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Research Article

DEVELOPMENT OF THE METHODOLOGY OF THE CHROMATOGRAPHIC DETERMINATION OF NIFEDIPINE IN MEDICINES

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ABSTRACT

Objective: The aim was to develop a simple, rapid, less expensive, linear, precise, and accurate reverse phase high performance liquid chromatography method for determination of nifedipine in tablets.

Methods: The chromatographic analysis of nifedipine was performed using liquid chromatograph Agilent 1290 Infinity II LC System. Selected conditions were isocratic elution with binary mobile phase consisting of solution methanol and 0.1% trifluoroacetic acid (55:45). Detection was carried out using spectrophotometric detector at 265 nm. The method was validated as per ICH guidelines.

Results: The retention time for nifedipine by proposed high performance liquid chromatography (HPLC) method is observed as 3.5 minutes. The correlation coefficients are 1.0000. The developed chromatographic method was found to be accurate with recovery 99.2-99.8% and was found within the acceptance criteria (i.e. 98.0-102.0%) with acceptable % relative standard deviation of not more than 2% at each level. The assay results of nifedipine in tablets by developed method are highly reproducible, reliable and are in good agreement with the label claim of the medicines (average 99.62 %).

Conclusion: Finally, it should be noted that a new simple, rapid, linear, precise, accurate HPLC method was developed and validated for the determination of nifedipine in medicines in accordance with the ICH guidelines. These results show the method are accurate, precise, sensitive, economic, and rugged. The proposed HPLC method is rapider (retention time is 3.5 minutes). This method can be useful for the routine analysis of nifedipine in pharmaceutical dosage form.

Keywords: Nifedipine, High-performance liquid chromatography, Validation, Linearity, Accuracy, Range of application.

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INTRODUCTION

One of the important problems of modern pharmaceutical science is to replenish the range of new medicines and scientific justification of their application in clinical medicine. Increasing the number of antihypertensive medicines and different chemical substances and the need to identify them in present another active pharmaceutical ingredients are constantly put before standardization of medicines and pharmaceutical chemistry task of improving existing and developing new methods of analysis. In this regard, increasing the role of chromatographic methods, and especially - highly efficient options for liquid chromatography (HPLC). Nowadays spectroscopy with liquid and gas chromatography are the most common instrumental methods of analysis. Over the past decade developed spectrophotometric methods for determining amlodipine, nifedipine, verapamil, captopril, fosinopril, diltiazem in substance and dosage forms, and conducted validation and verification spectrophotometric techniques developed in compliance with pharmacopoeias. Along with the unexplored issue is the development of chromatographic methods for analysis of antihypertensive medicines in the standardization of this pharmacological group of medicines [1-6].

Nifedipine, dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, is a potent dihydropyridine-type calcium channel blocker which is an antihypertensive medicine. The pharmacopoeial chromatographic conditions are column chromatographic categories L1 (with a fixed phase C18) size of 4.6 mm × 250 mm; mobile phase - acetonitrile:methanol:water (25:25:50); wavelength - 265 nm, flow rate - 1.0 ml/minutes [7]. Our goal was to improve HPLC methods of analysis of nifedipine in medicines [8,9].

The aim of work was the development of simple, expressive, accurate, and less expensive analytical methods for the determination of nifedipine in medicines by HPLC.

METHODS

Instrumental and analytical conditions

In developing this technique, column Ascentis C18 was used, which has several advantages from a number of columns L1 and provides high speed and high efficiency at a lower pressure system. This reduces the number of used mobile phase that according reduces the cost analysis.

Selected conditions were isocratic elution with binary mobile phase consisting of methanol and 0.1% trifluoroacetic acid solution in the ratio of 55:45 for optimum peak symmetry of the active ingredient. Besides reducing the analysis time are achieved by increasing the flow rate of 1.5 mL/minutes and column temperature increase to 35°C.

The chromatographic analysis of nifedipine performed on liquid chromatograph Agilent 1290 Infinity II LC System.

The brief scheme of the experiment:

- Column: Ascentis express C18 column size 4.6 mm × 150 mm with a particle size of 5 μ;
- Mobile phase: Methanol R - 0.1% solution of trifluoroacetic acid R (55:45);
- The rate of mobile phase: 1.5 ml/minutes;
- Column temperature: 35°C;
- Wavelength of the ultraviolet detector: 265 nm.

