# Sophisticated Planar Chromatographic Method Estimation and Validation for Newer Combination of Lidocaine HCl and Diltiazem HCl For Anal Fissure

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**Additional Declarations:** No competing interests reported.

## Abstract

Newer drugs and drugs combinations for treatment of different diseases are approved by regulatory authorities. Such new combination of existing drugs like Lidocaine HCl and Diltiazem HCl has been approved for anal fissure treatment. The present study represents validated method for estimation of Lidocaine HCl and Diltiazem HCl in a combined gel dosage form applying High- performance thin layer chromatography. The stationary phase used in this method was pre-coated silica gel G60 $F_{254}$ aluminium sheet (10 × 10 cm, 0.2 mm layer thickness), and the mobile phase was a mixture of toluene, methanol, ethyl acetate and two drops of ammonia (7: 2: 1% v/v/v). Resolved peaks of both drugs were obtained at the $R_f$ value 0.59 for Lidocaine HCl and 0.48 for Diltiazem HCl. Analytical wavelength of 220 nm was selected based on overlay UV spectra of both the drugs. The correlation coefficient ($r^2$) for Lidocaine HCl and Diltiazem HCl was found to be 0.9987 and 0.9980, respectively indicating a linear approach in the concentration range of 400−1200 ng/band for both. The method was found reproducible, accurate, precise, and robust for simultaneous estimation of Lidocaine HCl and Diltiazem HCl in bulk and pharmaceutical dosage form.

## 1. Introduction

Chemical name of Lidocaine HCl (LID) is 2- (diethyamino)-N-(2,6- dimethyphenyl) acetamide; hydrochloride and empirical formula is $C_{14}H_{23}ClN_2O$. (Fig. 1 **(a)**) [1]. Mechanism of action of LID is as local anesthetic and cardiac depressant used as an anti-arrhythmic agent.

Chemical name of Diltiazem HCl (DIL) is [(2S,3S)]-5-[3-(dimethylamino) ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3-dihydro-1,5-benzothiazepine-3-yl] acetate; hydrochloride and empirical formula is $C_{22}H_{27}ClN_2O_4S$. (Fig. 1 **(b)**) [2]. Mechanism of action of DIL is a calcium- channel blocker and vasodilator.

LID provides symptomatic relief by numbing the affected area and DIL works by relaxing the smooth muscles in the blood vessels. The combination of LID and DIL in rectal formulation offers both therapeutic and symptomatic relief in anal fissure; particularly beneficial for patients who are experiencing both pain and difficulty in healing [3].

Extensive study of the literature [4−21], it was discovered that many analytical techniques for LID estimation had been published, including ultraviolet, high performance liquid chromatography, RP-HPLC, HPLC-MS/MS, etc. Methods for analysing DIL had been documented, including Ultra violet, high performance liquid chromatography, high performance thin layer chromatography, etc. But only one UV spectroscopy method is available for estimation of LID and DIL simultaneously [22], no other method has been published for simultaneous estimation of LID and DIL by chromatographic method till date. The present study shows developed and validated high performance thin layer chromatographic method for simultaneous estimation of LID and DIL as per ICH Q2 (R1) guideline [23].

## 2. Experimental
## 2.1 Standard API, Chemicals and Materials

LID and DIL were provided as a gratis sample by reputed pharmaceutical companies of Gujarat, India. The marketed formulation available as a Gel Formulation is Crema-L gel (2% W/W of Lidocaine HCl and 2% W/W of Diltiazem HCl) was purchased from local pharmacy store. The acetonitrile, toluene, methanol, ethyl acetate and ammonia were purchased from SRL Chemicals Ltd. in Mumbai, Gujarat.

## 2.2 Analytical Wavelength Selection

LID and DIL were found freely soluble in acetonitrile. For analytical wavelength, individual solutions of LID and DIL at a concentration of 10 µg/ml were prepared and using UV- Visible double beam spectrometer UV-1900i, both solutions were scanned from 400 nm to 200 nm. After scanning the iso-absorptive point was found at 220 nm for determining both LID and DIL.

## 2.3 HPTLC System

A semi-automatic HPTLC instrument comprised of a Linomat 5 sample applicator (CAMAG, Muttenz, Switzerland), CAMAG TLC scanner IV and 100 µl applicator syringe (Hamilton, Bonaduz, Switzerland). HPTLC aluminium plates 10 cm × 10 cm, precoated with silica gel G60- $F_{254}$ (E. Merck, Darmstadt, Germany; provided by Anchrom Technologists, Mumbai, India) and Twin through chambers were used for chromatographic development.

