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Development and validation of RP-HPLC method for nifedipine and its application for a novel proniosomal formulation analysis and dissolution study

Krishna Sanka[a], Rakesh Gullapelli[a], Narmada Patil[a], Padmanabha Rao A.[b] and Prakash V Divan[a*]

[a]*Department of Pharmaceutics, School of Pharmacy (formerly Lalitha College of Pharmacy), Hyderabad, AP, India*
[b]*Department of Pharmaceutical Analysis and Quality Assurance, School of Pharmacy (formerly Lalitha College of Pharmacy), Hyderabad, AP, India*

ABSTRACT

The aim of the present study was to develop and validate a simple RP-HPLC method to determine nifedipine in a maltodextrin based oral proniosomes, bulk and marketed preparations. A method was carried out on a 5µm particle octadesyl silane (ODS) column (250 x 4.6 mm i.d) with acetonitrile, 0.1% (v/v) TEA (pH 7.4) 78:22 (v/v) as a mobile phase at a flow rate of 1mL/min and quantification was achieved at 326 nm. The linear range and correlation coefficient (r^2) was found 625 to10000 ng/mL and 0.999 respectively. The proposed method was found to be simple, precise, suitable and accurate for quantification of nifedipine in a novel maltodextrin based oral proniosomes, bulk drug and marketed tablets.

Keywords: Nifedipine; PDA detector; RP-HPLC method; Maltodextrin based proniosomal powder.

INTRODUCTION

Nifedipine (brand names Adalat CC and Procardia, according to FDA Orange Book) was developed by a German pharmaceutical company Bayer with initial name BAY a1040. It is chemically known as 1, 4-dihydro-2, 6-dimethyl-4-(2-nitrophenyl)-3, 5-pyridine dicarboxylic acid dimethyl ester [Fig.1], a dihydropyridine calcium channel antagonist.

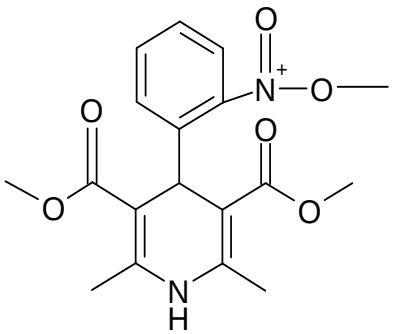


Figure1: Structure of Nifedipine

It has been formulated as both a long- and short-acting calcium channel blocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, nifedipine prevents calcium-dependent myocyte contraction and vasoconstriction. A second proposed mechanism for the drug's vasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition of smooth muscle carbonic anhydrase. It is widely used in the treatment of angina pectoris (especially in Prinzmetal's angina), hypertension, and other vascular disorders such as Ray-naud's phenomenon, premature labor, and painful spasms of the esophagus such as in cancer and tetanus patients. It is also commonly used for the small subset of pulmonary hypertension patients whose symptoms respond to calcium channel blockers.

Comprehensive literature survey revealed that several analytical methods have been reported for estimation of Nifedipine (NFD) individually or in combination of other drugs in plasma and formulations which include Gas-Liquid Chromatography and Electron-Capture Detection [1], Capillary gas chromatography with nitrogen detection [2], high-performance liquid chromatography with electrochemical detection [3], gas chromatography [4], capillary gas chromatography-automated electron capture detector [5], micellar electro kinetic chromatography [6], flow-injection tandem mass spectrometry (HPLC-MS-MS) [7], reverse phase high performance liquid chromatography [8,9,10], square wave adsorptive stripping voltametry [11], solid-phase extraction & HPLC [12,13], LC-MS [14], cation exchange monolithic column [15], spectrofluorimetry [16], ultra performance liquid chromatography-tandem mass spectrometry [17], colorimetric method [18], LCMS/MS [19].

