10 mL volumetric flask was

calculated according to Eq. (5) and was found to be 0.0108 mL. Standard relative uncertainty

was calculated and shown in Eq. (6).

$$u(V_{10}) = \sqrt{(u(V_{10-cal}))^2 + (u(V_{10-rep}))^2 + (u(V_{10-temp}))^2} \quad (5)$$

$$u(V_{10}) = 0.0108 \text{ mL}$$

The standard relative uncertainty will be:

$$\frac{u(V_{10})}{V_{10}} = 0.001089 \text{ mL} \quad (6)$$

Similarly the standard relative uncertainty due to volume of 1 mL pipette was found to be 0.00338 mL

Thus the standard uncertainty due to discharge of volume for 1 ml pipette and 10 ml volumetric flask was found to be 4.4×10^{-2} ml and standard relative uncertainty was found to be 4.9×10^{-3} ml

2) Uncertainty associated with the sample mass measurement (Msample)

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

2.1) sensitivity

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

2.2) linearity

As the manufacturer data indicated linearity value is 0.0001g thus, to determine overall

Uncertainty value, standard uncertainty due to linearity was considered. A rectangular distribution was assumed to convert contribution of linearity. It was calculated and is expressed in Eq (7)

$$u = \frac{0.0001 \times 10^{-3}}{\sqrt{3}} = 5.77 \times 10^{-8} \text{ kg} \quad (7)$$

2.3) Repeatability

Uncertainty due to repeatability was calculated for ointment, gel, aerosol, injection, transdermal patch by weighing the formulation 10 times and taking into account standard deviation.

2.4) Calculation of relative standard uncertainty due to sample mass

The standard uncertainty due to sample mass is calculated by Eq (8)

$$u(M_{sample}) = \sqrt{2 \times U(L)^2 + U(R)^2} \quad (8)$$

Standard uncertainty for ointment, gel, injection, aerosol, transdermal patch was found to be 1.42×10^{-7} , 1.35×10^{-7} ,1.02×10^{-7}, 2.08×10^{-7} , 1.60×10^{-7}.

From standard uncertainty relative standard uncertainty for ointment, gel, injection, aerosol, transdermal patch was found to be 2.7×10^{-2}, 2.6×10^{-2} , 2.0×10^{-2}, 4.1×10^{-2} , 3.3×10^{-2}.

3) Uncertainty due to Concentration (C)

Uncertainty due to concentration for Lidocaine 5 different formulation was expressed as concentration uncertainty from calibration curve and is given by Eq (9)

$$U(C) = \frac{Sr}{b}\sqrt{\frac{1}{n} + \frac{1}{p} + \frac{(c - c_{avg})^2}{sxx}} \quad (9)$$

Where

$$Sr = \sqrt{\sum_{j=1}^{n} \frac{[yj - (a + bcj)^2}{n-2}}$$

$$Sxx = \sum_{j=1}^{n} (cj - c_{avg})^2$$

Sr, residual standard deviation

n, number of measurements used for calibration curve (n=18)

p, number of measurements used to obtain concentration of the sample;

c, lidocaine concentration in sample (M)

c_{avg} average of standard solution (M)

Yj, analytical signal of the measurement;

j, index for number of measurements made in order to obtain the calibration curve;

i, index for number of solution for calibration; b, slope of calibration curve (L/mol); a, calibration curve intercept

The sample solution was measured ten times (p = 10) and concentration was obtained from the calibration curve regression equation Eq (10)

$$y = mx + c \quad (10)$$

Where Y, absorbance of sample; c, calibration curve intercept; m, calibration curve slope; x,

Concentration of Lidocaine.

For the determination of calibration curve, six solutions have been measured three times (n=18). The sample solution was measured ten times, and the analyte concentration (Lidocaine) from ointment, gel, injection, aerosol, transdermal patch was measured by using Eq 9.

Also relative standard uncertainty was calculated whose results are shown in table no 7

4) Uncertainty due to Precision, P

Precision is divided into repeatability, intermediate precision, and reproducibility. Repeatability expresses the precision under same operating conditions over a short period of time. Whereas intermediate precision expresses within-laboratories variations: different days, different analysts, and different equipment. And Reproducibility expresses the precision between laboratories.

