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**Der Pharmacia Lettre, 2016, 8 (11):236-242**
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ISSN 0975-5071
USA CODEN: DPLEB4

# Development and validation of stability indicating RP-LC method for estimation of calcium dobesilate in pharmaceutical formulations

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**ABSTRACT**

*A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for estimation of Calcium dobesilate in tablet formulations. The separation was achieved by using column Waters symmetry C18 (4.6x150mm), 5µ (Make: Waters), in mobile phase consisted of pH 2.5 Phosphate buffer Acetonitrile and in the ratio of (95:5, v/v). The flow rate was 1.0 mL.min-1 and column oven temperature 30°C, the injection volume was 20 µL the separated Calcium dobesilate was detected using UV detector at the wavelength of 300 nm. The retention time of Calcium dobesilate, was noted to be 4.22 min respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.*

**Keywords:** Liquid Chromatography; Calcium dobesilate, Validation

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## INTRODUCTION

Calcium dobesilate (**Figure: 1.1**, calcium 2, 5- dihydroxybenzenesulfonate) is a drug used for the treatment of diabetic retinopathy and chronic venous insufficiency. Calcium dobesilate shows anti-platelet and fibrinolytic activities by inhibiting platelet activation factor (PAF) and enhancing the release of tissue plasminogen activator (tPA) and acts selectively on the capillary walls regulating their physiological functions of resistance and permeability [1-3]. A detailed literature survey reveals that RP-HPLC/UV methods have been reported for the quantitative estimation of calcium dobesilate [4] individually in various matrixes such as human plasma and pharmaceutical dosage forms. RP-HPLC methods are also reported for calcium dobesilate with other drugs in suppositories and in ointment [5]. As per our detailed literature survey as on date, there are no RP-HPLC methods reported for the quantitative estimation of calcium dobesilate in any matrix either of pharmaceutical dosage forms, plasma, etc. Hence we here report a new, simple, sensitive, rapid, precise, accurate and linear RP-HPLC isocratic method for the quantitative estimation of calcium dobesilate in tablets as per ICH guidelines [6].

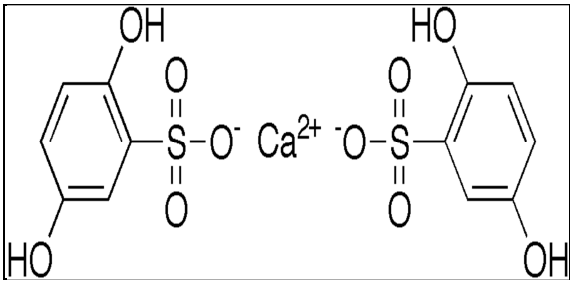


**Fig.1.1 The structure of Calcium dobesilate**

## MATERIALS AND METHODS

### 2.0 Experimental

### 2.1. Chemicals and Reagents

Analytical-grade Potassium dihydrogen phosphate, Orthophosphoric acid, Acetonitrile and Water HPLC-grade, were from Merck Chemicals. Mumbai, India.

### 2.2. Instrumentation

Waters 2489 U.V-Visible detector/2695 Separation Module, equipped with Empower 2 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were use in the present assay.

### 2.3 Buffer preparation

Dissolve about 3.40 g of Potassium di-hydrogen phosphate in 1000 mL of water and adjust the resulting pH of the solution to 2.5 with Orthophosphoric acid. Filter through 0.45µ Nylon membrane filter.

### 2.4 Mobile phase preparation

Accurately mixed pH 2.5 Phosphate buffer Acetonitrile and in the ratio of (95:5, v/v). Filter through 0.45µ Nylon membrane filter.

### 2.5 Diluent preparation

Acetonitrile: Water (10:90 %v/v).

### 2.6 Standard preparation:

Weigh accurately 50 mg of Calcium dobesilate working standard or reference standard into 100 mL volumetric flask. Add about 70 mL of diluent and sonicate for 2 minutes to dissolve. Make up to volume with diluent and mix well.

From the above solution pipette out 2 mL of standard stock solution in to 100 mL volumetric flask, diluent add and finally make up to the volume with diluent and mix well.

### 2.7 Sample preparation:

Weighed and crushed the ten tablets with mortar and pestle. Transfer the tablet powder equivalent to 100 mg of calcium dobesilate into a 200 mL volumetric flask. Add 170 mL of diluent, Sonicate for 30 minutes with intermediate shaking. Dilute to volume make up with diluent and mix well.