Reagents and chemicals

Nifedipine was obtained as gift sample form company "Zdorovja" (Ukraine). Trade name of nifedipine is "Fenigidin Zdorovja." Tablets were procured from local pharmacy containing 10 mg of nifedipine. Ultrapure water was obtained from a Millipore system. HPLC solvents were obtained from Merck Company. All other chemicals used were AR grade.

Preparation of sample solution

To 200.0 mg powder pounded tablets, add 10 ml of methanol R, shake in ultrasonic bath for 10 minutes and add methanol R to the volume of 20.0 ml. Filter through a membrane filter with a pore size of 0.45 μ, discarding the first 5 ml of filtrate. 2.0 ml of the resulting filtrate adjusted to 20.0 ml of solvent.

Preparation of standard solution

20.0 mg of nifedipine SPhU dissolve in methanol R and dilute with the same solvent to 20.0 ml volume. 2.0 ml of the resulting solution adjusted to 20.0 ml of solvent.

RESULTS

For elaboration of the method the chromatograms of the standard solution of nifedipine (Fig. 1) and the sample solution of nifedipine (Fig. 2), as well as the dependence of the intensity peaks on the retention time were obtained and analyzed.

The chromatographic system is considered suitable if the following conditions are performed:

- The effectiveness of the chromatographic column, calculated peak of nifedipine should be not <4000 theoretical plates;
- Relative standard deviation (RSD) calculated peak area for nifedipine should be not more than 1.0%;
- Symmetry factor calculated on the peak of nifedipine should be <1.5.

The content of nifedipine (X) in one tablet, in milligrams, was calculated by the formula. Calculation formula:

$$X = \frac{S_i \cdot m_o \cdot b \cdot}{S_o \cdot B_i \cdot 100}$$

Where,

S_i - Average of the peak areas of nifedipine, calculated from the chromatograms of the sample solutions;

S_0 - Average of the peak areas of nifedipine, calculated with the standard solutions chromatograms;

m_0 - Mass of the sample SPhU nifedipine, in milligrams;

m_i - Mass of the powder pounded tablets, in milligrams;

P - Content of the main substance in SPhU nifedipine as a percentage;

b - Average of weight tablets in milligrams.

Method validation

Validation of an analytical procedure involves analytical tasks are articulated clearly - for what purpose the analytical procedure is used. We have studied the following validation characteristics: Linearity, accuracy and precision, range of application [10-12].

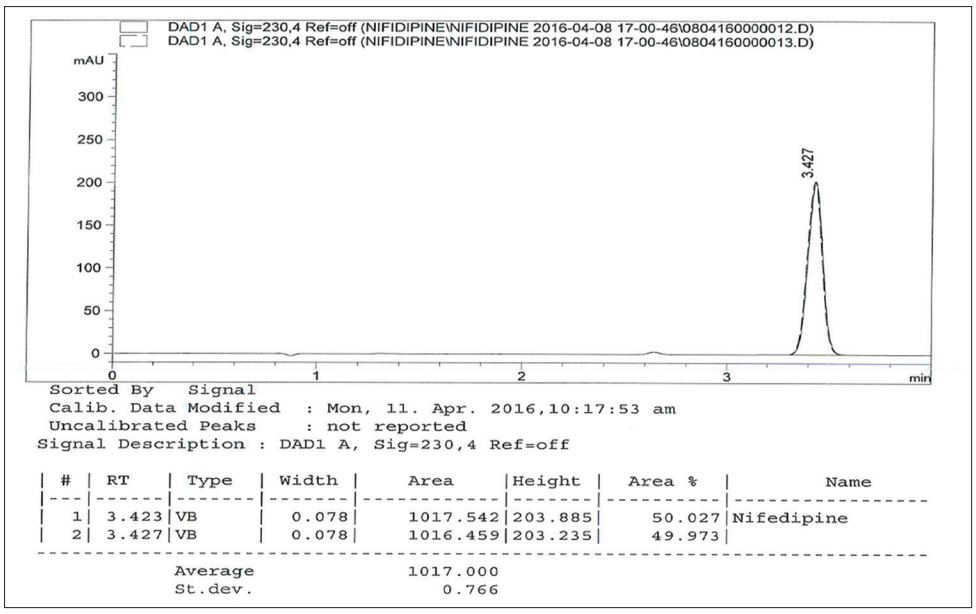
The statistical quantities and validation parameters were shown in Tables 1 and 2, Figs. 3 and 4.

DISCUSSION

In the work, a solution containing a mixture of the placebo was prepared using the sample preparation procedure to evaluate possible interfering peaks. The investigated chromatograms did not show any other substances peaks, which prove the specificity of the developed method.

