Validated High Performance Liquid Chromatographic Method for Estimation of Nisoldipine from Matrix Type Transdermal Films: Application to *Ex vivo* Skin Permeability Studies

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Abstract

Context: Assessment of nisoldipine (NSP) skin flux using validated high-performance liquid chromatographic (HPLC) method. **Aim:** A validated HPLC method was developed for estimation of NSP in skin permeated samples from matrix type transdermal films. **Materials and Methods:** Chromatographic separation was done using Phenomenex Luna C18 column (150 mm × 4.6 mm i.d. 5 µm) employing acetonitrile:water:methanol (40:35:25 v/v) as the mobile phase. **Settings and Design:** The calibration solutions were prepared in the linearity range of 0.250-50 µg/mL. The flow rate was maintained at 0.8 mL/min at 25°C. 10 µL samples were injected and were monitored using photodiode array detector at a wavelength of 275 nm. **Results:** The limit of detection and limit of quantification for NSP were recorded as 0.125 µg/mL and 0.150 µg/mL, respectively. The correlation coefficient (r^2) values for intraday and interday were >0.998 and % relative standard deviation values were <10%. **Conclusions:** The method was validated as per International Conference on Harmonization guidelines, found to be simple, sensitive, cost effective and was successfully used for *ex vivo* skin permeation studies of NSP from matrix type transdermal films.

Key words: *Ex vivo*, nisoldipine, transdermal films, skin flux

INTRODUCTION

Nisoldipine (NSP) (9) 3-isobutyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-(2-nitophenyl)-pyridine-3,5-dicarboxylate, is an orally active calcium blocking agent belonging to the dihydropyridine family [Figure 1]. It is indicated for the treatment of angina pectoris, hypertension and congestive heart failure.[1] Immediate-release (5 and 10 mg) and controlled-release (10, 20, 30, and 40 mg) oral preparations for NSP have been approved in a number of countries.[2] Absolute bioavailability of the oral tablet is only 5.5%, as a result of significant first-pass metabolism in the gastrointestinal tract and liver.[2,3] To overcome the problem of first pass effect an alternate transdermal[4,5] route of administration for NSP is reported.

Estimation of NSP by liquid chromatography - mass spectroscopy,[6] gas chromatography[7] and high performance liquid chromatographic (HPLC) with amperometry[8] in human plasma was reported. However, we have made an attempt to develop HPLC method for quantification of NSP in *ex vivo* skin permeated samples on application of transdermal films. The method is simple, robust, cost effective and has short run time.

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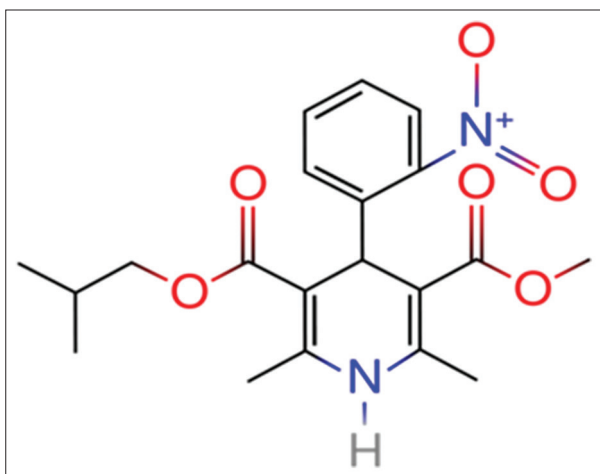E-mail: pgayatripharmaco@gmail.com

Figure 1: Structure of nisoldipine (obtained from file: Nisoldipine.svg from Wikipedia)

METHODS AND CHROMATOGRAPHIC CONDITIONS

Materials

NSP was a gift sample from Orchid Pharmaceuticals and Chemicals Ltd., Chennai, Tamil Nadu, India. HPLC grade methanol was purchased from Merck Ltd., Mumbai, India. Other chemicals used were of analytical grade.