Centrifuge a portion of the solution with lid at 4000 RPM for about 10 minutes. Pipette out 2 mL of above clear centrifuged solution into a 100 mL volumetric flask, dilute to volume with diluent and mix well and filter the solution through 0.45µ nylon membrane filter.

### 2.8 Chromatographic conditions

Chromatographic analysis was performed on Waters Symmetry C18 (4.6x150mm), 5µ (Make: Waters) column. The mobile phase consisted of pH 2.5 Phosphate buffer and Acetonitrile and in the ratio of (95:5, v/v). The flow rate was 1.0 mL/min, column oven temperature 30°C , the injection volume was 20 µL, and detection was performed at 300 nm using a photodiode array detector (PDA).

## RESULTS AND DISCUSSION

### Method development

Spectroscopic analysis of compound Calcium dobesilate showed that maximum UV absorbance (λmax) at 300 nm respectively. To develop a suitable and robust LC method for the determination of Calcium dobesilate, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Inertsil-ODS-3V, 150x4.6mm, 5µ with the following different mobile phase compositions like that 50:50 water, Acetonitrile mixture , 50:50 Phosphate buffer (pH 3.0), Acetonitrile mixture, 50:50 Phosphate buffer (pH 3.0), Acetonitrile mixture. It was observed that when Calcium dobesilate was injected, Peak Tailing, not satisfactory. For next trial the mobile phase composition was changed slightly. The mobile phase composition was 95:5 Phosphate buffer (pH 2.5), Acetonitrile mixture. Respectively as eluent at flow rate 1.0 mL/min. UV detection was performed at 300nm. The retention time of Calcium dobesilate is 4.22 minutes and the peak shape was good.

The chromatogram of Calcium dobesilate standard using the proposed method is shown in **(Fig: 1.2)** system suitability results of the method are presented in **Table-1.2.**

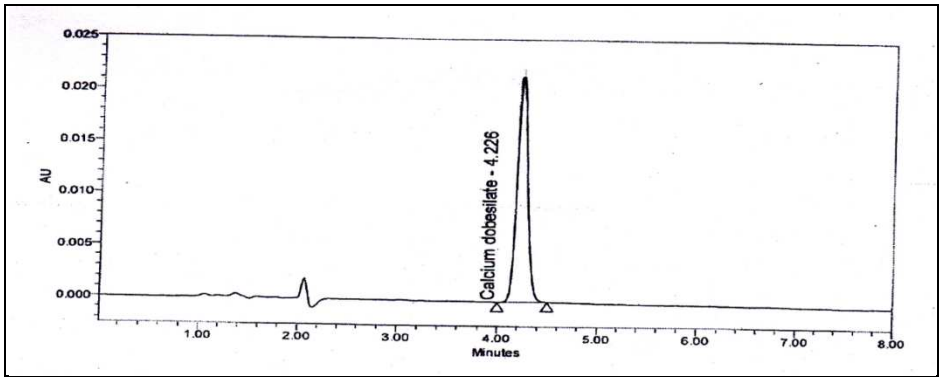


**Figure 1.2: Chromatogram showing the peak of Calcium dobesilate**

**4.0 Method validation**

The developed RP-LC method extensively validated for assay of Calcium dobesilate using the following parameters.

**4.1Specificity**

**Preparation of blank solution:**

Acetonitrile: Water (10:90 %v/v).

**Preparation of Placebo solution:**

Placebo solution was prepared in duplicate by weighing the equivalent amount of excipients present in the finished drug product and analysed as per proposed method. Interference due to placebo was evaluated for each of the placebo preparations.

**Blank and Placebo interference**

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **(Fig: 1.3)** showed no peak at the retention time of Calcium dobesilate peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Calcium dobesilate in Calcium dobesilate tablets. Similarly chromatogram of placebo solution **(Fig: 1.4)** showed no peaks at the retention time of Calcium dobesilate peak. This indicates that the placebo used in sample preparation do not interfere in estimation of Calcium dobesilate in Calcium dobesilate tablets.

**Impurity interference**:

Prepared and injected stressed blank, placebo and test solution for Acid degradation, Alkali degradation, Peroxide degradation, Water degradation, Thermal degradation, Humidity degradation, UV light degradation.