Evaluation of linearity was performed on the range of application of the method using standard method. The results obtained were statistically processed by the least squares method according to the requirements of the SPhU and ICH guidelines. Calculation of the linear relationship $Y=mX+b$ was conducted by the least square method. The linearity plot of nifedipine is found linear and correlation coefficient 1.00000 for nifedipine. This performs that the method is linear in the specified range for the analysis of nifedipine in tablets.

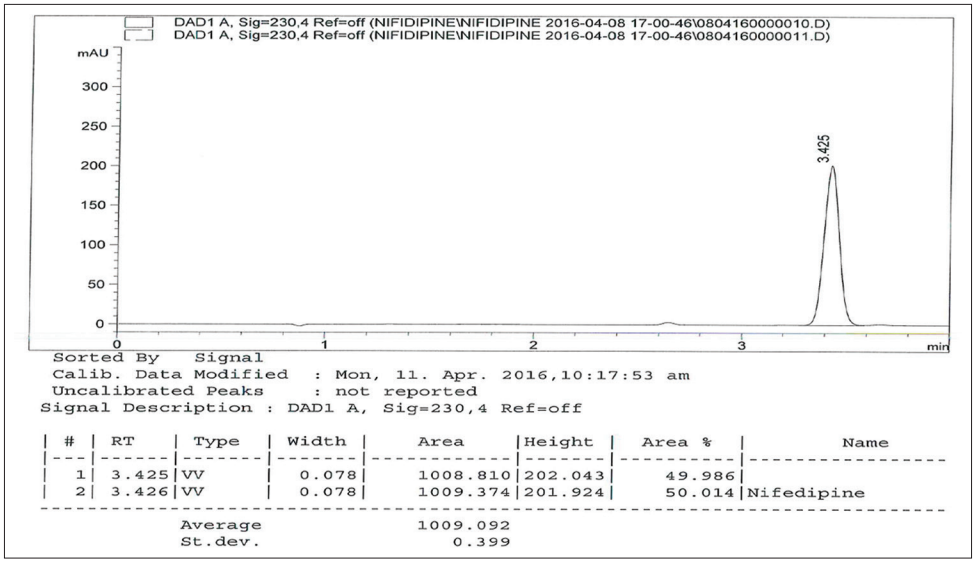
The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same sample under prescribed conditions. Accuracy and precision were studied by method "put-found" on standard solutions of nifedipine. The proposed chromatographic method was found to be accurate with recovery 99.2-99.8% and was found within the acceptance criteria (i.e., 98.0-102.0%) with acceptable % RSD of not more than 2% at each analyte level. Precision was calculated as repeatability and intraday and interday variation for nifedipine. The proposed method was found to be precise with coefficient of variance=0.5 for intraday and interday for nifedipine.



Sorted By Signal
Calib. Data Modified : Mon, 11. Apr. 2016,10:17:53 am
Uncalibrated Peaks : not reported
Signal Description : DAD1 A, Sig=230,4 Ref=off

#	RT	Type	Width	Area	Height	Area %	Name
1	3.423	VB	0.078	1017.542	203.885	50.027	Nifedipine
2	3.427	VB	0.078	1016.459	203.235	49.973	
	Average			1017.000			
	St.dev.			0.766			

Fig. 1: High performance liquid chromatography chromatograms of the standard solution of nifedipine in the terms of the quantification of nifedipine in tablets



Sorted By Signal
Calib. Data Modified : Mon, 11. Apr. 2016,10:17:53 am
Uncalibrated Peaks : not reported
Signal Description : DAD1 A, Sig=230,4 Ref=off

#	RT	Type	Width	Area	Height	Area %	Name
1	3.425	VV	0.078	1008.810	202.043	49.986	
2	3.426	VV	0.078	1009.374	201.924	50.014	Nifedipine
	Average			1009.092			
	St.dev.			0.399			

Fig. 2: High performance liquid chromatography chromatogram of the sample solution of nifedipine in the terms of the quantification of nifedipine in tablets

#	RT	Type	Width	Area	Height	Area %	Name
1	4.554	BB	0.135	1305.018	147.369	33.309	
2	4.559	VB	0.134	1305.667	146.356	33.325	
3	4.563	VB	0.136	1307.244	146.332	33.366	Amlodipine
	Average			1305.976			
	St.dev.			1.144			

#	RT	Type	Width	Area	Height	Area %	Name
1	4.470	VB	0.130	1258.198	146.335	50.023	
2	4.471	BB	0.130	1257.035	146.222	49.977	Amlodipine
	Average			1257.617			
	St.dev.			0.823			

