PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2021, **55**(3), p. 204–211

Chemistry

ISOLATION, IDENTIFICATION AND QUANTITATIVE DETERMINATION OF NIFEDIPINE FROM BIOLOGICAL MATERIAL

S. Gh. PETROSYAN [1*], L. Yu. SAHAKYAN [2**], V. G. KIRAKOSYAN [1***], E. V. MINASYAN [3****], S. E. SARDARYAN [3*****]

[1] *Chair of Pharmaceutical Chemistry and Pharmacognosy, YSU, Armenia*
[2] *Chair of Biomedicine, YSU, Armenia*
[3] *Laboratory of Asymmetric Synthesis, Institute of Pharmacy, YSU, Armenia*

The optimal conditions of the method, applicable during forensic chemical examinations, were elaborated. Particularly, the investigation of the conditions for the isolation of Nifedipine from liver and skeletal muscle tissues, the identification of the isolated Nifedipine by TLC method and checking the accuracy of the method using HPLC was carried out. As a result, a new alternative express method for the isolation and further identification of Nifedipine from different biomaterials was elaborated. The method is quite fast, accurate and much cheaper.

https://doi.org/10.46991/PYSU:B/2021.55.3.204

***Keywords*:** Nifedipine, isolation, biological material and identification.

Introduction. Nifedipine is a dihydropyridine calcium channel blocker indicated for the management of several subtypes of angina pectoris, and hypertension [1].

The use of calcium channel blockers is quite common in hypertension treatment, moreover, drugs of this group, as drugs of the first choice, are included in the guidelines of many countries [2, 3].

Nifedipine causes dilatation of vascular smooth muscle, vessels, dilates coronary and peripheral arteries, and reduces peripheral resistance, arterial pressure, and the oxygen supply of the heart.

Nifedipine is quite cheap, which contains the risk of its widespread use for self-medication. The lethal poisoning incidents due to incorrect acceptance of the Nifedipine are known in the world [4, 5]. Therefore, Nifedipine is a potential target for forensic chemical research.

The LD_{50} of Nifedipine after intragastric administration to laboratory animals is 1022 *mg*/*kg*. Studies have shown that Nifedipine is found in unchanged form both in organs and in the blood. There are recorded cases of fatal poisoning of people with Nifedipine as a result of maladministration or overdosing in the process of treatment or for committing suicide. In case of poisoning, the greatest amounts of Nifedipine is present in the muscles and liver [6].

* E-mail: satenikpetrosyan@ysu.am
** E-mail: lusine_sahakyan@ysu.am
*** E-mail: virab.kirakosyan@ysu.am
**** E-mail: ella.minasyan@yahoo.com
***** E-mail: sirsardayan1991@mail.ru

The present work is devoted to the elaboration of the method applicable in forensic chemical examinations, particularly, investigation of the conditions for the isolation of Nifedipine from the liver and muscles and the identification of the isolated Nifedipine by thin layer chromatography (TLC) method.

Experimental Part.

Obtaining a Model System. 10 *g* of beef liver (or muscle tissue) was cut and mashed in a mortar until a homogeneous mass. The well milled 0.01 *g* of Nifedipine was added to the mass and mixed with a magnetic stirrer (350 *r*/*min*) at room temperature. Then, 20 *mL* of ethanol was added to the mixture and mixed well, from which 5 μL of a sample was taken and dripped onto a TLC plate (Silufol).

Selection of Nifedipine Extraction Solvents. 20 *mL* of appropriate solvent (2 times more by mass) was added to the models of tissues saturated by Nifedipine and mixed at room temperature for 45 *min*.

Results and Discussion. For the identification of Nifedipine as a TLC eluent hexane : 1,4-dioxane : 2-propanol (15 : 5 : 2) was selected from systems known in the literature [7–9], because the spots were much clear and the outlines were much visible in UV light (254 *nm*). In the selected systems R_f-values of Nifedipine and its decomposition product were found by different concentration spots. For Nifedipine $R_f = 0.40$, and for the product of Nifedipine decomposition $R_f = 0.57$.

Obtaining a model system of two tissues saturated by Nifedipine was performed using the method mentioned in Experimental Part. The content of Nifedipine in the sample was tested by TLC method. The absorption of Nifedipine was checked by following the size of the spots in order to find the optimal retention time for Nifedipine-tissue mixture.