Here three different analysts were chosen to perform the precision uncertainty study shown in table 7

Table 7: Precision Uncertainty Measurement Data of Three Analysts

No of measurement, N	Analyst 1	Analyst 2	Analyst 3
1	0.101	0.102	0.099
2	0.098	0.094	0.105
3	0.105	0.098	0.104
4	0.098	0.102	0.098
5	0.1	0.099	0.099
6	0.102	0.1	0.102
7	0.104	0.101	0.104
8	0.098	0.105	0.101
9	0.105	0.102	0.102
10	0.101	0.101	0.1

Where the uncertainty due to precision was calculated from data obtained from intermediate precision measurement results of three analysts. One-way ANOVA test was also performed to evaluate the closeness of measurement results between three analysts whose results are shown in table 8

Table 8: Results Showing One-Way ANOVA Test

Source of variation	Sum of square	Degree of freedom	Mean square	F- value calculated	F-value tabulated
Between analyst	0.0000056	2	0.0000028	0.37	3.25
Within treatment	0.0002004	27	0.0000074		
Result	F tab > F cal				
Conclusion	No significant difference between analyst				

Uncertainty due to precision is calculated by equation (11)[10]

$$P = \frac{S.D}{Avg\sqrt{N}} \quad (11)$$

Where S.D = standard deviation of the analysts

Avg = average result of analyst with maximum deviation

N = Number of measurement made by analyst

Results for uncertainty due to precision for ointment, gel, injection, aerosol, transdermal patch is given in table no 9

5) Uncertainty due to Recovery of Method

Recovery (or bias) is a measure of the losses or interferences that arise from the difference between the amounts of analyze measured in the sample relative to that expected in the sample which gives an uncertainty that needs to be calculated [10]. Results of recovery are evaluated as percentage recovery from sample matrix of representative spiking. The value of recovery was obtained from validation of method as discussed earlier.

When a 'spike' is used to estimate recovery, the recovery of analyte from the sample may differ

from recovery of spike so that an uncertainty needs to be evaluated. So for all the formulations uncertainty associated with recovery of method was evaluated using Eq. (10) and recovery was simply calculated by Eq. (11).

$$R_m = \frac{C_{obs} - C_{native}}{C_{spike}} \quad (10)$$

Where Cobs: mean of replicate analysis of spiked sample

Cspike: nominal concentration of Lidocaine in spiked sample

C_{native} : observed concentration of analyte in the unspiked sample.

$$U(R) = R_m \times \sqrt{\frac{\frac{s^2_{obs}}{n+s^2_{native}}}{(C_{obs} - C_{native})2} + \left(\frac{u(C_{spike})}{C_{spike}}\right)^2} \quad (11)$$

Where S_{obs} = standard deviation of results from replicate analysis of spiked samples

N= number of replicates

U (C_{spike}) = standard uncertainty in concentration of spiked samples. It can be calculated by using Equation (12)

$$U(C_{spike}) = C_{spike} \times \sqrt{\frac{u(C_{bal})^2}{(C_{bal})^2} + \frac{u(v)^2}{(v)^2}} \quad (12)$$

U (C_{spike}) was calculated using uncertainty due to mass of Lidocaine (from balance), calibration of pipette, calibration of flask and temperature effect. Thus the combined uncertainty due to recovery of method, calculated by equation (11) for ointment, gel, injection, aerosol, transdermal system is given in table no 9

Table 9: Summary of all the parameters having impact on Lidocaine determination from its Formulations.

Parameters	Volume, V (ml)	Concentration, C (M)	Mass of sample, (Kg)	Recovery	Precision
Ointment					
Value	10	$4.39x10^{-10}$	$5.15x10^{-6}$	$103x10^{-2}$	
Std uncertainty	$4.4x10^{-2}$	$1.706x10^{-11}$	$1.42x10^{-7}$	$3.45x10^{-3}$	$5.0x10^{-3}$
Relative std uncertainty	$4.9x10^{-3}$	$3.8x10^{-2}$	$2.7x10^{-2}$	$3.33x10^{-3}$	$5.0x10^{-3}$
Gel					
Value	10	$4.35x10^{-10}$	$5.1x10^{-6}$	$102x10^{-2}$	
Std uncertainty	$4.4x10^{-2}$	$1.493x10^{-11}$	$1.35x10^{-7}$	$2.9x10^{-3}$	$5.2x10^{-3}$
Relative std uncertainty	$4.9x10^{-3}$	$3.4x10^{-2}$	$2.6x10^{-2}$	$2.8x10^{-3}$	$5.2x10^{-3}$
Injection					
Value	10	$4.224x10^{-10}$	$4.96x10^{-6}$	$99.29x10^{-2}$	
Std uncertainty	$4.4x10^{-2}$	$1.237x10^{-11}$	$1.02x10^{-7}$	$1.16x10^{-3}$	$4.0x10^{-3}$
Relative std uncertainty	$4.9x10^{-3}$	$2.9x10^{-2}$	$2.0x10^{-2}$	$1.10x10^{-3}$	$4.0x10^{-3}$
Aerosol					
Value	10	$4.309x10^{-10}$	$5.065x10^{-6}$	$101.3x10^{-2}$	
Std uncertainty	$4.4x10^{-2}$	$1.408x10^{-11}$	$2.08x10^{-7}$	$3.6x10^{-3}$	$5.0x10^{-3}$
Relative std uncertainty	$4.9x10^{-3}$	$3.2x10^{-2}$	$4.1x10^{-2}$	$3.5x10^{-3}$	$5.0x10^{-3}$
Transdermal patch					
Value	10	$4.055x10^{-10}$	$4.75x10^{-6}$	$95x10^{-2}$	
Std uncertainty	$4.4x10^{-2}$	$1.65x10^{-11}$	$1.60x10^{-7}$	$3.79x10^{-4}$	$6.0x10^{-3}$
Relative std uncertainty	$4.9x10^{-3}$	$4.0x10^{-2}$	$3.3x10^{-2}$	$3.9x10^{-3}$	$6.0x10^{-3}$

