ABSTRACT

Objective: To develop and validate stability indicating HPTLC method for determination of clevidipine butyrate in synthetic mixture.

Methods: The present study deals with development and validation of stability indicating HPTLC method for estimation of clevidipine butyrate. Chromatographic separation was performed on aluminum plate pre coated with Silica Gel 60 F254 using toluene: ethyl acetate (8:2) as mobile phase. TLC scanner was set at wavelength of 370 nm.

Results: Retention factor Rf of clevidipine was found to be 0.49. The method was validated as per ICH guidelines. Calibration curve was in the range of 1000-6000ng/band. The correlation coefficient was found to be 0.999. The precision expressed by RSD was less than 2%. The accuracy of method was confirmed by recovery studies using standard addition method and recovery was found to be 99.03-99.57%. The drug was subjected to ICH prescribed hydrolytic, oxidative, photolytic and thermal stress conditions. Clevidipine and its degradation products were well resolved under experimental conditions. The method was validated according to ICH guidelines. The drug showed significant degradation in alkaline and acidic condition and slight degradation in oxidative condition. The drug was stable in thermal condition.

Conclusion: A new, Simple, Accurate, Precise, Sensitive and economic stability indicating HPTLC method has been developed and validated for the determination of clevidipine and can be employed for stability indicating analysis.

Keywords: Clevidipine butyrate, Stability indicating HPTLC method, ICH Guidelines
Preparation of solutions

Standard solutions

Standard stock solutions were prepared by dissolving 10 mg of CLEVI in methanol in 10 ml of volumetric flask. Different volumes of stock solution (1, 2, 3, 4, 5 and 6 µl) solution were applied as band to the HPTLC plate to make concentration in the range of 1000-6000 ng/band.

Sample solution assay

CLEVI marketed formulation could not be imported and it was prepared as laboratory synthetic mixture as per the given formula [13] in table 1.

Forced degradation study

Stock solution of CLEVI was prepared by dissolving 250 mg of CLEVI in 25 ml of methanol. This stock was used for degradation studies.

| Table 1: Composition of CLEVI synthetic mixture |
|-----------------------------------------------|
| Composition        | %w/v |
| Clevidipine        | 0.05 |
| Soyabean oil       | 20   |
| Glycerin           | 2.25 |
| Disodium Edetate   | 0.005|
| Sodium citrate     | 0.1  |
| Egg yolk phospholipid | 1.2  |
| Water for injection| upto 100% |

Acid degradation

2.5 ml of stock solution of CLEVI was taken in 25 ml of volumetric flask, 1 ml of 1 N HNO₃ was added and solution was heated in a water bath at 60 °C for 1 hour. The solution was cooled and neutralized with 1 N NaOH. Volume was made upto 25 ml with methanol to make concentration of 1 mg/ml. The solution was filtered through 0.45µm Nylon 6,6 membrane syringe filter. From this solution 5 µl (5000 ng/band) was applied to HPTLC plate and development was carried out under optimized chromatographic conditions.

Base degradation

2.5 ml of stock solution of CLEVI was taken in 25 ml of volumetric flask, 1 ml of 0.01 N NaOH was added and solution was heated in a water bath at 60 °C for 1 hour. The solution was cooled and neutralized with 0.01 N HCl. Volume was made upto 25 ml with methanol to make concentration of 1 mg/ml. The solution was filtered through 0.45µm Nylon 6,6 membrane syringe filter. From this solution 5 µl (5000 ng/band) was applied to HPTLC plate and development was carried out under optimized chromatographic conditions.

Oxidative degradation

2.5 ml of stock solution of CLEVI was taken in 25 ml of volumetric flask, 1 ml of 3 % hydrogen peroxide was added. The solution was kept at room temperature for 1 hr. The solution was made upto volume with methanol. The solution was filtered through 0.45 µm Nylon 6,6 membrane syringe filter. From this solution 5 µl (5000 ng/band) was applied to HPTLC plate and development was carried out under optimized chromatographic conditions.

