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A validated stability-indicating HPLC assay method for Nicardipine Hydrochloride in bulk drug and dosage form

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ABSTRACT

An isocratic reversed phase stability-indicating high-performance liquid chromatographic (HPLC) assay method was developed and validated for quantitative determination of Nicardipine hydrochloride in bulk drugs and the degradation products generated from forced decomposition. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using an Waters Symmetry shield C18 (250 x 4.6)mm,5u column and the mobile phase containing the of mixture of triethylamine-phosphoric acid buffer (pH-3.5 by orthophosphoric acid, acetonitrile (35:65,v/v). The detection was carried out at wavelength 353 nm. The Nicardipine hydrochloride was subjected to stress conditions of hydrolysis (acid, base), oxidation (50 % H_2O_2).The degradation was observed for buclizine hydrochloride in base and negligible degradation observed 50 % H_2O_2. The mass balance was close to 100 in all the stress conditions. The degraded products were well resolved from main peak. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, selectivity, robustness and forced degradation studies prove the stability indicating ability of the method.

Introduction

Nicardipine hydrochloride is 2-(N-benzyl-N-methyl amino) ethyl methyl 1, 4-dihydro-2, 6-dimethyl-4-(m-nitrophenyl)-3, 5-pyridinedicarboxylatemonohydrochloride is a calcium antagonist with highly potent vasodilating activity and has been widely used for the treatment of hypertension and cerebrovascular disease [1, 2]. Literature surveys reveal, high-performance liquid chromatographic methods were reported for the determination of Nicardipine in bulk drugs and dosage form.[3,4]. We are gratified to report a stability indicating HPLC method for the analysis and separation of drugs from the degradation products formed under ICH suggested conditions hydrolysis, oxidations, and thermal stress. In present article, reversed phase HPLC method was developed for the separation of Nicardipine in bulk drug and the impurities formed from its forced degradation under stress conditions like acid hydrolysis, base hydrolysis, oxidation, heat. [5,6]

Experimental

Material and reagents

Nicardipine hydrochloride bulk drug was made available from Glen mark Ltd. India (purity 99.3). Orthophosphoric acid, triethylamine, and hydrochloric acid were obtained from Rankem fine chemicals, India Limited. Acetonitrile, hydrogen peroxide, sodium hydroxide were obtained from Rankem laboratories, India. All chemicals and reagent were used as HPLC grades, Milli-Q-Water was used throughout the experiment.

Chromatographic Conditions

A chromatographic system (Systronic) consisting of binary solvent delivery pump, a degasser, an auto- injector, column oven and UV detector, 10A-VP series with Cromoline software. The chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with Octadecyl silane Waters Symmetry Shield C18 (Water Ltd., USA) stationary phase with particle size 5 micron and pore size 100A° was used. The instrumental settings were a flow of 1 ml/min, the injection volume was 20 µl.

Mobile Phase

The Mobile phase consisted of a mixture of Triethylamine-phosphoric acid buffer (pH-3.5 by orthophosphoric acid, acetonitrile (35:65,v/v). The mobile phase was premixed and filtered through a 0.45 µm nylon filter and degassed.

Preparation of Standard stock solutions

Standard stock solutions of 50 ppm of Nicardipine hydrochloride in acetonitrile and water(1:1) were prepared in volumetric flasks. Working solutions were prepared by diluting the stock solutions with the same solvent (0.025 µg/ml).

Sample solution (Tablets)

Ten tablets of Cardene (25mg) were finely ground using agate mortar and pestle. The ground material, which was equivalent to 10 mg of the active pharmaceutical ingredient, was transferred accurately in to a 100ml calibrated dark flask containing acetonitrile and water mixture (1:1) the content of the flask was shaken for about 45 min and diluted to volume with same solvent. The solution was filtered through 0.45-micron filter, that to separate out the insoluble excipients, rejecting the first portion of the filtrate. The desired concentration for the drug was obtained by accurate dilution and the analysis was followed up as in the general analytical procedure [7, 8].