## 2.4 Standard solution preparation

Both LID and DIL were accurately weighed in 10 mg quantity each and transferred to two separate 10 ml volumetric flasks, and dissolved with a little amount of acetonitrile. To bring the volume up to 10 ml, acetonitrile was added which gave 1000 µg/ml concentration of each. Further dilutions were made to obtain mixture containing 100 µg/ml concentration of LID and DIL in mixture, as standard working solution.

## 2.5 Calibration curve determination

From standard working solution, 4µl, 6µl, 8µl, 10µl and 12µl were applied using Hamilton syringe through CAMAG Linomat 5 sample applicator to perform calibration curve on HPTLC silica gel aluminium plate G60- $F_{254}$.

## 2.6 Validation

The developed HPTLC method was validated according to International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2 (R1) guideline.

### 2.6.1 Linearity

The concentration range of 400 ng/band to 1200 ng/band for LID and DIL were conducted for five times to determine the linearity relationship. A straight- line equation was observed from plot of peak area v/s concentration during estimation of the calibration curve.

### 2.6.2 Precision

The closeness of measurements obtained from homogeneous sample is known as precision. Six injections of the linearity range's middle concentration of 800 ng/band were applied to confirm repeatability parameter.

Intraday and Interday, were carried out to evaluate intermediate precision. Intraday indicates three determinations on same day while interday indicates three determinations on different days. For intermediate precision, three concentrations from linearity range- the lowest, the middle and the highest – 400ng/band, 800ng/band and 1200ng/band were selected and determinations was carried out. From peak areas obtained, % RSD were calculated.

### 2.6.3 Accuracy

Nearness of observed measurement to true value indicates accuracy. Standard spiking method is frequently used to confirm accuracy of method in which the standard solution was spiked at 3 levels 80%, 100% and 120% concentrations in the LID and DIL sample solution. The middle concentration of 800 ng/band was used for this parameter as 100%. The study was performed in triplicate and mean of peak area was used to find out % recovery.

### 2.6.4 Limit of Detection and Quantification

The lowest concentration which can detect the analyte inside the matrix, without quantifying is called limit of detection. The lowest concentration which can detect the analyte inside the matrix, with quantifying the analyte along with precision and accuracy is called limit of quantification. The equation used to calculate LOD & LOQ are as shown below.

LOD = 3.3 × σ/S & LOD = 10 × σ/S

Where σ is the standard deviation of y- intercepts of regression lines and S is the average slope of calibration curves.

### 2.6.5 Robustness

Deliberate small changes to optimized conditions such as chamber saturation time, run distance, wavelength, and mobile phase composition was introduced and outcomes were o observed. For both LID and DIL, the robustness of the method was assessed at 800 ng/band concentration in triplicate manner. From $R_f$ values mean and % RSD values were computed.

### 2.6.6 System suitability

An essential component of developing a method is testing the system's appropriateness to ensure that it is suitable for performing LID and DIL analyses. Prior to every validation run, the chromatographic system underwent a system appropriateness test. There were six identical injections of a system suitability standard solutions were made. For each of the six suitable injections, the retention factor ($R_f$), peak purity, and resolution factor were obtained.

### 2.6.7 Assay of marketed formulation

After precisely weighing the gel, quantity equivalent to 10 mg of LID and DIL, was transferred to a 10 ml volumetric flask. The aforesaid volumetric flask was filled with a few ml of acetonitrile, vortexed for ten minutes, sonicated for ten minutes, and the mixture was filtered through Whatman filter paper No. 45 to a second volumetric flask of 10 ml capacity. Acetonitrile was used to get the volume up to the required level, yielding 1000 µg/ml LID and DIL. A further dilution was made to produce 100 µg/ml LID and DIL. Concentration of 800 ng/band of LID and DIL were obtained by applying 8 µl on a TLC plate using a Hamilton syringe. The plate was developed under optimised developed conditions and developed plate was densitometric scan was performed. The regression equation used to carry out the quantification process.

# 3. Results and Discussion

## 3.1 Wavelength Selection

The sensitivity of the approach depends on the selection of an appropriate detecting wavelength. Standard LID and DIL solutions were scanned in the 400−200 nm UV range in the currently presented investigation. The spectra that were superimposed revealed that both medications absorb noticeably at 220 nm and therefore chosen as the detecting wavelength as shown in Fig. 2.

## 3.2 Mobile Phase Optimization

Toluene: methanol: acetonitrile: two drops of ammonia (7:2:1 v/v/v) was the mobile phase that gave symmetrical and selective peak for LID at $R_f$ 0.59 and DIL at $R_f$ 0.48 as shown in Fig. 3.

## 3.3 Validation

International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2 (R1) was followed to validate developed HPTLC method for quantification of LID and DIL simultaneously in bulk and gel dosage form. The results are mentioned and discussed as in below;

## 3.3.1 Linearity

The developed HPTLC method shown that the correlation coefficient of for LID and DIL were linear within the specified concentration range 400 ng/ band to 1200 ng/band. The overlay of the densitogram shown in Fig. 4. The Table 1 represents the regression analysis.

