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RESEARCH ARTICLE

Development of Validated Reversed-phase High-performance Liquid Chromatography Method for the Estimation of Nifedipine *In situ* Gel Form

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ABSTRACT

The primary point of this research was to develop the reversed-phase high-performance liquid chromatography (RP-HPLC) method for the determination of nifedipine *in situ* gel. The method development was done using different solvents, mobile phase composition, flow rate, and column. The developed technique was approved according to the ICH guideline Q2R1. Chromatographic separation of nifedipine from gel was achieved on Primesil C-8, dimension: 250 mm × 4.6 mm, 5 µ, i.e. a stainless steel column 250 mm long, 4.6 mm id loaded with octadecyl silane chemically bonded to permeable silica particles of 5 µm diameter maintained column oven temperature at 25°C. Acetonitrile:methanol:water in the ratio of 9:36:55 (v/v/v) was selected, as it gave symmetrical peak of nifedipine with minimal tailing. The chromatograph were recorded with UV vis detector at 235 nm wavelength and flow rate was 1 ml/min. The accuracy and precision of the methods were determined and validated statistically. Every method demonstrated great reproducibility and observed to be fast, exact, precise, and accurate with percent relative standard deviation <2. The method was observed to be simple, rapid, accurate, and precise. These methods can be effectively connected for the routine investigation of standard preparation and sample preparations.

Keywords: ICH guideline, nifedipine, reversed-phase high-performance liquid chromatography, validation

INTRODUCTION

Nifedipine (3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) [Figure 1] is a dihydropyridine calcium channel blocker that principally squares L-type calcium channels.[1] Nifedipine is utilized to treat hypertension and to control angina (chest pain).[2] It acts basically on vascular smooth muscle cells by balancing out voltage-gated L-type calcium channels in their dormant compliance.[3] It brings down circulatory strain by loosening up the veins so the heart does not need to pump as hard. It controls chest pain by expanding the supply of blood and oxygen to the heart.[4] Literature survey for nifedipine uncovered a few logical strategies dependent on various methods, namely ultraviolet (UV) spectrophotometric,[5,6] method for simultaneous determination, method validation of nifedipine solid dosage form, high-performance liquid chromatography (HPLC)[7,8] investigation of nifedipine residues on stainless steel surfaces in the manufacture of pharmaceuticals, techniques for the determination of nifedipine in pharmaceutical preparation, and RP-HPLC.[9-13]

In situ framing gels are formulations, applied as a solution, which undergo gelation after instillation due to physicochemical changes intrinsic to the biological fluids. Along these lines, the polymers, which indicate sol-gel phase transition and in this way trigger drug release in due to external stimuli, are the most investigated. In the present study, an endeavor has been made to create simple, accurate, and precise HPLC techniques for the determination of nifedipine *in situ* gel form.

MATERIALS AND METHODS

Reagents and materials

Nifedipine bulk powder was kindly gifted by Helios Orgo Chem., Chennai, India. The nifedipine

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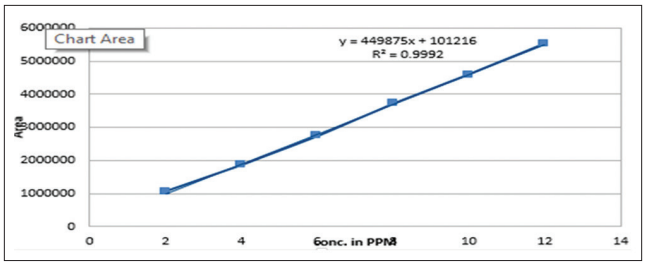

Figure 1: Linearity graph for nifedipine

Table 1: Linearity of nifedipine

Sr. No.	Concentration in ppm	Peak area
1	Linearity-2 ppm	1,062,342
2	Linearity-4 ppm	1,865,342
3	Linearity-6 ppm	2,735,648
4	Linearity-8 ppm	3,725,642
5	Linearity-10 ppm	4,578,213
6	Linearity-12 ppm	5,534,875
	Slope	44,987
	Intercept	10,121
	Correlation-coefficient	0.999

in situ gel was prepared in laboratory [Table 1]. HPLC grade acetonitrile and methanol were used for Mobile phase preparation. The water used was of HPLC grade by triple glass distillation and separated through nylon 0.45 µm-47 mm layer filter.

