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ORIGINAL ARTICLE **OPEN ACCESS**

Development and Validation of Rp-HPLC Method for Simultaneous Estimation of Calcium Dobesilte in Tablet Dosage Form

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ABSTRACT

A simple and accurate method has been developed, the High-Performance liquid chromatography (RP-HPLC) for simultaneous estimation of Calcium Dobesilate *in tablet dosage form. In HPLC method separation was done by* Kromasil C18**,** 250 mm X 4.6mm ID, 5 µm*, with mobile phase containing* Acetonitrile: 0.1% OPA in Water (80:20 %V/V)*. The flow rate was 1.0 ml/min &effluent was monitored at 300nm. The retention time of* Calcium Dobesilate *was in the range of 6 min. The linearity for* Calcium Dobesilate *was in the range of 2 to 30µg/ml. The recoveries of* Calcium Dobesilate *were found in the range of 99.48% respectively. The LOD (Limit of detection) of* Calcium Dobesilate *was found to be* 0.569 µg/Ml *and LOQ (Limit of Quantitation) of* Calcium Dobesilate *was found to be* 1.723 µg/mL*. The proposed methods were validated as per ICH guidelines by means of different parameter likes linearity, precision, accuracy a Limit of detection, limit of quantification, range, selectivity, Robustness Ruggedness, Solution stability as per ICH guidelines & successfully applied to the estimation of* Calcium Dobesilate *in tablet dosage form.*
Keywords: Calcium Dobesilate*, RP-HPLC, Simultaneous estimation, validation.*

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INTRODUCTION

Calcium dobesilate is **a** vasoprotective**.** It is the calcium salt of dobesilic acid. It is a synthetic molecule with the ability to reduce capillary permeability in the body. It is the calcium salt of dobesilic acid. It is a synthetic molecule with the ability to reduce capillary permeability in the body [1-3].

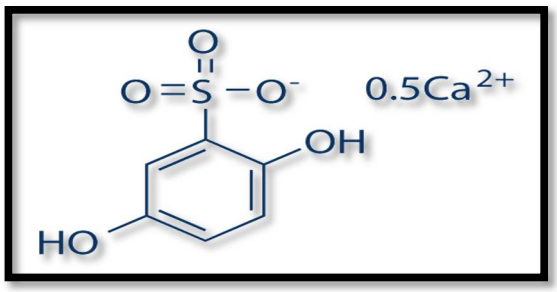


Fig.1: Structure of Calcium dobesilate

The literature review says that various analytical method reported for Calcium dobesilate in UV-spectrometry, HPLC, RP-HPLC individually and common.

MATERIAL AND METHODS

Materials: Calcium dobesilate pure API, Capsule of Dobesil, Distilled water, Methanol, Acetonitrile.
Instruments Electronic balance, pH meter, Ultrasonicator, HPLC system, UV-VIS spectrophotometer.
Methods Diluent: The mobile phase Acetonitrile: 0.1% OPA in Water (80:20 %V/V) was sonicate for 20 min and filter through 0.45 µm membrane nylon filter [4].
Preparation of Std. stock solution: In order to prepare stock solution, weighed accurately 10 mg Calcium Dobesilate and transferred into 20 ml volumetric flask, added 15 ml of Water and sonicated to dissolve the standard completely and diluted up to the mark with Water (500 PPM).
Further diluted 0.8 mL to 20 mL with Water. (20 PPM)

Solution scanned from 400 nm to 200 nm.

Preparation of Sample stock solutions [5]: Calculate the average weight of Dobesil 500 mg capsule. Weighed the powder material equivalent to 100 mg of Calcium Dobesilate. Transferred it in a clean and dried 100 mL of volumetric flask, added 70 ml of Water and sonicated to dissolve it completely. Made the volume up to the mark with Water. Filter the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of filtrate. Further dilute 0.4 ml of filtrate to 20 ml with mobile phase. (20 PPM of Calcium Dobesilate)

Determination of Analytical wavelength [6]: Water as a blank and Calcium Dobesilate standard solution (20 PPM) was scanned from 400 nm to 200 nm. Absorption maxima was determined for drug. 300 nm wavelength used for further analysis.