Methods

Chromatographic conditions

Chromatographic separation was performed on Phenomenex Luna C18 column (150 mm × 4.6 mm. i.d. 5 μm) with photodiode array detector. A 10 μL sample was injected into the column and eluents were analyzed using a composition of mobile phase acetonitrile:water:methanol (40:35:25 v/v) with a flow rate of 0.8 mL/min at 25°C.

Preparation of calibration standards and working sample solutions

NSP primary stock solutions 1 mg/mL^{-1} were prepared using methanol as solvent. The secondary stock solutions of 10 μg/mL were prepared by diluting the primary stock solutions. Secondary stock solution of NSP was diluted suitably to get calibration solutions (0.25, 0.5, 1, 2, 5, 10, 15, 25, and 50 μg/mL) and working standard solutions at different levels (0.75, 7.5, and 20 μg/mL). A nine-point calibration curve of NSP was constructed to get a linear graph. Prepared stock solution and working sample solutions were kept in a refrigerator at 4°C when it is not in use.

Preparation of ex vivo samples

The *ex vivo* samples permeated through albino rat abdominal skin were filtered using 0.45 μm membrane filter, diluted with methanol:water (60:40, v/v) and were injected into

HPLC for analysis. The amount of drug dispersed in a polymeric matrix of NSP transdermal films were estimated by dissolving the pieces of films in methanol, the solution was filtered through 0.45 μm membrane filters, and 10 μL was injected into HPLC system.

Method validation

Validation of an analytical method is the procedure by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the proposed analytical application of International Conference on Harmonization (ICH) guidelines.[9] Validation of the developed method is important to get reproducible and reliable data when the samples are analyzed number of times by the same or different operator using the same equipment in different laboratories. Analytical parameters for validation include linearity, suitability, specificity, robustness, precision, accuracy, limit of quantification (LOQ), and limit of detection (LOD).[10]

System suitability

System suitability solution 10 μg/mL of NSP was prepared by suitably diluting the respective stock solution. Six replicate injections were spiked into HPLC system before sample analysis to determine system suitability.

Linearity and range

Calibration curve of NSP was prepared using nine calibration solutions in the range of 0.25-50 μg/mL. Each calibration solution was injected 3 times. The data of the peak area versus drug concentration were treated by linear least square regression analysis.[11]

Precession and accuracy

Working sample solutions of NSP at three different levels (0.75, 7.5, and 20 μg/mL) were injected to study the reliability of the developed method.[12] Observed values and theoretical concentrations were compared. The precision of the method can be assed from the repeatability results of each working sample solution. Five replicates of each working sample solution at three levels were spiked. The intraday precision and interday precision were expressed from the results of % coefficient variation (% CV) values with corresponding peak area.[12]

LOQ and LOD

Sensitivity of the developed method was estimated by determining the LOD and LOQ of NSP based on standard deviation of response and slope of calibration curves.[10,11] The linearity study was conducted in triplicate. NSP was prepared in the range of 0.250-50 μg/mL. Average peak

area was plotted against concentration. LOD and LOQ of developed method were calculated using equations 1 and 2.

$$LOD = (3.3\ \sigma)/S \qquad (1)$$

$$LOQ = (10\ \sigma)/S \qquad (2)$$

Where *S* is slope and σ is standard deviation.

Specificity

Samples of buffer and NSP free films were injected to check the absence of any interfering peaks at the time of elution. Chromatograms were carefully checked for the absence of additional peaks.

Robustness

The experimental conditions were purposely altered to check the effect on the resolution of NSP was evaluated to check the robustness of the method. The flow rate was altered by ±0.1 mL/min composition of the mobile phase was altered by methanol ±2 mL, and column temperature was altered by ±5°C.