Instrumentation

An Agilent Technology 1220 infinity series RP-HPLC instrument (LC-2010CHT) equipped with a UV-visible detector and a photodiode array (PDA) detector, manual injector with 20 mL loop, C18 column (250 mm × 4.6 mm id, 5 mm particle size), EZ chrome elite software, with gradient pump, digital pH meter (LI 612 pH analyzer, Elico, Mumbai), analytical balance (Sartorius CP224S, Gottingen, Germany), and ultrasonic cleaner (Wenser WUC series 2.5 L) were used in the study.

Selection of analytical wavelength

Standard stock dilutions of nifedipine were prepared separately with mobile phase to get the last concentration of 20 µg/ml of nifedipine. Every solution was scanned utilizing UV-visible spectrophotometer in the spectrum mode of range between the wavelength scope of 400 and 200 nm and their spectra were overlaid. The wavelength selected was 235 nm.

Selection of mobile phase

The pure drug of nifedipine was injected into the HPLC framework and keeps running in various solvent systems. A mixture of various solvents was tried so as to decide ideal chromatographic conditions for compelling separation of nifedipine. After few stage and combination, it was discovered that blend of acetonitrile: methanol:water gives agreeable outcomes when contrasted to other mobile phases. As last, the optimal composition of the mobile phase acetonitrile:methanol: water in the ratio of 9:36:55 (v/v/v) was selected, as it gave high resolution of nifedipine with minimal tailing.

Preparation of mobile phase

The acetonitrile: methanol:water was mixed in ratio (9:36:55 v/v/v) and degassed with ultrasonic bath. The mobile phase was separated through nylon 0.45 µm–47 mm membrane channel and was degassed before utilize.
Diluent: Same as mobile phase.

Preparation of standard stock solution

About 10 mg of nifedipine working standard was accurately weighed and transferred it into a 100 ml volumetric flagon. 30 ml of diluent was added and sonicated for 5–10 min to dissolve the drug completely, and finally, the volume was made with diluent and mixed. The concentration of nifedipine is 100 mcg/ml.

Application of proposed technique for examination of nifedipine *in situ* gel formulation

About 1.6 ml of nifedipine *in situ* gel was taken and diluted in 100 ml methanol. This solution was sonicated for 30 min. Once the solution was obtained it was sifted through 0.45 µ layer filter membrane filter. The concentration of sample solution is 100 mcg/ml.

Validation of the proposed HPLC method

Calibration curve (linearity)
Calibration curves were built by plotting peak areas versus concentrations of nifedipine, and the regression equations were determined. The calibration curves were plotted over the focus