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Selectivity

Selectivity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradants, matrix etc. The selectivity of the developed LC method for buclizine was carried out in the presence of its degradation products. Stress studies were performed for Nicardipine bulk drug to provide an indication of the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (0.05 N Hydrochloric acid), alkali (0.025N NaOH), hydrogen peroxide (50%), heat (60 ^{o}C) to evaluate the ability of the proposed method to separate Nicardipine from its degraded products. For heat study, study period was 7 days where as for acid, oxidation 48 hr and for base 2 hour. Assay studies were carried out for stress samples against Nicardipine reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degraded products) was calculated. The excipient mixture present in Nicardipine tablets was injected in the optimized conditions to show the selectivity of the method in formulation of Nicardipine

Results and discussion

Optimization of chromatographic conditions

The main target for the development of chromatographic method was to get the reliable method for the quantification of Nicardipine from bulk drug and which will be also applicable for the degradable products. Initially, we took the effort for the development of HPLC method quantification of standard Nicardipine from bulk. For this purpose, we have used Water nova pack C18(150X4.6)mm, 5µ, Kromasil C18(150X4.6)mm,5µ, Inertsil ODS 3V C18(250X4.6)mm,5µ and Kromasil C18(250X4.6)mm,5µ,Star ODS-II C18 (250X4.6)mm,5µ and Grace Alpha C18 (250mm x 4.6)mm,5u Out of these used HPLC column, Grace Alpha C18 (250mm x 4.6)mm,5u found to comparatively better and gave the graph with better gaussian shape at retention time 4.392 min. To improve the shape and width of the graph, for the above columns different solvents and buffer taken for trials such as 0.1M KH_2PO_4 and Acetonitrile (60:40,v/v) in these trials peak shape is not good,another trials 0.01M Ammonium acetate P^H-5.9 and acetonitrile (20:80,v/v) peak shape not found well, trials Acetonitrile and water (80:20, v/v) column temperature 35^{o}C peak shape not found good, trials K_2HPO_4,Methanol and water (10:70:20,v/v/v)column temperature 35^{o}C, trials 1.0gm KH_2PO_4 and 0.45gm 1-Hexa sulphonic acid sodium salt make P^H-3.5 Ortho phosphoric acid and methanol(25:75, v/v) peak shape obtained but retention is not good, finally try for triethylamine-phosphoric acid buffer (P^H-3.5 by orthophosphoric acid), acetonitrile (35:65,v/v) good peak shape and retention observed.

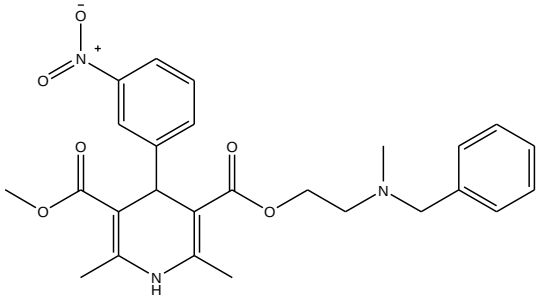

Figure 1. Chemical structure of Nicardipine

Result of forced degradation experiments

Considerable degradation was not observed in Nicardipine hydrochloride bulk samples, under stress conditions such acid (fig.3), Considerable degradation of Nicardipine hydrochloride was observed under stress condition such as base (fig.4) and oxidative hydrolysis (fig.5) leads to the formation of some unknown degradation peaks. The mass balance of Nicardipine hydrochloride in stress samples was close to 100% and moreover, the unaffected assay of Nicardipine hydrochloride in the Tablets confirms the stability indicating power of the method. The summary of forced degradation studies is given in Table I.