Application to *ex vivo* transdermal skin flux studies

The *ex vivo* skin permeation studies for estimation of flux were conducted with the permission (IAEC/14/VIPS/2016) of Institutional Animal Ethical Committee, Vaagdevi Institute of Pharmaceutical Sciences, Warangal, India. The study was conducted using vertical Franz diffusion cells. Albino rat abdominal skin was exercised carefully and was mounted between donor and receptor compartment. Transdermal film containing NSP was placed on the skin. The receptor compartment was filled with pH 7.4 phosphate buffered saline as release media. A small bead was placed in the receptor compartment for uniform stirring of the contents. The entire assembly was placed on the magnetic stirrer, and the content of the receptor media was stirred at 400 rpm. The temperature was maintained at 37 ± 2°C. 1.5 mL of sample was collected carefully for 24 h at different time points. Sample was filtered through 0.25 μm membrane filter, suitably diluted and was injected into HPLC system. The cumulative amount of NSP permeated, and transdermal flux was calculated.

RESULTS AND DISCUSSION

Method development (chromatographic conditions)

The proposed HPLC method was effectively developed, and optimized conditions of developed method were validated for linearity, range specificity, precision, accuracy, LOD and LOQ, and robustness. Chromatograms of blank, blank containing 2 μg/mL of NSP, sample collected from *ex vivo* study at 4 and 12 h after application of NSP transdermal film

are shown in Figure 2. The retention time was 9.6 min for NSP, theoretical plates of NSP were 3224 with a complete run time of 12 min. The tailing factor was <2.0 for NSP.

Method validation for linearity and range

The calibration curve plotted in the range of 0.25-50 μg/mL was found to be linear with good correlation $r^2 > 0.998$. The parameters are represented in Table 1.

Specificity

The peaks obtained after spiking the *ex vivo* samples of transdermal films were clear, and there were no other peaks at the retention times of NSP. The complete separation of the drug was shown in Figure 2. The components of transdermal films or *ex vivo* samples did not interfere with the eluents indicating the specificity of the developed method. Figures 3 and 4 chromatograms of *ex vivo* permeation studies at 4 and 12 h authenticate the above statement of non-interference of formulation components and samples of *ex vivo*.

Precession and accuracy

The intraday and interday precession and accuracy values are represented in Table 2 indicating the method to be reliable. The % CV values for intraday and interday precision were noted as 5 and 4, respectively. The interday precision was
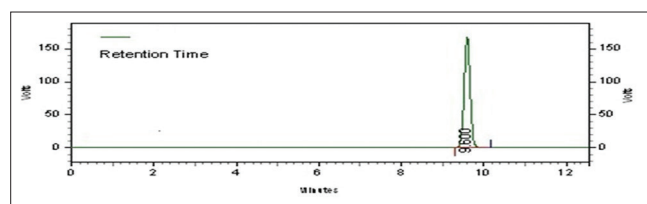

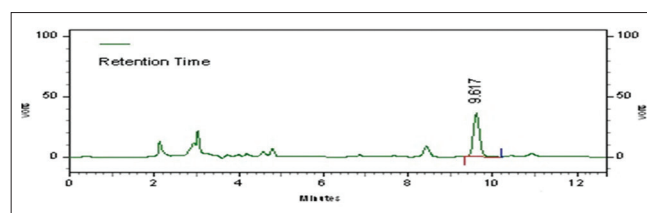**Figure 2:** Chromatogram showing no interference at retention time of nisoldipine

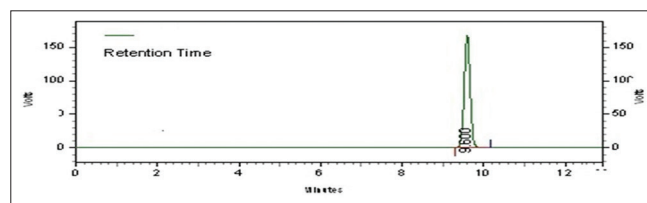**Figure 3:** Chromatogram of *ex vivo* skin permeated sample at 4 h

Figure 4: Chromatogram of *ex vivo* skin permeated sample at 12 h

Table 1: Nisoldipine calibration curve for linearity

Sample	Concentrations range for calibration µg/mL	r^2	Slope	Intercept	LOD µg/mL	LOQ µg/mL
Nisoldipine	0.250-50	0.9985	31884	18448	0.125	0.150

Mean±SD *n*=5 replicates for each point in regression line. SD: Standard deviation, LOQ: Limit of quantification, LOD: Limit of detection