Table 1
Regression analysis of calibration curve

| Parameter | LID | DIL |
|---|---|---|
| Range (ng/band) | 400−1200 | 400−1200 |
| Regression coefficient ($R^2$) | 0.9987 | 0.9980 |
| Slope of regression | 3.5817 | 2.1483 |
| Standard deviation of slope | 0.04 | 0.01 |
| Intercept of regression equation | 1049 | 997.68 |
| Standard deviation of Intercept | 31.40 | 12.18 |

## 3.3.2 Precision

Lower % RSD values for LID and DIL were 0.12−0.47% and 0.36−0.58%, respectively for Intraday precision and Interday precision, % RSD values for LID and DIL were 0.16−0.70% and 0.47−0.80%, respectively indicates that method is precise for quantifying LID and DIL simultaneously. Table 2 displays the summary of validation parameters of proposed HPTLC method for LID and DIL.

Table 2
Result of validation parameter of RP-HPTLC method for Lidocaine HCl
and Diltiazem HCl

| Parameters | LID | DIL |
|---|---|---|
| Linearity (ng/band) | 400−1200 | 400−1200 |
| Retention factor | 0.59 | 0.48 |
| Detection limit (ng/band) | 28.93 | 18.72 |
| Quantitation limit (ng/band) | 87.67 | 56.74 |
| Accuracy (%) | 99.15% − 101.27 | 99.02% − 100.59% |
| Precision (%RSD) | | |
| Intra-day (n = 3) | 0.12−0.47 | 0.36−0.58 |
| Inter-day (n = 3) | 0.16−0.70 | 0.47−0.80 |
| Robustness | Robust | Robust |
| Solution stability | Stable for 24 hr | Stable for 24 hr |

### 3.3.3 Accuracy

Accuracy study using standard spiking method and through regression equation, % recoveries for LID and DIL were found to be 99.15-101.27% and 99.02-100.59%, repectively which were between the range of 98−102% indicating method is accurate.

### 3.3.4 Limit of Detection and Limit of Quantification

The LOD for LID and DIL were 28.93 ng/band and 18.72 ng/band, respectively. The LOQ values for LID and DIL were 87.67 ng/band and 56.74 ng/band, respectively. Lower values indicate the approached method is highly sensitive for both detection and quantification of LID and DIL in small quantities.

### 3.3.5 Robustness

The variation included to conduct robustness study was a minor shift in the chamber saturation time, run distance, wavelength, and mobile phase composition. For both medications LID and DIL, no significant change in the $R_f$ value or the bands' compactness was observed. The analysis of both medications is unaffected by the presence of excipients as well showing specificity of method. The robustness was assessed in triplicate manner at concentration of 800 ng/band for both LID and DIL. % RSD values were computed using obtained data as shown in Table 3.

Table 3
Robustness study for LID and DIL

| Parameters | Normal condition | Change in condition | Mean $R_f$ (n = 3) ± SD | | % RSD | | Mean Area (AU) (n = 3) ± SD | | % RSD | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | LID (800 ng/band) | DIL (800 ng/band) | LID (800 ng/band) | DIL (800 ng/band) | LID (800 ng/band) | DIL (800 ng/band) | LID (800 ng/band) | DIL (800 ng/band) |
| Change in wavelength (± 2) | 220 nm | 218 nm | 0.64 ± 0.005 | 0.43 ± 0.005 | 0.89 | 1.32 | 3928.0 ± 28.61 | 2730.0 ± 25.94 | 0.72 | 0.95 |
| | | 222 nm | 0.62 ± 0.005 | 0.41 ± 0.005 | 0.92 | 1.38 | 3934.3 ± 24.37 | 2763.6 ± 23.11 | 0.61 | 0.83 |
| Change in saturation time (± 5) | 30 mins | 25 mins | 0.61 ± 0.005 | 0.49 ± 0.005 | 0.93 | 1.17 | 3974.3± 18.77 | 2757.6 ± 39.87 | 0.47 | 1.44 |
| | | 35 mins | 0.61 ± 0.005 | 0.46 ± 0.005 | 0.93 | 1.24 | 3956.6 ± 10.69 | 2759.3± 32.25 | 0.27 | 1.16 |
| Change in run distance (± 5) | 80 cm | 75 cm | 0.64 ± 0.005 | 0.41 ± 0.005 | 0.89 | 1.38 | 3969.6 ± 5.50 | 2765.3 ± 31.02 | 0.13 | 1.12 |
| | | 85 cm | 0.60 ± 0.005 | 0.41 ± 0.005 | 0.95 | 1.38 | 3949.3 ± 10.78 | 2741.3 ± 33.23 | 0.27 | 1.21 |
| Change in mobile phase ratio (± 0.2) | 7:2:1: 2 drops ammonia | 6.8:2:1.2: 2 drops | 0.60 ± 0.005 | 0.42 ± 0.005 | 0.95 | 1.35 | 3850.6 ± 24.44 | 2808 ± 28.58 | 0.63 | 1.01 |
| | | 7.2:2:0.8: 2 drops | 0.64 ± 0.005 | 0.42 ± 0.005 | 0.89 | 1.36 | 3960.6 ± 23.62 | 2806.6 ± 33.20 | 0.59 | 1.18 |