Table 1

The penetration of Nifedipine in liver and muscle tissue cells

№	Retention, time	Diameter of spot, *cm* (length / width)	
		liver tissue	muscle tissue
1	after 15 *min*	0.9/0.4	1.0/0.5
2	after 30 *min*	0.4/0.2	0.9/0.4
3	after 45 *min*	0.4/0.2	0.6/0.3
4	after 60 *min*	–	0.4/0.2
5	after 75 *min*	–	0.4/0.2

As seen from the Tab. 1 the amount of non-absorbed Nifedipine has not been changed in the case of the liver-after 30 *min*, and in the case of the muscle tissue-after 60 *min*. Therefore, for obtaining models it is enough to mix the liver-Nifedipine mixture at room temperature for 30 *min*, and the muscle-Nifedipine mixture for 60 *min*.

Selection of Nifedipine Extraction Solvents. At the next stage, when there were models of poisoning and the TLC solvent system was selected, the effectiveness of Nifedipine extraction with different solvents from the model of forensic sample was checked using the method mentioned in Experimental Part. The solvents shown in Tab. 2 were tested.

As seen from the Tab. 2 for both tissues the best extraction solvents were acetone and ethyl acetate.

Table 2

The effectiveness of the extraction of Nifedipine by different solvents

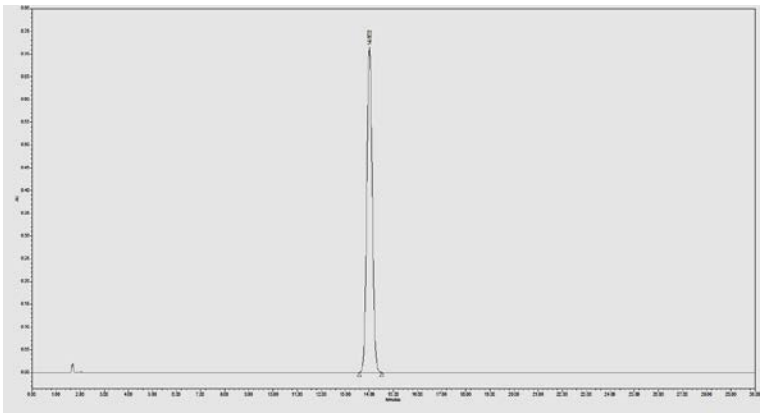
№	Solvent	Diameter of spot, *cm* (length / width)	
		liver tissue	muscle tissue
1	Acetone	**0.9/0.4**	**0.7/0.3**
2	Hexane	0.1/0.1	0.2/0.1
3	Chloroform	0.7/0.3	0.6/0.3
4	Acetonitrile	0.5/0.2	0.4/0.2
5	Isopropanol	0.4/0.1	0.3/0.1
6	Ethyl acetate	**0.8/0.3**	**0.6/0.3**
7	Methanol	0.4/0.2	0.4/0.2
8	Ethanol	0.5/0.2	0.4/0.2
9	1,4-dioxane	0.2/0.1	0.2/0.1
10	Methylene chloride	0.2/0.2	0.2/0.2

Next, the study of the dependence of the extraction efficiency on the extraction time was performed.

For this purpose, the extraction process with the best solvents acetone and ethyl acetate was followed by taking TLC samples every 15 *min*.

It was shown that after 30 *min* the amount of the extracted Nifedipine practically does not change.

In the last stage, the accuracy of the suggested method was checked by HPLC analysis [10]. For this purpose, for the two best solvents, liver and muscle tissues the sampling at 30th and 45th minutes of extraction was performed. The results are shown in Figs. 1–5 and in Tab 4. As can be seen in the case of liver tissues, solvents after 30 *min* already provide up to 80% extraction, which grows very little over the time. The chromatographic conditions are presented in Tab. 3.