However, the exhaustive literature survey revealed that most of the earlier analytical methods suffer from a lack of specificity and selectivity. Some of the methods like GC and HPLC include laborious liquid-liquid and solid-phase extraction procedures to give adequate sample clean-up, thereby increasing the complexity and time required for analysis. Liquid chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry (LC/MS/MS) are expensive and thus unavailable in many laboratories. Though a number of methods are reported for estimation of NFD individually or in combination with other drugs, to the best of our knowledge there are no reports available for RP-HPLC method development and its validation for estimation of NFD in novel pharmaceutical dosage form (maltodextrin based proniosomes), bulk and marketed tablets. Because RP-HPLC methods have been widely used for routine quality control assessment of drugs in many laboratories owing to its sensitivity, repeatability and specificity, we felt it is essential to develop a RP-HPLC method in this area of novel research foreseeing the need for an analytical method for estimation of drugs in novel dosage forms.

The aim of the study is to develop a new, simple, rapid, economical, precise, accurate analytical method by using RP-HPLC for quantification of NFD in a novel maltodextrin based oral proniosomes, bulk drugs and marketed tablets. Analytical methods must be validated before use by the pharmaceutical industry; the proposed RP-HPLC method describes the validation parameters in accordance with ICH guidelines, by assessing its selectivity, specificity, linearity, precision, accuracy, and limit of quantification, limit of detection, robustness and system suitability.

MATERIALS AND METHODS

2.1 Materials

Nifedipine working standard powder was a gift sample from Abbot Laboratories, Mumbai, India and was used without further purification. NIFEDINE® 5 tablets containing 5mg NFD as per labels claim (manufactured by Reddy's laboratories, Hyderabad, India) were obtained from a local pharmacy. Methanols, Acetonitrile, Sodium dihydrogen phosphate, Sodium hydroxide, Ortho phosphoric acid, TEA were obtained from Merck (Darmstadt, Germany). HPLC grade water was obtained by reverse osmosis and filtration through a Milli-Q® system (Millipore, Milford, MA, USA) and the same was used to prepare all solutions.

2.2 HPLC instrumentation and chromatographic conditions

The HPLC analysis was carried out on Shimadzu HPLC-LC-20AD series binary gradient pump with Shimadzu SPD-M20A detector. The specifications of the column are Phenomenex Luna C18(2) 250 × 4.6 mm i.d, packed with 5 µm. The injection volume of sample 20µL was used in all the experiments. An isocratic mobile phase containing acetonitrile and 0.1% v/v TEA pH(7.4) 78:22(v/v) was pumped through the column with the flow rate of 1mL/min and the quantification was achieved at 326 nm. The mobile phase was filtered through 0.4µm membrane filter and degassed before use.

2.3 Preparation of maltodextrin based proniosomes

Proniosomes with maltodextrin as the carrier were prepared by a slurry method. The formulation comprised of 250 mol of Span 60, 250 mol of cholesterol, and 20 mg of NFD dissolved in chloroform and methanol mixture (2:1). The mixture was added to a 250 mL round bottom flask containing 500 mg of maltodextrin powder. The flask was attached to the rotary evaporator (Hei-VAP advantage/561-01300, Heidolph, Germany) maintained at a temperature of 65°C using water bath and the flask was rotated at 60 rpm under vacuum generated by a mechanical vacuum pump until the powder appeared to be dry and free flowing. The flask was removed from the evaporator and kept in a vacuum desiccator for 12 h. Proniosome powder was stored in well tight container at 4°C for further evaluation.

2.4 Preparation of stock and standard solutions:

A stock solution of (1 mg/mL) was prepared in HPLC grade methanol. The stock solution was protected from light using aluminum foil and aliquots of the standard stock solution of NFD were transferred using A-grade bulb pipettes into 10 mL volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations of 625, 1250, 2500, 5000 and 10000 ng/mL.

2.5 Preparation of sample solution

2.5.1 For assay method

2.5.1.1 Marketed tablets

Twenty tablets were weighed, crushed and mixed in a mortar and pestle. A portion of powder equivalent to the weight of one tablet was accurately weighed and transferred into 20 mL A-grade volumetric flask and 8mL of mobile phase was added to it. The volumetric flask was sonicated for 20 min to complete dissolution of the NFD and the solutions were then made up to the volume with mobile phase. The samples were diluted appropriately to concentration of NFD in the range of linearity previously described.