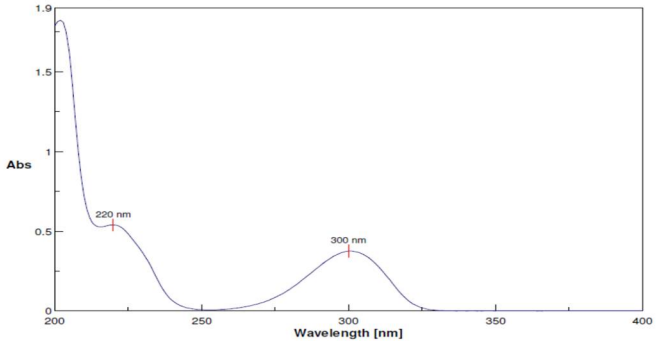


Fig. 2 UV spectra of Calcium Dobesilate

RESULTS AND DISCUSSION

All the system suitability parameters were within the range as per ICH guidelines.

Linearity: The various concentrations 2-30 **µg/ml** of Calcium Dobesilate were made. correlation coefficient of drugs was 0.99988.

Precision: The Precision is an analytical method that express the closeness series of measurements obtained from multiple sampling from the same sample under the maintained conditions. The measurement of precision of an analytical method is performed on three replicated of standard preparation. The results for the same are usually expressed as the variance, standard deviations or confidence level of a series of measurements.

Accuracy: The (recovery) accuracy of an analytical procedure expresses the closeness of agreement between the value which is either as a conventional true value or an accepted reference value and the value found. To check the accuracy of proposed method, level of recovery carried out at 50,100 and 150 % of the concentrations as per standard addition method.

LOD (Limit of detection:): LOD (Limit of detection) and LOQ (Limit of Quantitation) of Calcium Dobesilate determine by calibration curve method. Dilutions were prepared in linearity range. The average area was plotted against concentrations and they are calculated by following formula.

Robustness: Robustness is the capacity of a method to remain not affected by deliberate variations in method parameters. The robustness of a method was evaluated by varying method parameters such as percentage organic solvent, ionic strength, or temp, and determining the effect (if any) on the results of the method. The optimized system suitability parameter was unaffected by small variation in percentage organic solvent, ionic strength and temperature.

Linearity: Linearity of the Calcium Dobesilate in which relationship was established at these ranges between.

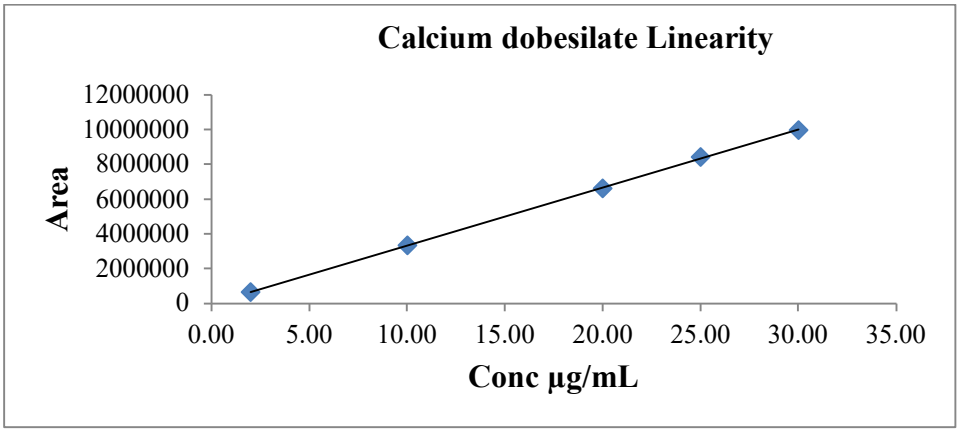


Fig 3. Calibration curve for Calcium Dobesilate

Precisions: The precision of the Calcium dobesilate was found to be within the limits (RSD<2)

	Sample	**Test Sample (mg)**	**Area**	**% Assay**
Repeatability	Sample 1	123.7	6515935	98.72
	Sample 2	123.5	6509563	98.78
	Sample 3	123.2	6424639	97.73
	Sample 4	123.4	6516894	98.97
	Sample 5	123.6	6506539	98.66
	Sample 6	123.5	6545938	99.33
	Mean			**98.70**
	STD DEV			**0.5327**
	% RSD			**0.540**
Intermediate precision (Inter-Day)	Sample 1	123.4	6415639	97.44
	Sample 2	123.5	6633639	100.67
	Sample 3	123.8	6549651	99.15
	Sample 4	123.6	6535130	99.09
	Sample 5	123.4	6451630	97.98
	Sample 6	123.5	6582130	99.88
	Mean			**99.04**
	STD DEV			**1.1883**
	% RSD			**1.200**
Repeatability Plus Inter-day	**Mean**			**98.867**
	STD DEV			**0.8954**
	% RSD			**0.906**

Tablet 1: The precision of the Calcium Dobesilate

Accuracy: The (recovery) accuracy of an analytical procedure expresses the closeness of agreement between the value which is either as a conventional true value or an accepted reference value and the value found. To check the accuracy of proposed method, level of recovery carried out at 50,100 and 150 % of the concentrations as per standard addition method.