## 3.3.6 Assay of Marketed formulation

The presented method was applied to the marketed gel dosage form for qualitative and quantitative estimation. It was discovered that the percentage of LID was 99.98% ± 0.28 and of DIL was 99.97% ± 1.40. Figure 5 shown the specificity of LID and DIL. Peak purity spectra of LID and DIL shown in Fig. 6.

## 4. Conclusion

The current economic study, emphasis on HPTLC approach is a straightforward, accurate, precise, and robust measurement of LID and DIL in bulk and in combination. The combination is useful to treat anal fissures. At 220 nm analytical wavelength, the method was found to be linear for LID and DIL in the concentration range of 400–1200 ng/band under the optimum mobile phase comprised toluene: methanol: ethyl Acetate: 2 drops of ammonia (7: 2: 1%v/v/v) along with silica gel $G60F_{254}$. ICH Q2 (R1) guideline was employed to validate the parameter and all parameters were found to be validated by proposed HPTLC method. The limit of quantitation was found to be 87.67 ng/band for LID, and for DIL was found to be 56.74 ng/band. % recovery for LID was found to be 99.15–101.27% and for DIL was determined to be 99.02–100.59%. The presented HPTLC method is beneficial in terms of less solvent consumption, multiple analysis in single run, and economic as compared to liquid chromatographic. Validated HPTLC method can be easily successfully utilize for quantification of LID and DIL in bulk and pharmaceutical dosage form.

## Declarations

## Author Contribution

Maitri Shah and Hetaben Kachhiya contributed to design of the research work and wrote the main manuscript.Jinal Tandel, Usmangani Chhalotiya and Dimal Shah prepared all figures and tables.Adarsh Patelia and Mehul Patel reviewed the tables and approved the version to be published.All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Acknowledgement

## Data Availability

Data is provided within the manuscript and can be available on request basis.

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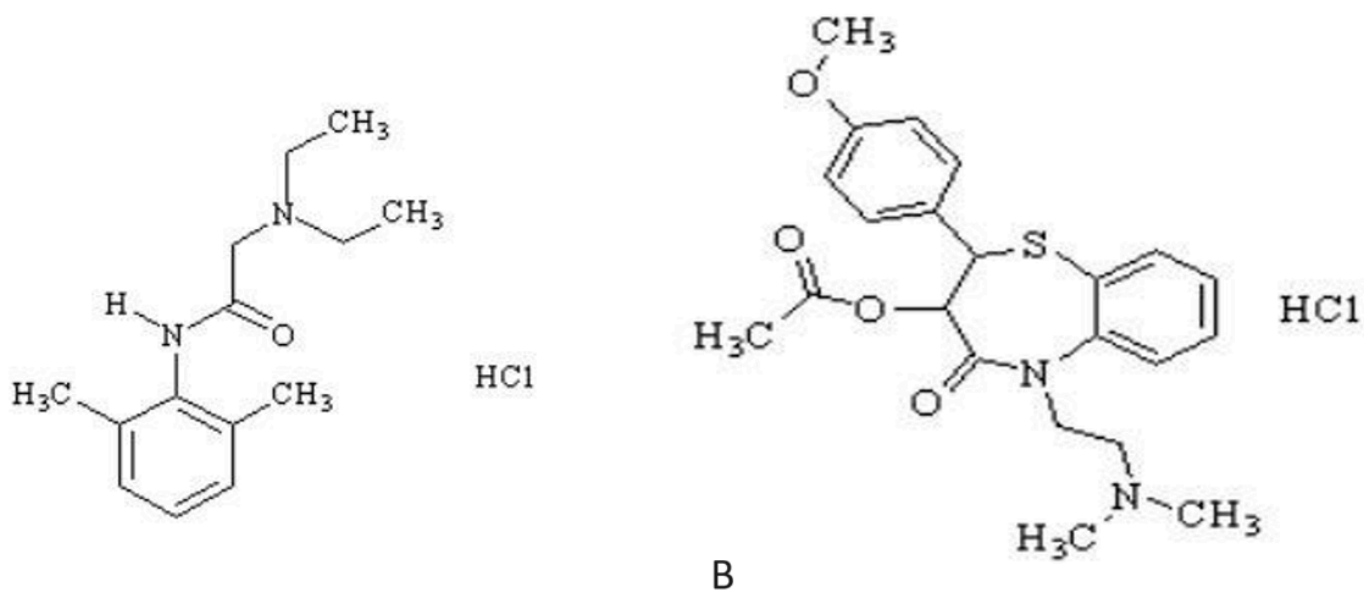
## Figures