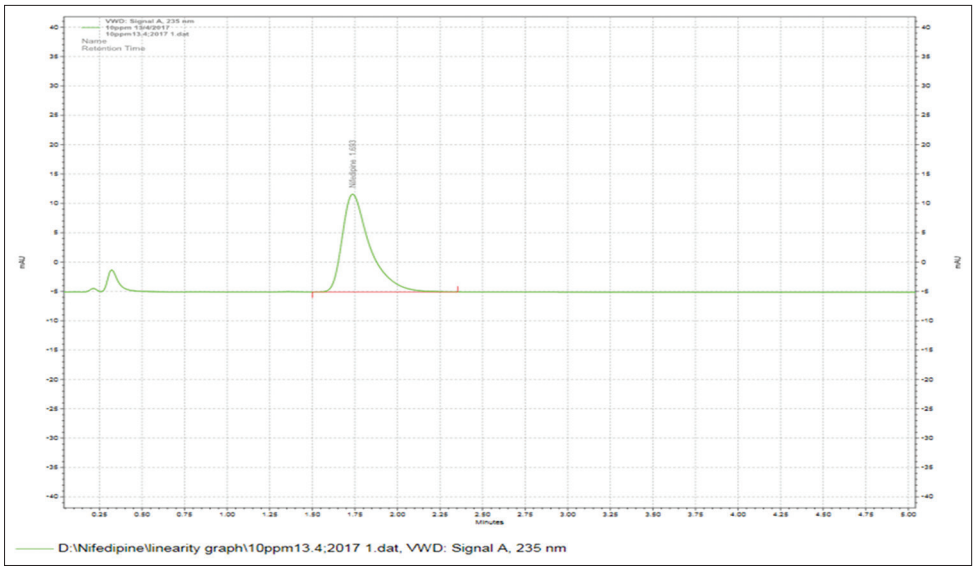

Figure 2: Completely resolved chromatogram

Table 2: Accuracy of nifedipine *in situ* gel

Sample identity	Area	Amount recovered in %
Accuracy_80% Set-I	8,423,566	101.2
Accuracy_80% Set-II	8,423,452	99.92
Accuracy_80% Set-III	8,423,461	99.97
Accuracy_100% Set-I	9,532,642	98.95
Accuracy_100% Set-II	9,532,812	100.7
Accuracy_100% Set-III	9,532,711	100.1
Accuracy_120% Set-I	10,245,678	99.98
Accuracy_120% Set-II	10,245,773	100.9
Accuracy_120% Set-III	10,245,832	101.3
	Average	100.6
	% RSD	1.262

% RSD: Percent relative standard deviation

go 2–12 µg/ml. Accurately estimated standard working solutions of nifedipine (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ml) were exchanged to a progression of 10 mL of the volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µl) of every solution were injected under the working chromatographic conditions portrayed previously.

Accuracy (% recovery)

The accuracy of the method was dictated by calculating recoveries of nifedipine by the standard addition technique. Known measures of standard solutions of nifedipine were added at 80, 100, and 120% level to prequantified test solutions of nifedipine. The measures of nifedipine were evaluated by applying obtained values to the regression equation of the calibration curve.

Precision (% repeatability)

The framework precision of the instrument was checked by over and over-preparing and injecting (n = 6) standard solutions of nifedipine (100 µg/ml) under the equivalent chromatographic condition, tailing factor, peak area, and retention time were estimated. The method precision was determined by injecting duplicate injection of each test solution (n = 6) for two different days. The outcomes were reported for as far as percent relative standard deviation (% RSD).

Ruggedness

Level of reproducibility of the test outcome was determined obtained by injecting standard solutions of nifedipine samples, under assortment of condition such as different laboratories, analyst, instrument, passed time, different time, temp, and days. Standard solutions of nifedipine samples prepared by 1.6 ml of nifedipine *in situ* gel were taken and diluted in 100 ml methanol (100 µg/ml). The results were accounted for as far as % RSD.

Robustness

It is the measure of capacity of the method to stay unaffected by little but purposeful variation in method parameter and provides a sign of its reliability under normal usage. It is performed by changing flow rate by ±0.1ml/min. The concentration of standard sample solution (100 µg/ml). Robustness of the method was assessed by determining the % RSD values.

Table 3: Precision of nifedipine *in situ* gel (day1)

Sample identity	Peak area	Amount recover in %	
		Day 1	Day 2
Precision_set_1	4,456,862	101	100.8
Precision_set_2	4,403,909	99.8	100.6
Precision_set_3	4,421,560	100.2	99.9
Precision_set_4	4,408,322	99.9	99.8
Precision_set_5	4,452,450	100.9	101
Precision_set_6	4,443,624	100.7	99.7
	SD	0.519294	0.565685
	% RSD	0.51714	0.563993

% RSD: Percent relative standard deviation

Table 4: Ruggedness of nifedipine *in situ* gel

Sr. No	Peak area	Amount recover in %	
		Analyst 1	Analyst 2
1	4,465,688	101.2	100.8
2	4,411,852	99.98	100.6
3	4,410,970	99.96	99.9
4	4,452,450	100.9	99.89
5	4,448,037	100.8	101
6	4,470,100	101.3	99.7
	SD	25921.9	0.550
	% RSD	0.583410	0.55

% RSD: Percent relative standard deviation

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD as 3.3 σ/*S* and LOQ 10 σ/*S* were calculated as per ICH guidelines, where σ is the SD of the response (*y*-intercept) and *S* is the slope of the calibration plot [Figure 1].