Calculated the % net degradation and evaluated the Calcium dobesilate peak purity in each stressed condition and found to be within the acceptable limits data were shown in **Table: 1.1.**

**Table 1.1: Forced degradation (% Net Degradation) of drug product of Calcium dobesilate**

| S. No. | Condition | % Assay | % difference | Peak Purity |
|---|---|---|---|---|
| 1 | Control sample | 99.6 | NA | 1 |
| 1 | U.V | 98.5 | 1.1 | 1 |
| 2 | Heat | 97.5 | 2.1 | 1 |
| 3 | Acid | 89.2 | 10.4 | 1 |
| 4 | Basic | 93 | 6.6 | 1 |
| 5 | Oxidation | 96.3 | 3.3 | 1 |
| 6 | Water | 92.5 | 7.1 | 1 |
| 7 | Humidity | 97.6 | 3 | 0.99 |

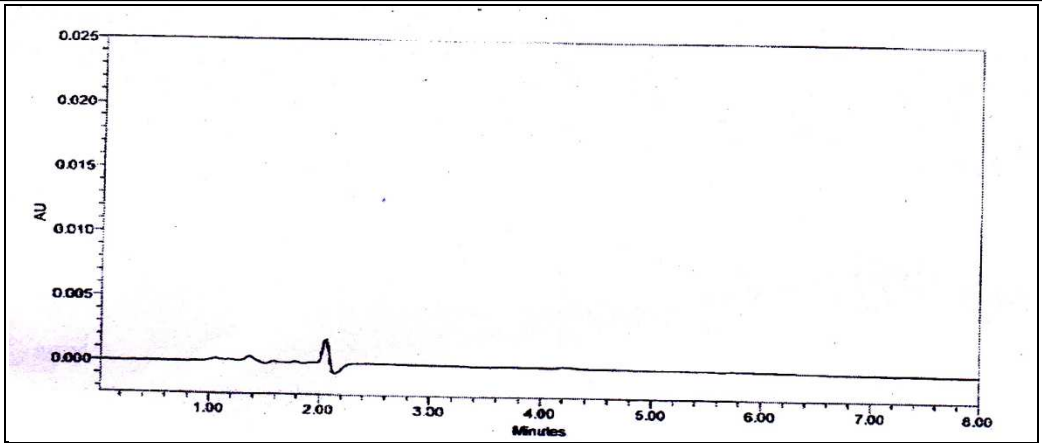


**Fig: 1.3 Chromatogram showing the no interference of diluent for Calcium dobesilate**

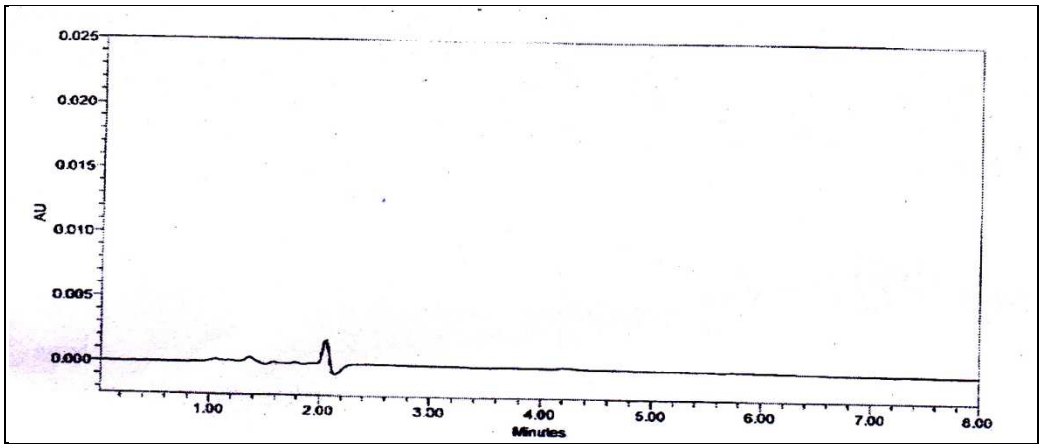


**Fig: 1.4 Chromatogram showing the no interference of placebo for Calcium dobesilate**

**Table 1.2: System suitability parameters for Calcium dobesilate by proposed method**

| Name of the Compound | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|
| **Calcium dobesilate** | 4.22 | 8031 | 1.2 |

**4.2 System precision:**

The standard solution was prepared as per the test method, injected into the HPLC system for six times and evaluated the % RSD for the area responses. The chromatogram was shown in **Figure: 1.5** and data were shown in **Table: 1.3**