**Figure 1**

(a): Chemical structure of Lidocaine HCl

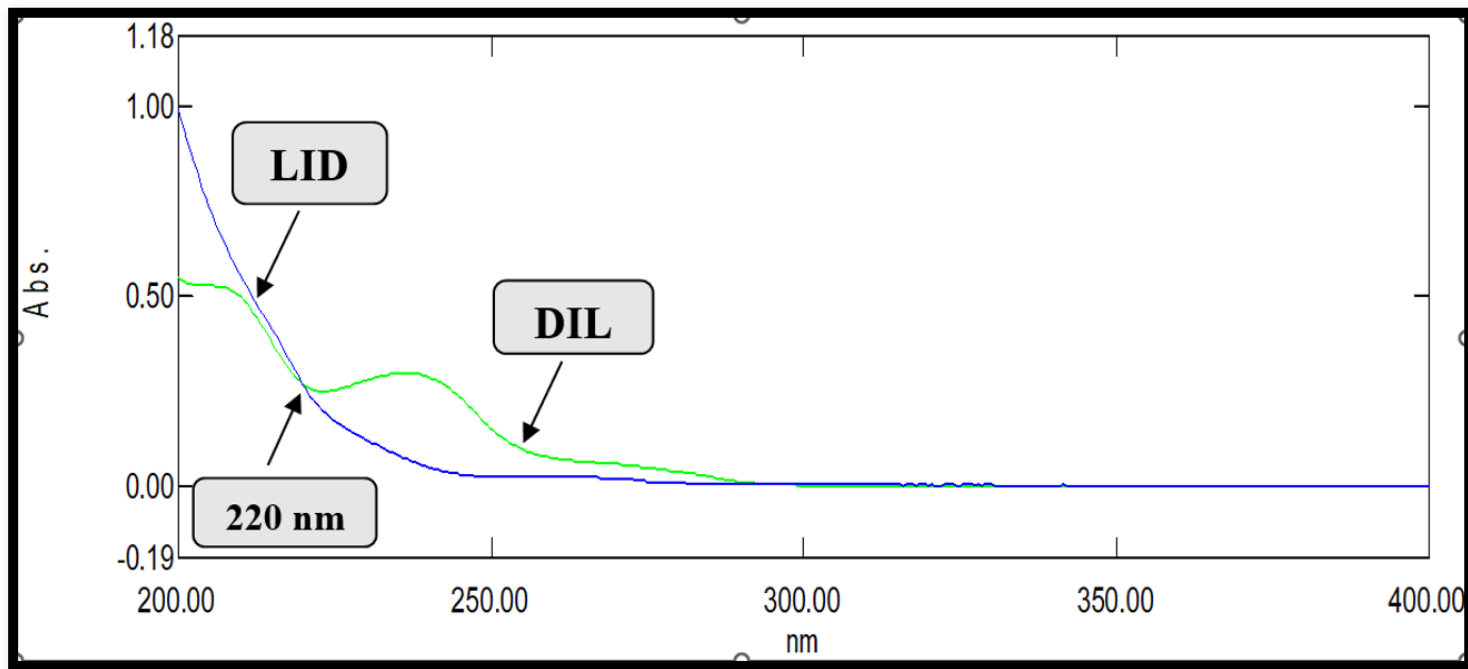(b): Chemical structure of Diltiazem HCl