2.5.1.2 Maltodextrin based proniosomal powder

The powder equivalent to one dose was into a 20mL volumetric flask containing 8mL of mobile phase and mixed well. The volumetric flask was sonicated for 20 min to complete dissolution of the NFD and the solutions were then made up to the volume with mobile phase. The samples were diluted appropriately to concentration of NFD in the range of linearity previously described.

2.5.2 For in vitro dissolution studies

Drug release studies were performed using a USP dissolution test apparatus-II at 50 rpm and bath temperature maintained at 37 ±5 °C. 900 mL of freshly prepared and degassed pH 6.8 phosphate buffer with 0.5%w/w SLS was used as dissolution medium. Dissolution samples were collected at 5, 10, 15, 30, 45, 60, 90 and 120 min. At each point of time, aliquots of samples were withdrawn from vessels and replaced with equal volume of pre-warmed buffer to maintain sink conditions. The samples were filtered through a 0.45µm nylon filter into labeled glass tubes and further analyzed by HPLC.

2.6 Preparation of placebo solution

2.6.1 Marketed tablet formulation

The solution for placebo was prepared with common excipients which were used in tablet formulation such as lactose, starch, methyl cellulose, hydroxyl propyl methyl cellulose, microcrystalline cellulose, talc, magnesium stearate using mobile phase.

2.6.2 Proniosomal powder formulation

The placebo solution for proniosomal powder formulation was prepared by mixing cholesterol, span 60 and maltodextrin in mobile phase.

2.7 Validation of the method

The optimized chromatographic method was completely validated according to the procedures described in ICH guidelines Q2 (R1) for the validation of analytical methods (ICH, 2005).

2.7.1 Linearity and range

The standard stock solution was diluted to prepare solutions containing 625-10000 ng/mL of the NFD. Finally, the solutions were injected in triplicate into the HPLC column, using hamilton syringe with constant volume of 20µl per injection.

2.7.2 System suitability
20 µL of the standard solution (2500 ng/mL) was injected six times under optimized chromatographic conditions to evaluate the suitability of system.

2.7.3 Precision
Three injections, of two different concentrations (1500 and 2500 ng/mL), were given on the same day and the values of relative standard deviation (R.S.D.) were calculated for determining intra-day precision. These studies were also repeatedly conducted on different days to determine inter-day precision.

2.7.4 Accuracy
Accuracy was evaluated by fortifying a mixture of common excipient solutions with two known NFD reference standards. The recovery of the drug was determined.

2.7.5 Specificity
To ascertain specificity, a placebo solution was prepared using the same excipients as those are present in the marketed tablet without NFD. Placebo solution was injected into the HPLC system under the optimized test conditions and the chromatogram was recorded. Responses of the peaks were noted for any possible interferences of the excipient at the retention time of the NFD.

2.7.6 Limit of detection (LOD) and Limit of quantification (LOQ)
The limit of detection (LOD) is the lowest amount of analyte that can be detected in a sample, but not necessarily quantified, under the stated experimental conditions. The LOQ was identified as the lowest plasma concentration of the standard curve that could be quantified with acceptable accuracy, precision and variability. They are determined by signal-to-noise method.

RESULTS AND DISCUSSION

3.1 Method development: The introduction of new HPLC methods are often useful in regular quality control assessment of pharmaceuticals which may convey relevant information in establishing optimal experimental conditions for the better usage of pharmaceutical preparations. In the present study, RP-HPLC method for the determination of NFD in novel maltodextrin based proniosomes, bulk and marketed tablets was developed and validated.

Different organic solvents such as methanol, acetonitrile and buffers such as 20Mm orthophosphoric acid and aqueous TEA were used as mobile phase. A mixture of acetonitrile : 0.1% v/v TEA solution (78:22) was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and free from tailing factor. An optimized chromatogram showing the separation is given in Fig. 2 and optimized chromatographic conditions were tabulated in Table 1.