Fig. 3: Resolution of nifedipine in different areas high performance liquid chromatography

Table 1: The results of HPLC determination of nifedipine

Model solution	Amount added (solution concentration), mg/ml	Amount found (concentration solution), mg/ml	Recovery, Z, %
RS 1	0.1080	-	-
RS 2	0.0995	-	-
MS 70%	0.0700	0.0690	98.6
MS 100%	0.0995	0.0993	99.8
MS 130%	0.1305	0.1300	99.6
The average			99.3
The RSD, Sz%			0.4137
Relative confidence interval, Δz%			0.99
The critical value for convergence results Δz%≤3.2			Conform
The criterion of statistical insignificance systematic error $\delta\% = \lvert\bar{Z} - 100\rvert \leq \Delta_z / \sqrt{n}$	≤0.33	Conform	
Criterion practical insignificance systematic error $\delta\% = \lvert\bar{Z} - 100\rvert \leq 0.32\Delta_{AS}$	≤2.048	Conform	
General conclusion about procedure		Conform	

HPLC: High performance liquid chromatography

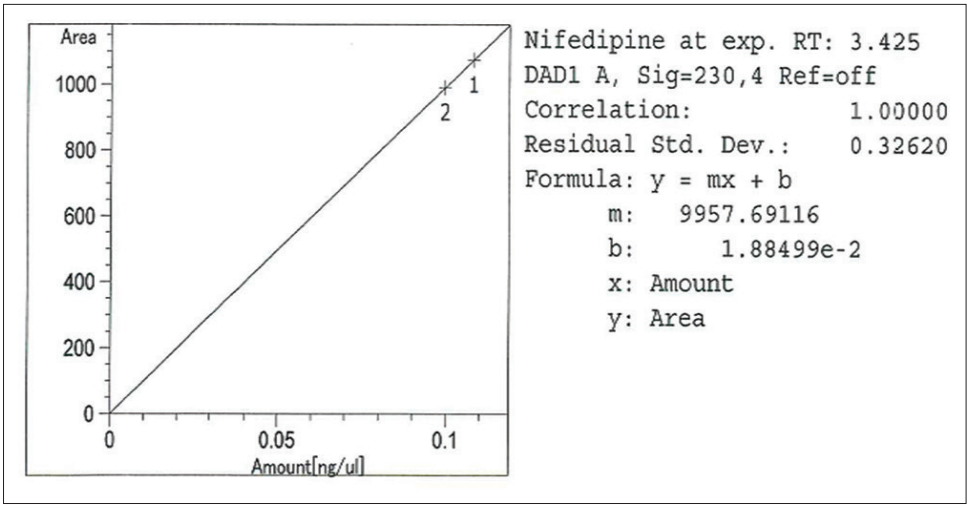


Fig. 4: The linear relationship of the area on nifedipine concentration

Table 2: Summary of validation parameters of nifedipine by proposed HPLC method

The slope of the linear relationship *b*	1.88499e-2
The constant term of the linear dependence *m*	9957.69116
The residual standard deviation *Sr*	0.32620
The correlation coefficient method *r*	1.00000
Accuracy, %	99.2-99.8
Repeatability, (% assay, n=6)	99.2-100
Precision (% assay)	
Interday (n=3)	101.2-101.8 (% RSD=0.5)
Intraday (n=3)	100.3-101.4 (% RSD=0.5)

RSD: Relative standard deviation, HPLC: High performance liquid chromatography

Table 3: Assay results

Sample no	Label claim (mg)	% Amount found	Average
1	10	99.8	99.62
2	10	99.6	
3	10	99.6	
4	10	99.7	
5	10	99.4	
6	10	99.6	

Our study describes new HPLC method using simple chromatographic conditions for the determination of nifedipine in tablets. Under this conditions, the peak of nifedipine elution is about 3.5 minutes.

The assay results of the tablets "Fenigidin Zdorovja" by developed chromatographic method are highly reproducible, reliable and are in good agreement with the label claim of the medicines (average 99.62 %) (Table 3). This demonstrated excellent result.

CONCLUSION

Finally, it should be noted that a new simple, rapid, linear, precise, accurate HPLC method was developed and validated for the determination of nifedipine in medicines in accordance with the ICH guidelines. These results show the method is accurate, precise, sensitive, economic, and rugged. The proposed HPLC method is rapider (retention time is 3.5 minutes). This method can be useful for the routine analysis of nifedipine in pharmaceutical dosage forms.

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