**Figure 2**

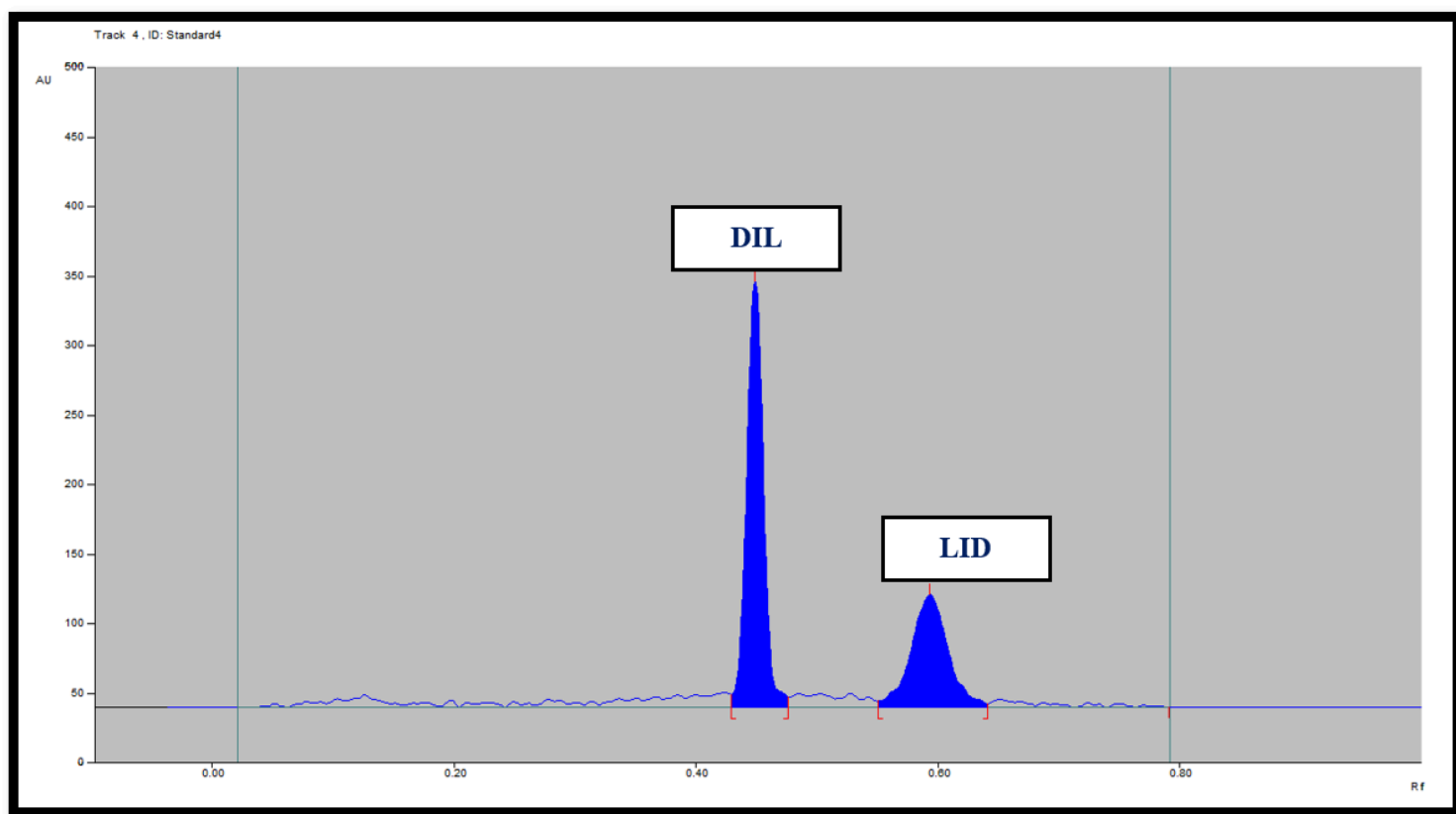Overlay UV-Visible spectra of Lidocaine HCl and Diltiazem HCl (10 µg/ml)