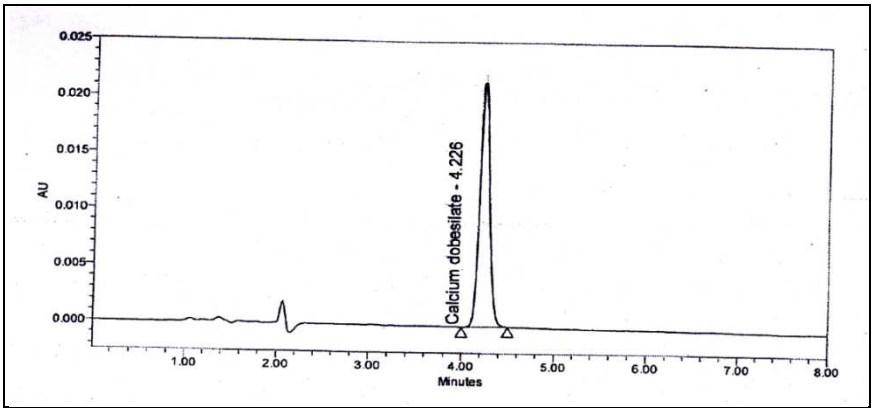


**Fig: 1.5 System precision standard chromatogram**

**Table: 1.3 System precision data for Calcium dobesilate**

| No. of injections | Peak area response |
|---|---|
| **1** | 3388949 |
| **2** | 3387682 |
| **3** | 3424050 |
| **4** | 3394325 |
| **5** | 3402068 |
| **6** | 3398582 |
| **Average** | **3399276** |
| **% RSD** | **0.4** |

### 4.3 Method precision:

The precision of test method was evaluated by doing assay for six samples of Calcium dobesilate tablet as per test method. The content in mg and % label claim for Calcium dobesilate for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The chromatogram was shown in **Figure: 1.6** and data were shown in **Table: 1.4**

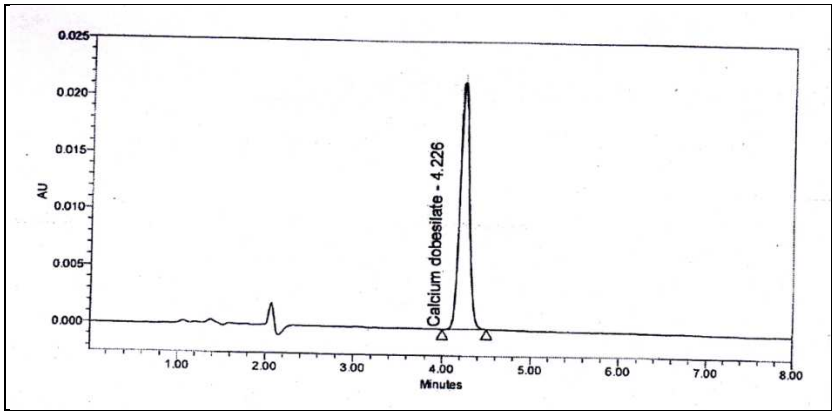


**Fig: 1.6 Method precision sample chromatogram**

**Table: 1.4 Method precision data for Calcium dobesilate**

| Sample Number | % Assay |
|---|---|
| 1 | 99.5 |
| 2 | 100.2 |
| 3 | 99.6 |
| 4 | 100.4 |
| 5 | 99.0 |
| 6 | 99.4 |
| **Mean** | **99.7** |
| **% RSD** | **0.5** |

### 4.4 INTERMEDIATE PRECISION

To evaluate intermediate precision for assay method, six samples were prepared and analyzed by using different HPLC system, different column, by different analyst on different day. Calculated % assay, % RSD for % assay results of Calcium dobesilate in method precision and intermediate precision (n=6 and n=12) and found to be within the acceptable limits. The results are summarized in following **Table: 1.5.**

**Table: 1.5 Intermediate Precision data for Calcium dobesilate**

| Sample No. | % Assay |
|---|---|
| 1 | 99.3 |
| 2 | 101 |
| 3 | 99.8 |
| 4 | 100.5 |
| 5 | 99.7 |
| 6 | 99.4 |
| **Average** | **100** |
| **%RSD** | **0.7** |