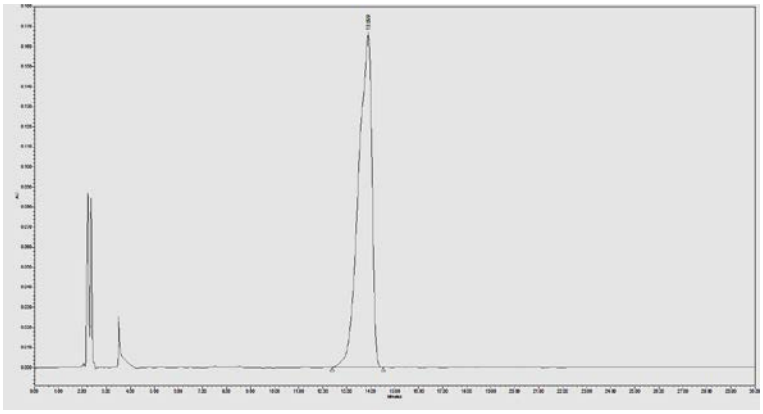
Name	Nifedipine
Retention time, *min*	14.002
Area, AU	11346016
% Area	100.00
Height	708819
Conc., %	100

Fig. 1. Chromatogram of Nifedipine (standard sample).

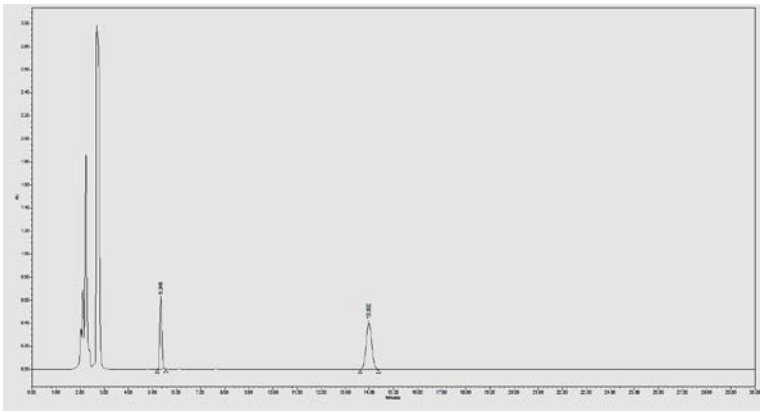
Table 3

Chromatographic conditions of HPLC analysis

Chromatographic column	Zorbax Eclipse C_{18}, 5 μm, 4.6 mm × 250 mm
Detector wavelength	254 nm
Flow rate	1 mL/min
Injection volume	20 μL
Column temperature	30°C
Pump operating mode	isocratic
Mobile phase	methanol : acetonitrile : water (1 : 1 : 2)



a



b

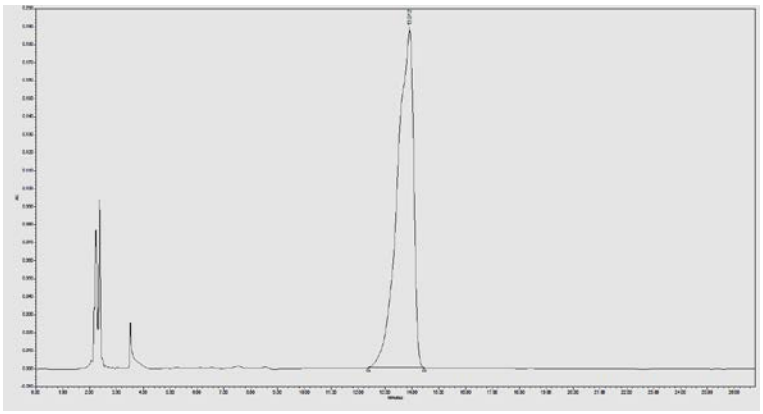
a

Name	Nifedipine
Retention time, *min*	13.899
Area, AU	6444867
% Area	100.00
Height	165915
Conc., %	56.8