**Figure 3**

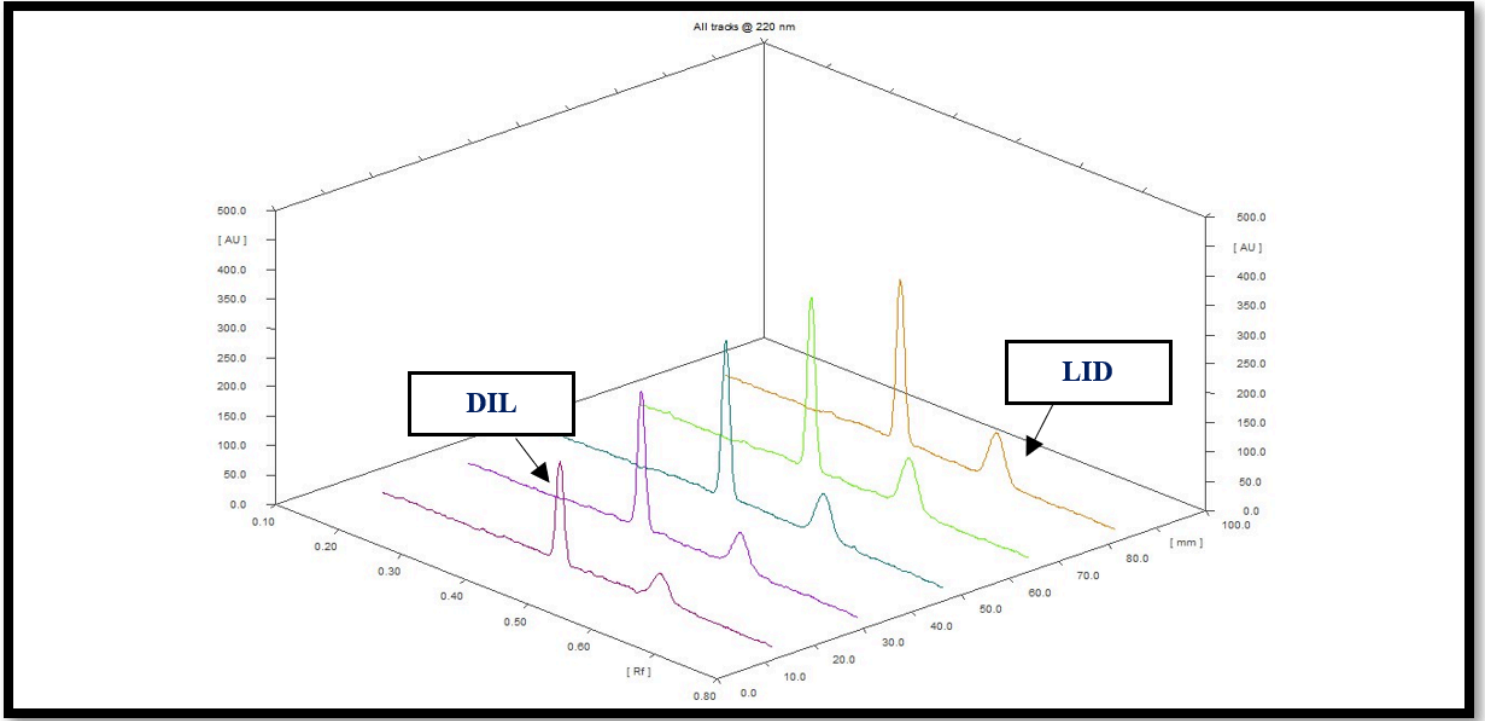Densitogram of Lidocaine HCl and Diltiazem HCl (800 ng/band)

**Figure 4**

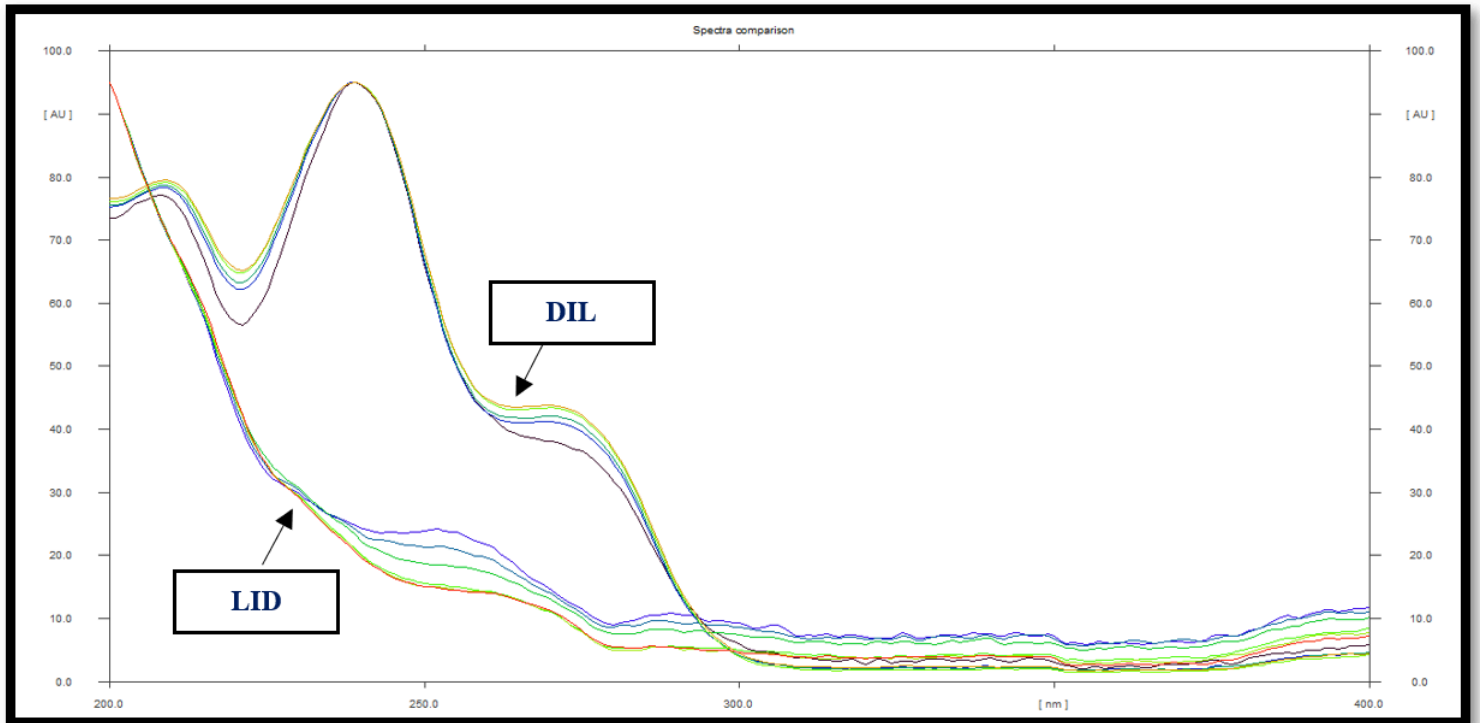3D Overlay densitogram of Lidocaine HCl and Diltiazem HCl (400 – 1200 ng/band)