**Table: 1.6 Overall RSD (twelve assay results) data for Calcium dobesilate**

| Sample No. | % Assay | |
|---|---|---|
| | For Method Precision | For Intermediate Precision |
| 1 | 99.5 | 99.3 |
| 2 | 100.2 | 101.0 |
| 3 | 99.6 | 99.8 |
| 4 | 100.4 | 100.5 |
| 5 | 99.0 | 99.7 |
| 6 | 99.4 | 99.4 |
| **Average** | **99.7** | **100** |
| **%RSD** | **0.5** | **0.7** |
| **% RSD (n=12)** | **0.6** | |

### 4.5 Linearity of detector response

The standard curve was obtained in the concentration range of 1.0-15.0µg/ml for Calcium dobesilate. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in **Figure: 1.7** to demonstrate the linearity of the proposed method. From the data obtained which given in **Table: 1.6** the method was found to be linear within the proposed range.

**Table: 1.6 Linearity studies for Calcium dobesilate by proposed method**

| Level no. | Linearity concentration | Drug concentration (in ppm) | Response for Calcium dobesilate |
|---|---|---|---|
| **1** | 10 | 200 | 1355584 |
| **2** | 25 | 300 | 2053915 |
| **3** | 50 | 400 | 2711167 |
| **4** | 75 | 500 | 3388949 |
| **5** | 100 | 600 | 4063500 |
| **6** | 125 | 700 | 4659774 |
| **7** | 150 | 800 | 5422334 |

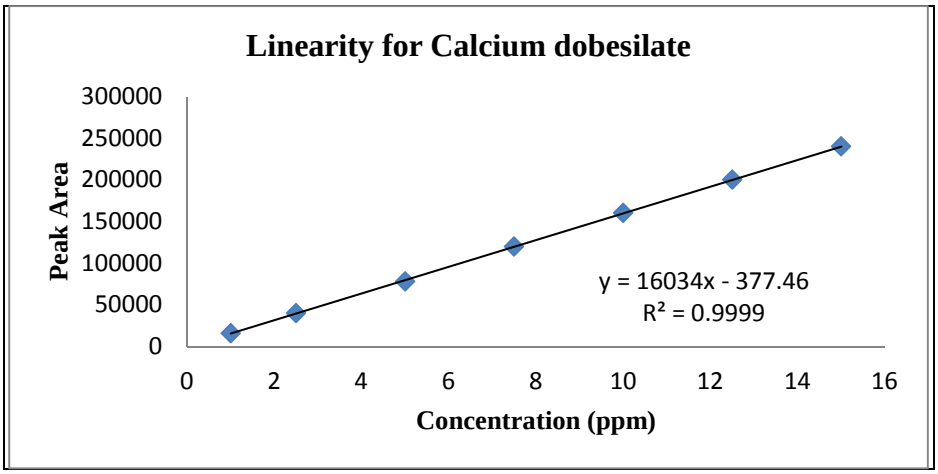


**Figure: 1.7 Calibration curve for Calcium dobesilate**

### 4.6 Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Calcium dobesilate, analyzed as per the proposed method. The percentage recoveries with found in the range of 99.1 to 100.7 for Calcium dobesilate. The data obtained which given in **Table: 1.8** the method was found to be accurate.

**Table: 1.8 Recovery studies for Calcium dobesilate by proposed method**

| S. No. | % spike level | Amount added (ppm) | Amount recovered (ppm) | % Recovery | % Mean recovery | % RSD |
|---|---|---|---|---|---|---|
| 1. | 50 | 5.02 | 5.05 | 99.7 | 100.2 | 0.5 |
| 2. | | 5.05 | 5.06 | 100.3 | | |
| 3. | | 5.08 | 5.02 | 100.7 | | |
| 1. | 100 | 10.03 | 10.03 | 99.2 | 99.7 | 0.5 |
| 2. | | 10.05 | 10.05 | 100.2 | | |
| 3. | | 10.07 | 10.15 | 99.5 | | |
| 1. | 150 | 15.10 | 15.08 | 99.1 | 99.6 | 0.7 |
| 2. | | 15.05 | 15.02 | 99.4 | | |
| 3. | | 15.01 | 15.00 | 100.4 | | |

## CONCLUSION

An RP-HPLC method for estimation of Calcium dobesilate was developed and validated as per ICH guidelines like Accuracy, Precision, Linearity, Specificity, and System suitability. The results obtained were within the acceptance criteria.

The proposed method was applied for determination of Calcium dobesilate in marketed formulation. Hence the proposed method was found to be satisfactory and could be used for the routine analysis of Calcium dobesilate in tablet dosage form.

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