Figure 2. A typical blank chromatogram of the tablet Nicardipine

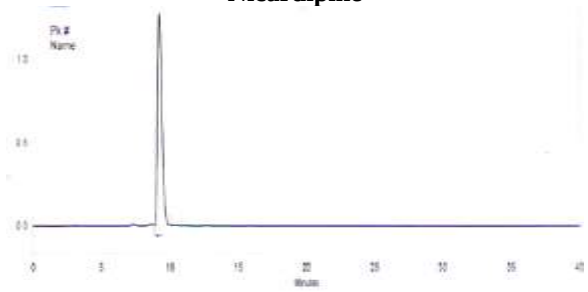

Figure 3. A typical chromatogram of the tablet: Nicardipine

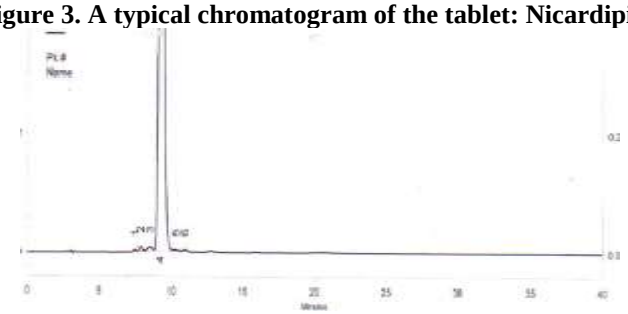

Figure 4. Chromatogram of Nicardipine in Acid degradation

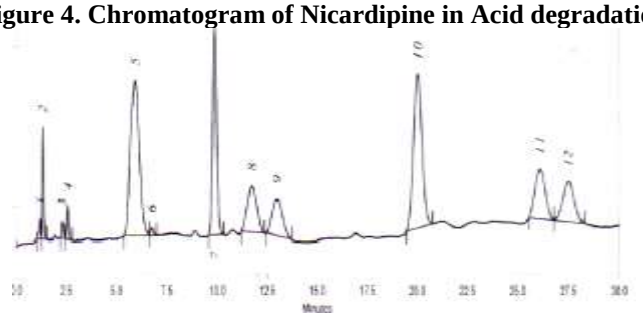

Figure 5. Chromatogram of Nicardipine in Base degradation

Method Validation

System suitability

For system suitability studies, five replicate injections of acid, base and oxidative degraded solutions were used and the RSD of peak area ratio, resolutions, tailing factor and number of theoretical plates of the peak were calculated. The system suitability results are shown in Table II.

Precision

The precision of the method was studied by determining the concentrations of the drug Nicardipine hydrochloride in the

tablet for six times [9,10]. The results of the precision study (Table III) indicate the reliability of the method (RSD %< 2).

Intermediate precision (reproducibility)

Intermediate precision of the method was determined by analyzing the samples for six times on different days, by different chemists, by using different analytical columns of the same make and different HPLC systems. The percentage assay was calculated using calibration curves. The assay results are shown in Table IV.

Accuracy (Recovery test)

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80%, 100% and 120% of the label claim of the tablet (25mg). and the amounts of Nicardipine hydrochloride at 80%, 100% and 120% of the label claim of the tablet were added to it. The recovery samples were prepared as aforementioned procedure. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Nicardipine hydrochloride ranged from 101.66 % to 103.11% (Table V). The average recoveries of three levels nine determinations for Nicardipine hydrochloride were 98.73 - 102.11%.

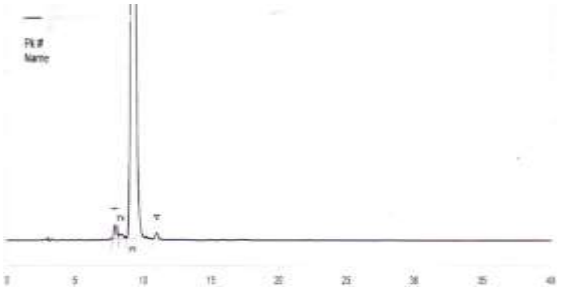

Figure6. Chromatogram of Nicardipine in Oxidative degradation

Calibration and linearity

Linearity test solutions for the method were prepared from Nicardipine hydrochloride stock solutions at six concentrations levels from tested from 10% to 150% of the targeted level (0.025 µg/ml), of the assay concentration Nicardipine hydrochloride. Standard solutions containing 10-150 µg/ml of Nicardipine hydrochloride in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration data was treated by least-squares linear regression analysis, the calibration graphs were found to be linear in the mentioned concentrations the slopes and correlation coefficients are shown in Table –III.