RESULTS AND DISCUSSION

Method development

To upgrade the RP-HPLC parameters, few mobile phase compositions were attempted. A good peak symmetry and satisfactory separation for nifedipine was acquired with a mobile phase acetonitrile:methanol:water in the ratio of 9:36:55 (v/v/v) at a flow rate of 1.0 ml/min to show sign of better reproducibility and repeatability. Based on peak area, quantification was achieved with PDA detection at 235 nm. Complete resolution of the peaks with clear baseline was obtained [Figure 2]. Peak purity of drugs was affirmed by looking the spectra of standard and sample solutions. System suitability parameters were inside the acceptance criteria as per the guidelines.

Table 5: Data for change in flow rate

Sr. No.	Description	%LC of nifedipine	
		Low flow (−0.1 ml)	High flow (+ 0.1 ml)
1	Precision set-1	100.1	100.1
2	Precision set-2	101.9	101.9
3	Precision set-3	99.9	99.9
4	Precision set-4	99.8	99.8
5	Precision set-5	100.0	100.0
6	Precision set-6	102.0	102.0
7	Robustness set-1	99.9	99.2
8	Robustness set-2	100.3	99.8
9	Robustness set-3	100.1	98.9
	Mean	100.5143	100.4143
	SD	0.985611	1.088468
	%RSD	0.9805	1.083

% RSD: Percent relative standard deviation

Table 6: Limit of detection and limit of quantitation

Parameter	Nifedipine
Limit of detection (μg/ml)	0.473
Limit of quantitation (μg/ml)	1.435

Validation of the proposed method

Linearity

Linear correlation was acquired between peak area and concentrations of nifedipine in the ranges of 2–12 μg/ml. The linearity of the calibration curve was validated by the high estimations of the correlation coefficient of regression and it was found to be 0.998 [Table 1].

Accuracy

Accuracy performed at level 80%, 100%, and 120%. The mean of recovery is 100.6% and RSD is 1.262 for nifedipine *in situ* gel. Along these lines, the HPLC technique for the estimation of nifedipine *in situ* gel is accurate [Table 2].

Precision

System precision

The RSD of system precision was observed to be 0.002854% [Table3]. Therefore, the HPLC technique for the determination nifedipine is precise.

Precision (Day 1)

The RSD of precision is 0.517%. Therefore, the HPLC method for the determination of nifedipine *in situ* gel is precise [Table 3].

Precision (Day 2)

The RSD of system precision is 0.561%. Therefore, the HPLC method for the determination of nifedipine *in situ* gel is precise [Table 3].

Ruggedness (intermediate precision analyst II)

Overall, RSD for 12 results obtained from two different analysts is within limits. Therefore, the HPLC method for the assurance of nifedipine *in situ* gel is rugged [Table 4].

Robustness

Change in flow rate

%RSD of nine replicate injections for flow rate (±0.1 ml/min) is 0.9805 and 1.083. Therefore, the HPLC strategy for the determination nifedipine *in situ* gel in tablet is robust [Table 5].

LOD and LOQ

LOD and LOQ were calculated as 3.3 σ/*S* and 10 σ/*S,* where σ is the SD of the response (*y*-intercept) and *S* is the slope of the calibration plot which was found to be 0.473 µg/ml and 1.435 µg/ml, respectively, as per ICH guidelines [Table 6].

CONCLUSION

A new RP-HPLC method has been created according to the ICH guidelines for the determination of nifedipine in novel gastroretentive oral *in situ* gel. The proposed method is simple, accurate, precise, rapid, and specific. In this way, it very well may be reasoned that the created technique is suitable for routine investigation of nifedipine in pharmaceutical dosage form.

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