**Figure 5**

Specificity spectra of Lidocaine HCl and Diltiazem HCl
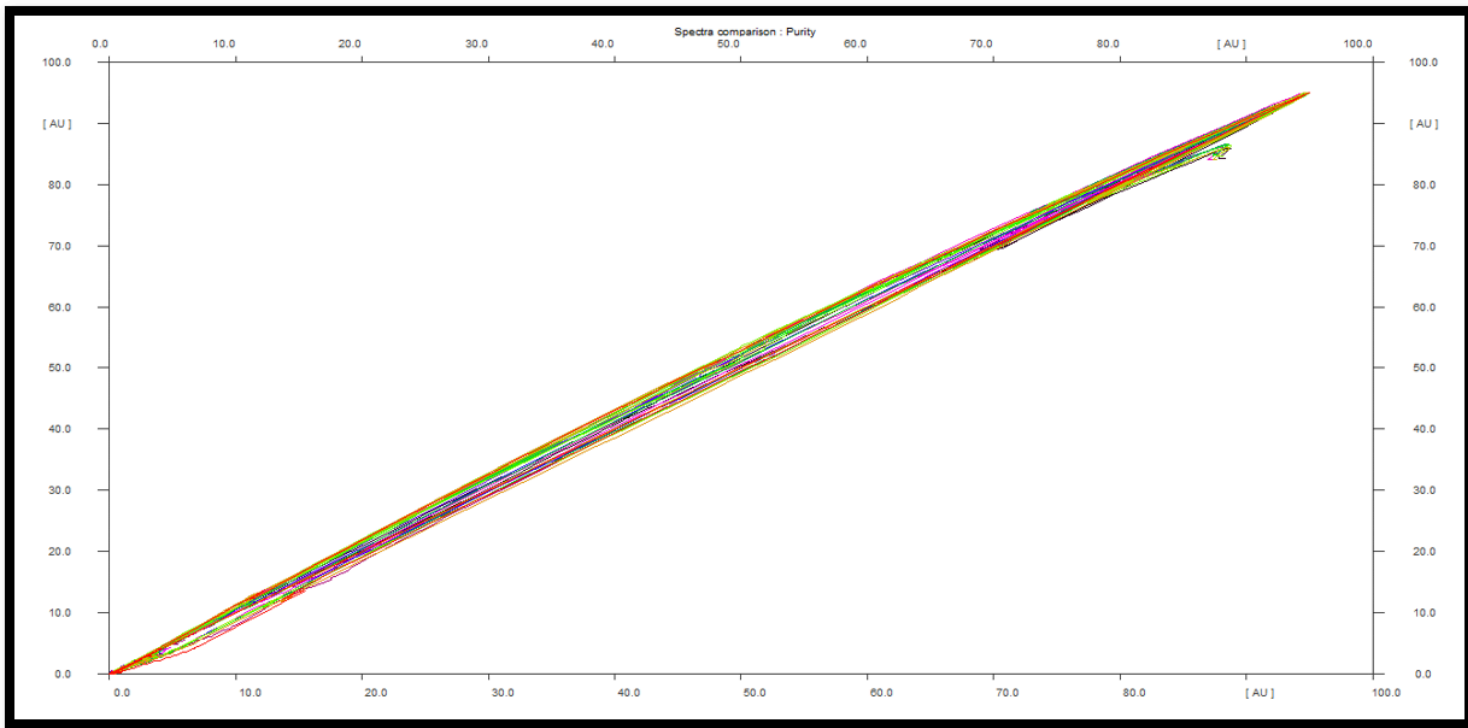
**Figure 6**

Peak purity spectra of Lidocaine HCl and Diltiazem HCl

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