Table 2: Determination of NSP-HPLC method intra- and interday variations (mean±SD *n*=5)

Concentration added (µg/mL)	Calculated concentration mean µg/mL	%CV	Accuracy
Intraday precision and accuracy			
0.75	0.74±0.022	2.98	98.22±1.3
7.5	7.5±0.142	1.89	100.1±1.1
20.0	19.9±0.201	1.01	100.4±0.7
Interday precision and accuracy			
0.75	0.73±0.028	3.83	99.42±1.8
7.5	7.6±0.153	2.01	101.3±0.9
20.0	19.9±0.188	0.94	99.8±1.0

NSP: Nisoldipine, HPLC: High performance liquid chromatography, CV: coefficient of variation, SD: Standard deviation

**Table 3: Impact of modifications in investigational parameters on the performance of chromatographic system**

Parameter	Modification	RT (min)	Recovery of NSP (%)
Methanol ratio in mobile phase (v/v) ±2 mL	23	9.42	98.22
	25	9.26	100.13
	27	9.02	99.53
Flow rate (mL/min) ±0.2 mL/min	0.6	10.20`	97.18
	0.8	9.26	100.13
	1.0	9.11	101.00
Temperature of column oven (°C) ±5°C	20	9.34	101.25
	25	9.26	100.13
	30	9.18	98.76

NSP: Nisoldipine, RT: Retention time

Table 4: *Ex vivo* permeation parameters of NSP transdermal films

Parameters	% Assay	Amount permeated at T_{24} (µg)	Flux µg/cm^{-2} h^{-1}	Permeability coefficient (%)
Values	92-98%	953	11.6	0.3

NSP: Nisoldipine
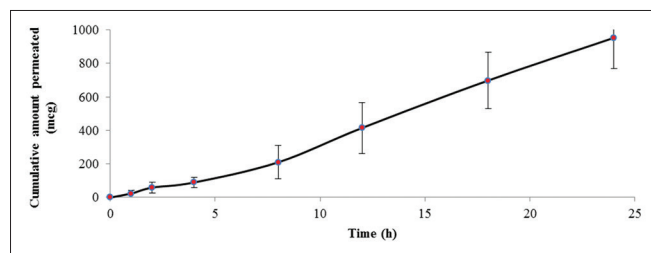
Figure 5: *Ex vivo* permeation profiles of nisoldipine from transdermal film

carried out for 3 days by injecting five replicates of NSP working sample solutions at three different levels. The accuracy of the method was determined by injecting the buffer solution with standard solutions. The recovery for all the samples was $100 \pm 2\%$ with a relative error of $< \pm 6\%$.

LOQ and LOD

The LOD was 0.125 µg/mL based on s/n ratio of 2:1. The LOQ was 0.150 µg/mL. The CV was <10% with an accuracy of 92-103% values of LOD and LOQ shown in Table 1.

Robustness

The small changes made in flow rate, mobile flow composition and temperature of the column does not affect

the chromatographic performance of the method indicating the method was robust [Table 3]. The retention time and tailing factor of NSP were found to be within the acceptable limits under every employed condition, the chromatographic parameters are in accordance with the established value.[12]

Estimation of NSP in the *ex vivo* skin permeated samples

The validated method was used for quantification of NSP in the skin permeated samples from matrix type transdermal films. The amount of drug dispersed in the transdermal film was 3.98 mg. The cumulative amount of NSP permeated across the albino rat abdominal skin was 953 µg for 24 h showing a flux of 11.6 µg cm^{-2} h^{-1} [Table 4]. The permeation profile from films across the skin is represented in Figure 5.

CONCLUSION

A validated HPLC method was developed for the estimation of NSP in *ex vivo* skin permeated samples. The method validated as per ICH guidelines was found to be simple, sensitive, and reproducible. The method was cost effective due to the use of inexpensive chemicals. The absence of interfering peaks at the time of elution of drug emphasizes this method as a useful tool for estimation of NSP in *ex vivo* transdermal delivery of different dosage forms.

ACKNOWLEDGMENT

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