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

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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. 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

INTRODUCTION

Lidocaine (LID) is chemically acetamide, 2-(diethylamino)-N-(2,6-dimethylphenyl)-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]

Depending on the type of aromatic chain, local anesthetics can be divided into two main classes: amide (eg, articaine, bupivacaine, lidocaine, & ropivacaine) and ester (eg, benzocaine, cocaine, prorparacaine, & 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 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 1.2%w/v, 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.

NaNO₂ (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 distill 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](image)

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](image)

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: Effect of Concentration of sodium nitrite](image)
4) Effect of sodium nitrite Volume (ml)

The effect of different volumes of NaNO₃ to obtain maximum sensitivity was investigated. The volume of the NaNO₃ required to obtain maximum absorption was 1 ml, as shown in Figure 5.

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.

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.

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.

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.

Recommended Procedure

From standard stock solution (500μg/ml) of LID, series of dilutions was done to obtain 10μg/ml of LID. Aliquots ranging from 0.05-0.8μg/ml were transferred into series of 10 ml volumetric flask. To each flask 0.5ml HCl (0.4%w/v), 1ml of NaNO₃ (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
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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**

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](image)

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

**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=0.9977x+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      |

**Table 2: Precision Data of Lidocaine**

|                     | SD     | %RSD  |
|---------------------|--------|-------|
| Intraday precision  | 0.002082 | 1.46 |
| 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.\(^\text{[10]}\)

Table no 4: Robustness Data of Lidocaine

| S.N. | FACTOR          | SD    | %RSD |
|------|-----------------|-------|------|
| 1    | Wavelength (±2nm) | 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.\(^\text{[10]}\)

Table 5: Ruggedness Data of Lidocaine

| Sr.no | Parameters | SD    | %RSD |
|-------|------------|-------|------|
| 1     | Analyst    | 0.0005 | 0.41 |
| 2     | Instrument | 0.002  | 1.49 |

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 5mg(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\cite{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\cite{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:

\[ \text{Lidocaine}_{\text{sample}} = C \times V \times 10^{-3} / M_{\text{sample}} R \] \hspace{1cm} (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.\cite{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

\[ \text{u (V}_{\text{cal}}) = \frac{\text{SD}}{\sqrt{n}} = 0.009 \text{ mL} \] \hspace{1cm} (2)

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)

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 \( \Delta t = \pm 4°C \). 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, \( \gamma \), of water is \( 2.1 \times 10^{-4} /°C \). Thus uncertainty for 10 mL volumetric flask \( \Delta V_{10} \) was calculated by Eq. (3)

\[ \Delta V_{10} = V_{10} \times \gamma \times \Delta t \] \hspace{1cm} (3)
Where $\Delta V_{10}$, uncertainty of the 10 mL volumetric flask; $V_{10}$, volume of the 10 mL volumetric flask; $\gamma$, volume dilatation coefficient; $\Delta t$, temperature variation in the laboratory. Thus, we obtain that uncertainty for volumetric flask of 10 mL is 0.0004 mL, also assuming temperature variation is rectangular distribution, standard uncertainty for 10 mL volumetric flask due to the temperature effect will be $u (V_{10-temp})$ as shown in Eq. (4).

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

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).

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

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

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 $4.4 \times 10^{-2}$ mL and standard relative uncertainty was found to be $4.9 \times 10^{-3}$ 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)

$$u (M_{\text{sample}}) = \sqrt{2 \times 10}^2 + U(R)^2 \quad (8)$$

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

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

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)

$$U (C) = \frac{S_r}{p \sqrt{n} + \frac{1}{p} + \left(\frac{c - c_{\text{avg}}}{S_{xx}}\right)^2} \quad (9)$$

Where

- $S_r$, 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)
- $c_{\text{avg}}$, average of standard solution (M)
- $Y_j$, 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)\(^{10}\)

\[
P = \frac{s_D}{\text{Avg}^\text{N}} \tag{11}\]

Where \( s_D \) = standard deviation of the analysts
\( \text{Avg} \) = average result of analyst with maximum deviation
\( N \) = number of measurements 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).

\[
R_m = \frac{C_{\text{obs}} - C_{\text{native}}}{C_{\text{spike}}} \tag{10}\]

Where \( C_{\text{obs}} \) = mean of replicate analysis of spiked sample
\( C_{\text{spike}} \) = nominal concentration of Lidocaine in spiked sample
\( C_{\text{native}} \) = observed concentration of analyze in the unspiked sample.

\[
U(R) = R_m \times \sqrt{\frac{S_{\text{obs}}^2}{n(C_{\text{obs}} - C_{\text{native}})^2} + \frac{u(C_{\text{spike}})^2}{C_{\text{spike}}}} \tag{11}\]

Where \( S_{\text{obs}} = \) standard deviation of results from replicate analysis of spiked samples
\( n = \) number of replicates

\[
U(C_{\text{spike}}) = C_{\text{spike}} \times \sqrt{\frac{(u(C_{\text{spike}}))^2}{(C_{\text{spike}})^2} + \frac{u(C_{\text{native}})^2}{C_{\text{native}}}} \tag{12}\]

\( U(C_{\text{spike}}) \) 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**     | 10             | 4.39x10^-10          | 5.15x10^-6           | 103x10^-2| 5.0x10^-3 |
| Value            |                |                      |                      |          |           |
| 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**          | 10             | 4.35x10^-10          | 5.1x10^-6            | 102x10^-2| 5.0x10^-3 |
| Value            |                |                      |                      |          |           |
| Std uncertainty  | 4.4x10^-2      | 1.93x10^-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**    | 10             | 4.224x10^-10         | 4.96x10^-6           | 99.29x10^-2| 4.0x10^-3 |
| Value            |                |                      |                      |          |           |
| 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**      | 10             | 4.309x10^-10         | 5.065x10^-6          | 101.3x10^-2| 5.0x10^-3 |
| Value            |                |                      |                      |          |           |
| 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** | 10           | 4.055x10^-10         | 4.75x10^-6           | 95x10^-2  |           |
| Value            |                |                      |                      |          |           |
| 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^-4| 6.0x10^-3 |

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

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

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

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

\[
\begin{align*}
\text{u(Csample)} & = \sqrt{\left(\frac{\text{u(Vm)}}{V_{10}}\right)^2 + \left(\frac{\text{u(m)}}{\text{C}}\right)^2 + \left(\frac{\text{u(Msample)}}{M_{\text{sample}}}\right)^2 + \left(\frac{\text{u(Msample)}}{M_{\text{sample}}}\right)^2 + \left(\frac{\text{u(p)}}{p}\right)^2} \\
\text{(13)}
\end{align*}
\]

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 \(^{[12,15]}\).

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 for Ointment = 7.80x10^{-8} Mol/kg
- EU for Gel = 7.23x10^{-8} Mol/kg
- EU for Injection = 6.08x10^{-8} Mol/kg
- EU for Aerosol = 8.82x10^{-8} Mol/kg
- EU for Transdermal patch = 8.93x10^{-8} Mol/kg

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
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