Thermal conditions

25 mg of CLEVI was placed in oven at 80 for 8 d under dry heat conditions in the dark and then cooled to room temperature. Volume was made with methanol to get the concentration of 1 mg/ml. From this solution 5 µl (5000 ng/band) was applied to HPTLC plate and development was carried out under optimized chromatographic conditions.

Photolytic degradation

For the photochemical study, CLEVI equivalent to 25 mg was spread in 1 mm thickness on a petridish and exposed to 5383 Lux and 144 UV/cm²for 11 d and volume was made upto 25 ml with methanol to make concentration of 1 mg/ml. From this solution 5 µl (5000 ng/band) was applied to HPTLC plate and development was carried out under optimized chromatographic conditions.

Validation

The method was validated as per ICH guidelines.
RESULTS

Development and optimization

For selection of appropriate mobile phase for the development of CLEVI, trials were made by using solvents of different polarity, at different concentration levels. Several different composition of mobile phase system like water: methanol, methanol: acetonitrile, toluene: ethyl acetate were tried. Out of these, mobile phase toluene: ethyl acetate in a ratio of 8:2 was found to give sharp well defined peak at \( R_f \) value of 0.49±0.0057 for CLEVI which is shown in fig. 2. Densitogram with sharp defined peaks was obtained without any interference from analytes or excipients.

Force degradation studies

The results of force degradation study are summarized in table 1. During stress degradation experiments it was observed that CLEVI was more susceptible to alkaline and acidic hydrolysis than oxidative, thermal and photolytic degradation.

![Fig. 1: UV spectra of CLEVI](image1.png)

![Fig. 2: Optimised densitogram of CLEVI](image2.png)

![Fig. 3: Representative 3D densitogram of linearity band of CLEVI](image3.png)

![Fig. 4: Representative densitogram of CLEVI standard](image4.png)

![Fig. 5: Representative densitogram of CLEVI synthetic mixture](image5.png)

![Fig. 6: Representative densitogram of 1 N HCl at 60°C for 1 h](image6.png)
Validation of developed stability indicating method

The developed method was validated as per ICH guidelines for parameters like linearity, accuracy, specificity, sensitivity and robustness. The 3D densitograms showed well resolved peaks. The linearity was evaluated by regression analysis. The linearity of densitograms is shown in Fig. 3. The correlation coefficient was found to be greater than 0.999. The low values of LOD and LOQ indicate that developed method is sensitive to be used as stability indicating (table 2). Satisfactory % recovery studies and lower % RSD obtained in accuracy, precision, robustness (table 3, 4).

Thus this indicated the proposed method is robust to minor changes in the experimental conditions and can give accurate and precise results without any interference from injectable emulsion for the analysis of drug in the formulation.

Table 2: Forced degradation study of CLEVI

| Stress condition                  | CLEVI (API) R_f | % Degradation | CLEVI (synthetic mixture) R_f | % Degradation |
|----------------------------------|----------------|---------------|------------------------------|---------------|
| 1 N HCl 60°C for 1 hr            | 0.01, 0.02     | 22.5%         | 0.01, 0.02                   | 19.5%         |
| 0.01 N NaOH 60°C for 1 h         | 0.27           | 45%           | 0.27                         | 41%           |
| 3% H₂O₂ RT for 1 hour            | 0.27           | 8.5%          | 0.27                         | 6.3%          |
| Thermal 80°C for 8 d             | —              | No degradation| —                            | No degradation|
| Photolytic                       | 0.01           | 0.6%          | 0.01                         | 0.4%          |