b

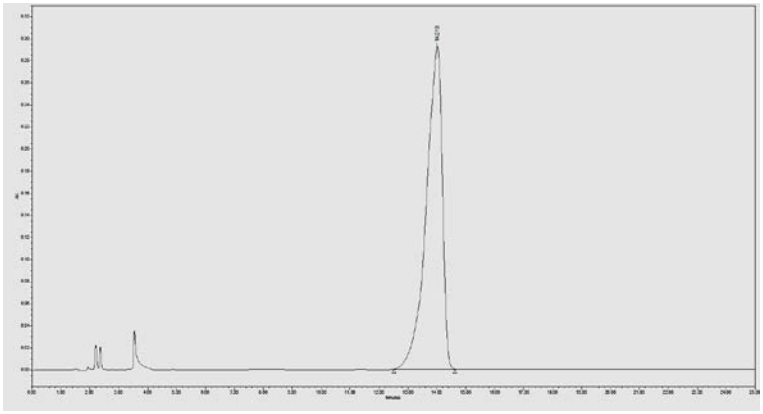
Name	Nifedipine
Retention time, *min*	13.980
Area, AU	6495876
% Area	63.98
Height	402150
Conc., %	57.25

Fig. 2. Chromatogram of Nifedipine extracted from muscle tissue by ethyl acetate (a) and acetone (b) after 30 *min*.

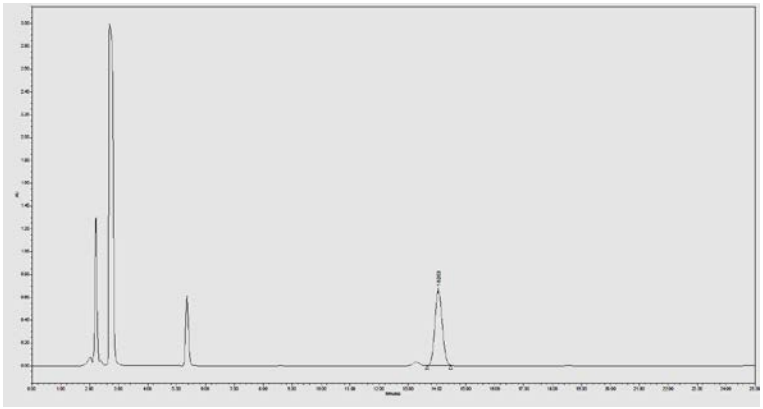


Name	Nifedipine
Retention time, *min*	13.912
Area, AU	7885283
% Area	100.00
Height	187491
Conc., %	69.49

Fig. 3. Chromatogram of Nifedipine extracted from muscle tissue by ethyl acetate after 45 *min*.



a



b

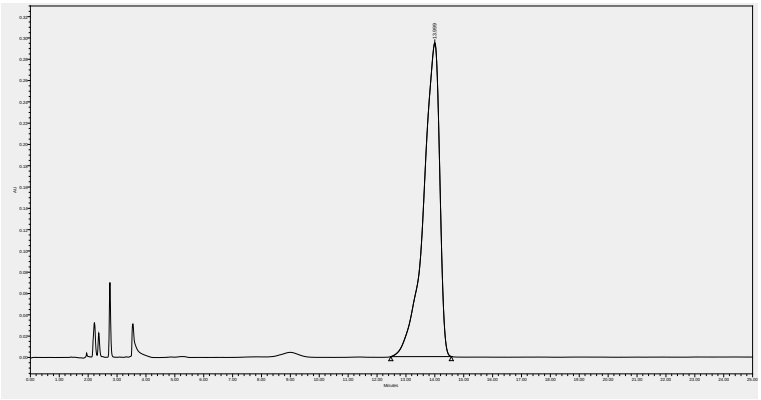
a

Name	Nifedipine
Retention time, *min*	14.018
Area, AU	11293582
% Area	100.00
Height	292621
Conc., %	81.3

b

Name	Nifedipine
Retention time, *min*	14.053
Area, AU	11654090
% Area	100.00
Height	655467
Conc., %	83.88

Fig. 4. Chromatogram of Nifedipine extracted from liver tissue by ethyl acetate (a) and acetone (b) after 30 *min*.



Name	Nifedipine
Retention time, *min*	13.999
Area, AU	11467353
% Area	100.00
Height	294576
Conc., %	82.54

Fig. 5. Chromatogram of Nifedipine extracted from liver tissue by ethyl acetate after 45 *min*.