Robustness

To determine the robustness of the developed method experimental condition were purposely altered and the resolution between Nicardipine hydrochloride and acid degraded product were evaluated. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, it was changed by 0.2 unit from 0.8 to 1.2ml/min while the other mobile phase component were held as stated in chromatographic conditions. The effect of percent organic strength on resolution was studied by varying acetonitrile from –10 to +10 % while other mobile phase components were held constant as stated in chromatographic condition. The effect of column temperature on resolution was studied at 25 and 35^{o}C instead of 30^{o}C while the other mobile phase components were held constant stated in chromatographic condition. The results are shown in table-VI

Stability of analytical solution

The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 72 h for Nicardipine hydrochloride was 0.35 %. The assay values were within $\pm$ 2 % after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

Determination of active ingredients in tablets

The contents of drug in tablets were determined by the proposed method using the calibration curve. The results are shown in Table IV. The chromatogram of the tablet sample is shown in (Fig. 1).

Conclusion

The method developed for quantitative determination of Nicardipine hydrochloride is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all method-validated parameters tested. The developed method is stability indicating and can be used for assessing the stability of Nicardipine hydrochloride as bulk drugs. The developed method can be conveniently used for the assay determination of Nicardipine hydrochloride in bulk drugs and pharmaceutical dosage form. The developed method can be conveniently used for dissolution of tablets of the pharmaceutical dosage forms containing Nicardipine hydrochloride in quality control laboratory.

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Table I Summary of Forced degradation results

Stress condition	Time	Assay of active Substance %	Mass balance (% Assay + % Impurity)	Remarks
Acid Hydrolysis (0.05 N HCl)	48 Hrs	98.77	99.78	negligible degradation
Base Hydrolysis (0.025 N NaOH)	2 Hrs	70.01	99.65	Degradation
Oxidation (30% H_2O_2)	48 Hrs	96.44	99.68	Degradation

Table II System suitability reports

Compound (n=3)	Retention Time	% RSD	USP tailing	Theoretical plates
Nicardipine	9.867	0.35	1.02	8600
Acid Degraded Product	9.834	0.43	1.12	7888
Base Degraded Product	9.857	0.72	1.13	6000
H_2O_2 Degraded	9.853	0.83	1.43	7345

Table III Results of the Linearity study and Precision

Ingredient	Precision (% RSD)	Linearity (µg/ml)	Slopes* (n= 3)	Coefficients of correlations
Nicardipine	0.66	10-150	5311.2	0.99994

*Standard deviation shown in parentheses

Table IV Assay Results of Active Ingredients in Tablets

Set (n= 3)	Label value(mg)	Found (mg)*	% assay	SD	RSD%
1	25	25.44	99.54	0.567	0.78
2	25	25.47	99.78	0.047	0.67

*Average of six analyses

Table V Results of the Recovery Tests for the Nicardipine

Level of Addition (%)	Amount added (n = 3) (mg)	% Recovery*	% Average recovery^
80	20	98.22	99.56
100	25	101.77	100.11
120	30	102.33	101.32

* RSD shown in parenthesis.
^ Average recovery = the average of three levels, nine determinations

Table VI Results of robustness study

Sr. No.	Parameters	Variations	
1	Temperature	a)	at 25 °C
		b)	at 35 °C
2	Flow rate	a)	0.8 ml/min
		b)	1.2 ml/min
3	Mobile phase	a)	58.5 ml
		b)	71.5 ml