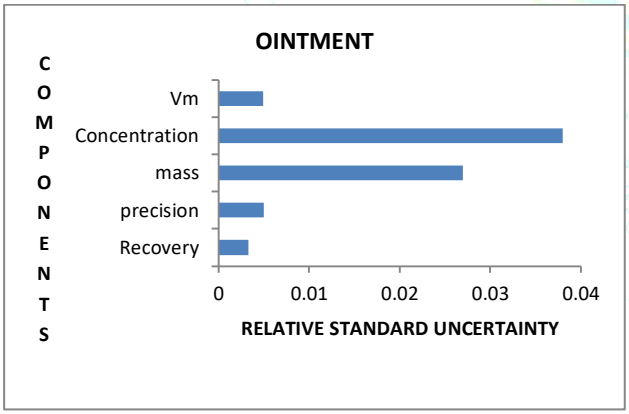

Figure 14: Graph Showing Contributions of Various Parameters towards Uncertainty of Ointment

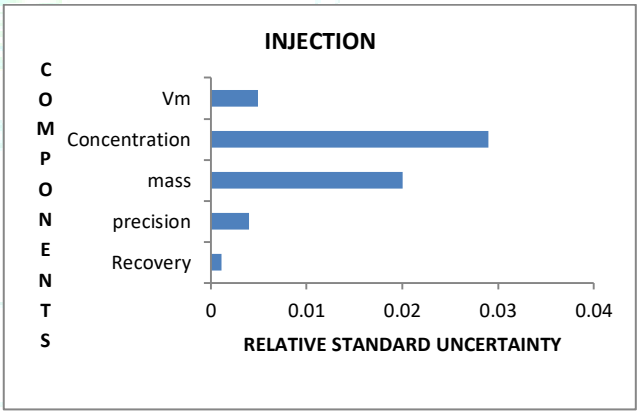

Figure 16: Graph Showing Contributions of Various Parameters towards Uncertainty of Injection

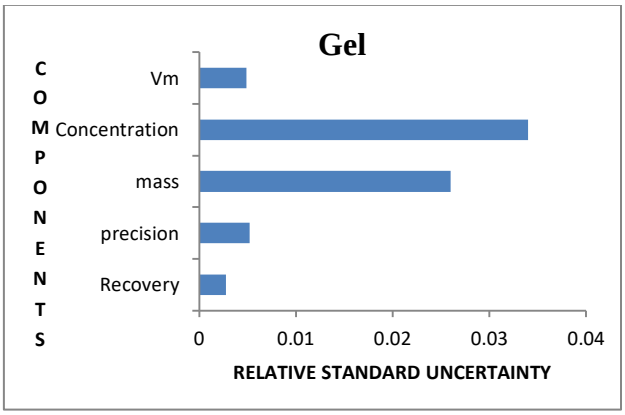

Figure 15: Graph Showing Contributions of Various Parameters towards Uncertainty of Gel