Table 4

Nifedipine extraction process according to HPLC

№	Sample	Extraction yield, % The amount of Nifedipine in solution compared to initial added Nifedipine
1	Nifedipine extracted from *liver tissue* by *acetone after 30 min*	83.88
2	Nifedipine extracted from *liver tissue* by *ethyl acetate* after 30 *min*	81.3
3	Nifedipine extracted from *liver tissue* by *ethyl acetate* after 45 *min*	82.54
4	Nifedipine extracted from *muscle tissue* by *acetone* after 30 *min*	57.25
5	Nifedipine extracted from *muscle tissue* by *ethyl acetate* after 30 *min*	56.8
6	Nifedipine extracted from *muscle tissue* by *ethyl acetate* after 45 *min*	69.49

Conclusion. A method for the separation and determination of Nifedipine from beef liver and muscle tissues has been developed.

It has been shown that:

1. In the solvent system of hexane : 1,4-dioxane : 2-propanol (15 : 5 : 2) TLC can be used as an express method for the detection of Nifedipine.
2. For getting model system, the tissue-Nifedipine mixture is enough to keep for 30 *min* in the case of liver, and 60 *min* in the case of muscle tissue.
3. The best extraction result was observed when the volume of acetone or ethyl acetate added was twice the mass of the raw material and 30 *min* of extraction was enough.
4. The data obtained by the TLC method match the data of the HPLC analysis.

Thus, separation of Nifedipine from biomaterials with acetone is recommended and TLC method can be used for detection. Undoubtedly, after process validation it can be used during forensic chemical examinations as an alternative express method, which is quite fast, accurate and much cheaper.

The authors want to thank students Sh. Sargsyan and T. Gevorgyan for their help in the experimental part.

The study was partially supported by Drug Quality Control and Monitoring Center of Institute of Pharmacy of Yerevan State University.

Received 18.11.2021
Reviewed 09.12.2021
Accepted 16.12.2021

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Ս. Ղ. ՊԵՏՐՈՍՅԱՆ, Լ. Յու. ՍԱՀԱԿՅԱՆ, Վ. Գ. ԿԻՐԱԿՈՍՅԱՆ,
Է. Վ. ՄԻՆԱՍՅԱՆ, Ս. Ե. ՍԱՐԴԱՐՅԱՆ

ԿԵՆՍԱՀՈՒՄՔԻՑ ՆԻՖԵԴԻՊԻՆԻ ԱՆՋԱՏՈՒՄԸ, ՆՈՒՅՆԱԿԱՆԱՑՈՒՄԸ ԵՎ ՔԱՆԱԿԱՆԱՆ ՈՐՈՇՈՒՄԸ

Իրականացվել է դատաքիմիական փորձաքննությունների ժամանակ կիրառելի մեթոդի օպտիմալ պայմանների մշակում: Մասնավորապես, իրականացվել է լյարդից և մկանային հյուսվածքներից նիֆեդիպինի անջատման պայմանների հետազոտում, անջատված նիֆեդիպինի հայտնաբերում նրբաշերտ քրոմատոգրաֆիայի մեթոդով և առաջարկվող մեթոդի ճշգրտության ստուգում ԲԱՀՔ անալիզի կիրառմամբ: Արդյունքում մշակվել է տարբեր կենսահումքերից նիֆեդիպինի անջատման և հայտնաբերման նոր այլընտրանքային էքսպրես մեթոդ, որը բավականին արագ է, ճշգրիտ և անհամեմատ ավելի էժան:

С. Г. ПЕТРОСЯН, Л. Ю. СААКЯН, В. Г. КИРАКОСЯН,
Э. В. МИНАСЯН, С. Е. САРДАРЯН

ВЫДЕЛЕНИЕ, ИДЕНТИФИКАЦИЯ И КОЛИЧЕСТВЕННОЕ ОПРЕДЕЛЕНИЕ НИФЕДИПИНА ИЗ БИОЛОГИЧЕСКОГО МАТЕРИАЛА

Была осуществлена разработка оптимальных методов анализа, применяемых в судебно-химических экспертизах. В частности, были осуществлены исследования условий выделения нифедипина из печени и мышечной ткани, идентификация выделенного нифедипина методом ТСХ, проверка точности предлагаемого метода с помощью ВЭЖХ-анализа. В результате разработан новый альтернативный достаточно быстрый, точный и намного более доступный экспресс-метод выделения и обнаружения нифедипина из различных биоматериалов.