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	3302950	10.03	10.04	99.90	99.50	0.969
	3246072	9.86	10.02	98.40		
	3310139	10.06	10.04	100.20		
100	6530411	19.84	20.02	99.10	99.18	0.484
	6568702	19.96	20.02	99.70		
	6515351	19.79	20.04	98.75		
150	9935024	30.18	30.02	100.53	99.76	0.682
	9811291	29.81	30.04	99.23		
	9837012	29.88	30.02	99.53		

Table 2: The Recovery data of Calcium Dobesilate

LOD & LOD: The limit of detection (LOD) The LOD for Calcium Dobesilate was found 0.569 µg/mL
The limit of quantitation (LOQ) The LOQ for Calcium Dobesilate was found to be 1.723 µg/mL The all value was found to be within the limit

Robustness: - This study of developed method was done using various parameters like change in wavelength and change in flow rate, the %RSD value of these observations in the specified range and method robustness is confirmed.

Table No 3: Change in wavelength, Flow rate by, Column oven temperature.

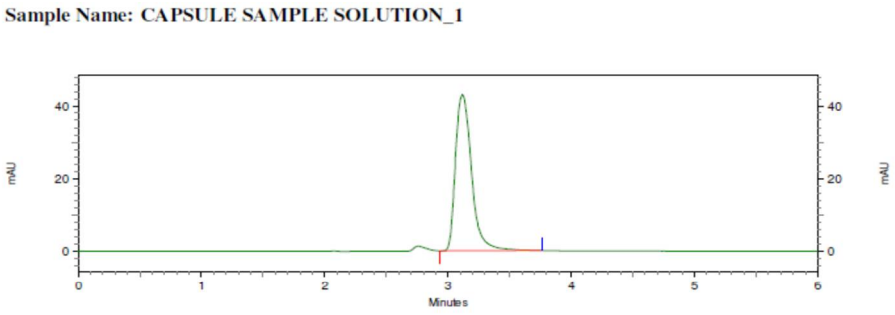
Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (303 NM)	3.11	6538534	1.48	3060
Wavelength by -3 NM (297 NM)	3.11	6143303	1.50	3060
Flow rate by +10% (1.1mL/min)	2.82	5893011	1.44	2861
Flow rate by -10% (0.9mL/min)	3.46	7208288	1.62	3340
Column oven temp by +2ºC (37 ºC)	3.11	6590471	1.43	3136
Column oven temp by -2ºC (33 ºC)	3.12	6611704	1.46	3067

Assay

Each Dobesil capsule tablet contains: Calcium Dobesilate 500mg

Table 4: Assay results of Dobesil 500 mg Capsule.

Sample	Area	% Assay	Mean Assay
Sample 1	6510263	98.87	98.98
Sample 2	6534261	99.08	
Sample	Area	% Assay	Mean Assay



VWD: Signal A, 300 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Calcium dobesilate	3.12	6510263	1.45	3039
Totals		6510263		

Fig.4 Chromatogram of Tablet formulation of Calcium Dobesilate

CONCLUSION

In this paper a new RP-HPLC method described for simultaneous estimation of Calcium Dobesilate. In RP-HPLC method, the estimation of Calcium Dobesilate carried out by Acetonitrile: 0.1% OPA in water (20:80 % v/v) as mobile phase, flow rate of 1.0 mL/min & Kromasil C18, 250 mm X 4.6mm ID, 5 µm column. The detection of Calcium Dobesilate was carried out at 300 nm. Retention time of Calcium Dobesilate was found at 6 min respectively. The LOD(Limit of detection) of Calcium Dobesilate was found to be 0.569 µg/mL respectively and LOQ (Limit of Quantitation) of Calcium Dobesilate was found to be 1.723 µg/mL .The results of analysis is both the methods were validated by ICH guidelines in terms of linearity & range, accuracy, precision, robustness &LOD, LOQ, Ruggedness solution stability form the studies it is concluded that developed method of RP-HPLC can be successfully used for the estimation of Calcium Dobesilate in their tablet formulations. The developed RP-HPLC method are accurate, precise, sensitive, reliable, specific, reproducible, rapid & economical.

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