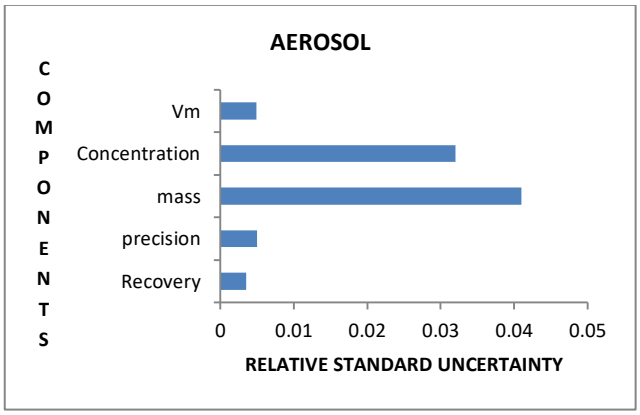

Figure 17: Graph Showing Contributions of Various Parameters towards Uncertainty of Aerosol

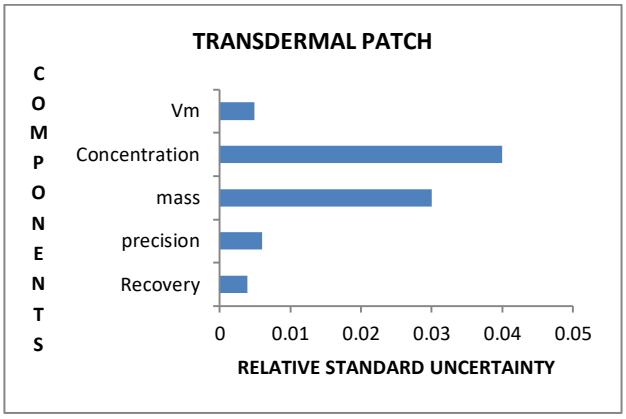

Figure 18: Graph Showing Contributions of Various Parameters towards Uncertainty of Transdermal patch

6) Combined Standard Uncertainty (CSU)

Following the estimation of individual or groups of components of uncertainty and expressing them as standard uncertainties, the next stage is to calculate the combined standard uncertainty [12, 13].

For determining the CSU of the measurement result, individual standard uncertainties were combined using the usual "root-sum-of-squares" method[12] shown in Equation (13)

The values of all the parameters having impact on Lidocaine determination from its formulation is complied in table no 7.

Further these values using equation (1) were used to quantify Lidocaine from Ointment, Gel, Injection, Aerosol, Transdermal patch and thus, we obtain quantity of $8.28x10^{-7}$, $8.32x10^{-7}$, $8.5x10^{-7}$, $8.39x10^{-7}$, $8.8x10^{-7}$ mol/kg respectively.

$$\frac{u(Q_{sample})}{Q_{sample}} = \sqrt{(\frac{u(V_{10})}{V_{10}})^2 + (\frac{u(C)}{C})^2 + (\frac{u(M_{sample})}{M_{sample}})^2 + (\frac{u(R_m)}{R_m})^2 + (\frac{u(P)}{P})^2} \quad (13)$$

7) Expanded Standard Uncertainty (EU)

The final stage is to multiply the combined standard uncertainty by the chosen coverage factor (k=2) in order to obtain an expanded uncertainty. The expanded uncertainty is required to provide an interval which may be expected to encompass a large fraction of the distribution of values which could reasonably be attributed to the measurand [12, 13].

Thus expanded uncertainty of Lidocaine from its different pharmaceutical formulations was estimated by multiplying the combined standard uncertainty by coverage factor, k = 2, at confidence level of 95%, the results were found to be as follows:

EU $_{ointment}$= $7.80x10^{-8}$ Mol/kg

EU $_{gel}$ = $7.23x10^{-8}$ Mol/kg

EU $_{injection}$ = $6.08x10^{-8}$ Mol/kg

EU $_{aerosol}$ = $8.82x10^{-8}$ Mol/kg

EU $_{transdermal\ patch}$ = $8.93x10^{-8}$ Mol/kg

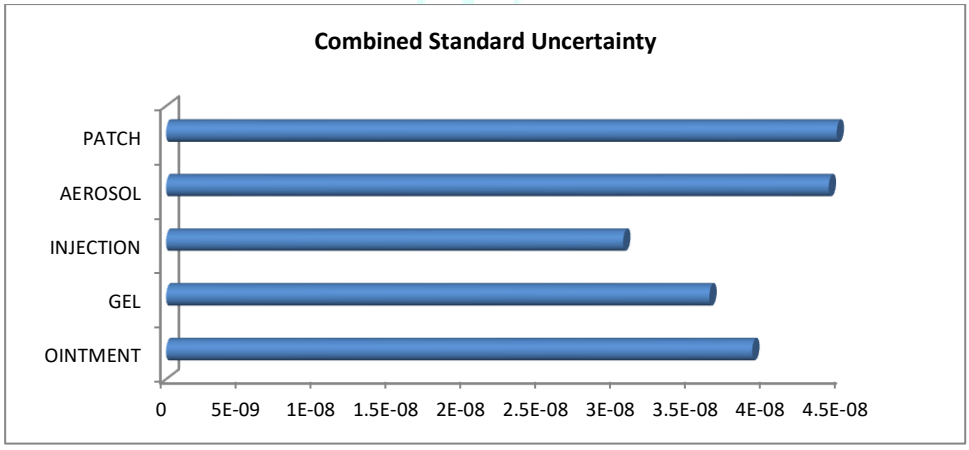

Figure 19 : Comparison Shown Between Combined Standard Uncertainties of Five Lidocaine Formulations

CONCLUSION

Colorimetric method developed for estimation of Lidocaine in five pharmaceutical Formulations was found to be accurate, precise, specific which was proved from validation data. Color kit was developed for On-Spot detection and quantification of Lidocaine. The estimation of uncertainty components proved to be a good way for the experimental model to obtain contribution of the uncertainty in the analytical results. In the present experiment, Concentration of sample and mass of sample was the major contributor towards uncertainty for all five Formulations which was proved from Figure 14-18. Also, from the five formulations combined standard uncertainty of Transdermal patch was high, followed by Aerosol, Ointment, Gel, Injection as depicted by Figure 19.

Injection < Gel < Ointment < Aerosol < Transdermal patch

REFERENCES

1) Lazeeza Sattar Omer, Rasul Jameel Ali, Extraction-Spectrophotometric Determination of Lidocaine Hydrochloride in Pharmaceuticals, International Journal of Chemistry; 2017; 9(4).

2) Edhorn, G. A; Pharx, M. Determination of lidocaine in whole blood by gas chromatography. Canad. Anaesth. Sot.. J. 1971; 18(2).

3) Sporkert , F; Pragst , F. Determination of Lidocaine in Hair of Drug Fatalities by Headspace Solid-Phase Microextraction. Journal of Analytical Toxicology. 2000; Vol. 24, July/August.

4) Janguo, L; Huangxia, J. Simultaneous determination of ethamsylate, tramadol and lidocaine in human urine by capillary electrophoresis with electrochemiluminescence detection. Electrophoresis. 2006; 27:3467–3474.

5) Zonghui, Q; Guangming, S; Rong, T; Lijun, P. Determination of the Content of Lidocaine Hydrochloride in Different Medium by Spectrophotometric Method. China Pharmacy. 2007; 34.

6) Sasa, I ; Dada, Y; Lnln, G. Spectrophotometric determination of lidocaine in some pharmaceutical preparations using bromocresol green. Pharmazie. 1975; 408. 30, 6, PMID 1161799.

7) Putta, M; Gurupadayya, B. M; Disha, N. S; Sama, S. Spectrophotometric determination of lidocaine in bulk and pharmaceutical formulations. Pharm Analysis & Quality Assurance. 2013; 02:573.

8) Qin, Z; Pu, L; Tan, R. Spectrophotometric Method for Determining Lidocaine Hydrochloride with Eosin Methylene Blue. Journal of Jiangxi Normal University. 2007; 03

9) Dr. A.J.Prataparedddy ,New Spectrophotometric Determination Ofmetoclopramide In Bulk And Pharmaceutical Dosage Form, Journal of Drug Discovery and Therapeutics, 2015; 3(35):01-04

10) ICH Q2 (R1): Validation of analytical procedure, Text and methodology, Geneva, International conference on Harmonization, 2005.

11) Sevtap Karakurt, Serdar Karakurt , Measurement Uncertainty Of Spectrophotometric Determination Of Hexavalent Chromium In Water , U.P.B. Sci. Bull., Series B, 2017; 79(4).

12) Karan Mittal, Riddhish Patadia, Chintan Vora, Rajashree C Mashru , **A** newfangled study using risk silhouette and uncertainty approximation for quantification of acyclovir in diverse formulation , Journal of Pharmaceutical Analysis, June 2014.

13) Krishna Jadav, Rajashree C. Mashru, Risk assessment and uncertainty appraisal for quantification of Aripiprazole and lamotrigine in combination: A neoteric validation approach, The Pharma Innovation Journal 2019; 8(4):538-548.

14) Eurachem/Citac, CG 4 Quantifying Uncertainty in Analytical Measurement Third Edition, 2012.