Table 3: Result of linearity, range, precision, method sensitivity study

| Parameter                        | CLEVI           |
|----------------------------------|-----------------|
| Concentration range (ng/band)    | 1000-6000       |
| Retention factor                 | 0.493±0.005     |
| Regression equation              | Y=1.877x+2410   |
| Correlation coefficient (R²)     | 0.999           |
| Precision                        |                 |
| Inter-day (% RSD)                | 1.33            |
| Intra-day (%RSD)                 | 1.16            |
| Method sensitivity               |                 |
| LOD(ng/band)                     | 403.22          |
| LOQ(ng/band)                     | 1222.492        |
Table 4: Results of accuracy (recovery) study by standard addition method (n=3)

| Concentration (μg/band) | % Spiking amount of CLEV1 added | Amount of CLEV1 recovered | mean±SD | % RSD | % Recovery mean±SD | % RSD |
|-------------------------|--------------------------------|--------------------------|---------|-------|-------------------|-------|
| 2500                    | 1250                           | 1237.6                   | 1237.8±1.71 | 0.142 | 99.02±0.94       | 0.14  |
| 2500                    | 2500                           | 2488.2                   | 2488.6±1.59 | 0.069 | 99.5±0.94        | 0.06  |
| 2500                    | 3750                           | 3732.5                   | 3733.9±1.55 | 0.041 | 99.57±0.94       | 0.04  |

n = number of determinations, SD= Standard Deviation, %RSD=% Relative Standard Deviation

Table 5: Robustness study for the developed method (n=3)

| Condition                          | Parameter   | Rf (Mean)±SD | % RSD | Area (mean±SD) | % RSD |
|------------------------------------|-------------|--------------|-------|----------------|-------|
| Mobile phase composition           | 7.8:2.2     | 0.47±0.005   | 1.21  | 8085.6±111.18  | 1.37  |
| Saturation of mobile phase         | 8.2:1.8     | 0.52±0.005   | 1.10  | 8023.6±75.9    | 0.94  |
| Solvent front                      | 17 min      | 0.48±0.005   | 1.19  | 8052.4±99.1    | 1.23  |
| Wavelength                         | 23 min      | 0.49±0.005   | 1.17  | 7911.8±65.74   | 0.82  |
|                                    | 7.5 cm      | 0.46±0.005   | 1.12  | 8054.6±101.24  | 1.25  |
|                                    | 8.5 cm      | 0.52±0.005   | 1.10  | 7939.5±106.37  | 1.3   |
|                                    | 368         | 0.48±0.005   | 1.19  | 8075.2±61.42   | 0.76  |
|                                    | 372         | 0.49±0.005   | 1.07  | 8022.3±62.98   | 0.78  |

n = number of determinations, SD= Standard Deviation, %RSD=% Relative Standard Deviation

DISCUSSION

Force degradation studies play an important role in the development of pharmaceuticals. The results of degradation studies helps in development of stability indicating method. The ICH Q1A guideline states that the validated stability indicating test methods must be performed to monitor the shelf life of drug substance which are susceptible to change during storage and which are likely to affect the quality, safety, efficacy of formulation. HPTLC method has an advantage over HPLC methods in the form that the reported HPLC methods [9], method is developed by gradient method which is time-consuming and method [10, 11] is isocratic with longer retention time while in hptlc method standard and sample can be analysed simultaneously on same TLC plate. Short equilibrium time, low solvent consumption, low volume of sample requirement, no preliminary treatment like filtration and degassing, more number of samples can be applied on TLC plate, shorter run time and less expensive are advantages of developed HPTLC method over reported HPLC method. From the development and validation studies it was found that the proposed method can resolve drug substances and degradation products in shorter time with optimum resolution.

CONCLUSION

The HPTLC method was developed on pre-coated silica gel using toluene: ethyl acetate (8:2) as mobile phase with densitometric detection at 370 nm. This study found that HPTLC method development for determination of CLEV1 in synthetic mixture is accurate, precise, linear, highly sensitive, specific and robust. The developed method was found to be suitable for determination of CLEV1 in bulk and synthetic mixture.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally to this manuscript.

CONFLICT OF INTEREST

None

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