Table 1: Optimized chromatographic conditions

Stationary phase (column)	Phenomenex Luna C18(2) (250x4.5mm i.d., packed with 5 µm)
Mobile phase	Acetonitrile, 0.1% (v/v) TEA (pH 7.4) 78:22 (v/v)
Detection wave length (nm)	326
Run time (min)	10
Flow rate (mL/min)	1
Volume of injection loop (all)	20
Column temperature	Ambient
Nifedipine R_t (min)	3.783

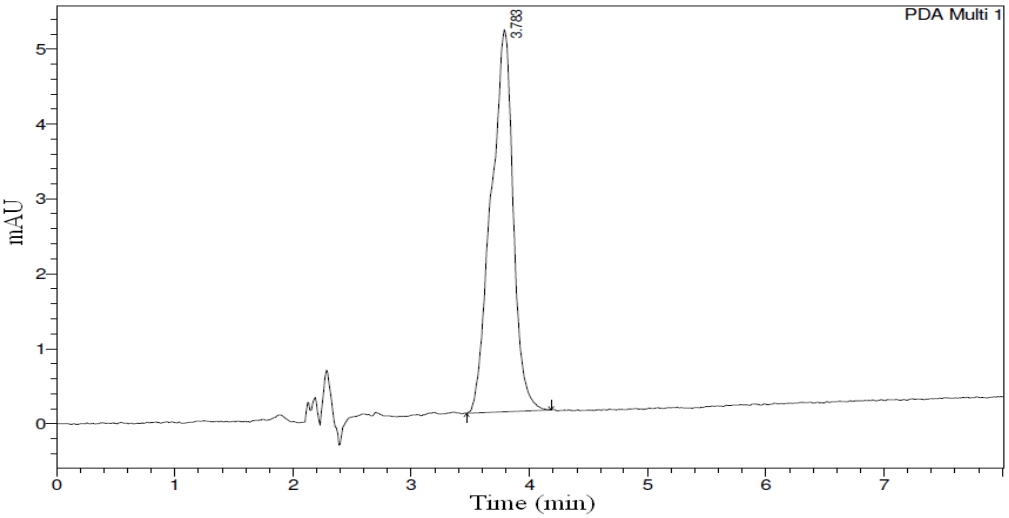


Figure 2: Standard chromatogram of Nifedipine

3.2 Linearity

From the standard stock solution, the various dilutions of NFD in the concentration of 625, 1250, 2500, 5000, and 10000 ng/mL were prepared. The solutions were injected using 20µL injection volume in to the chromatographic system at a flow rate of 1 mL/min and the effluents were monitored at 326 nm with PDA detector and chromatograms were recorded. Calibration curve was done by plotting the peak area ratio versus the applied concentrations of NFD. The linear correlation coefficient was found to be 0.999 and shown in Fig. 3. Linearity parameters were tabulated in Table 2.

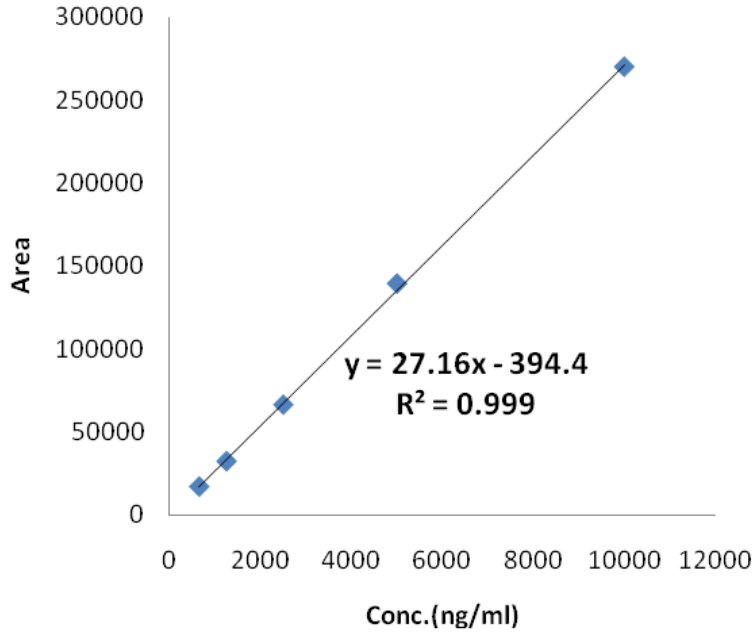


Figure 3: Linearity curve of Nifedipine

Table 2: Linearity parameter for Nifedipine

Conc. (ng/mL)	Area
625	17157
1250	32385
2500	66439
5000	139301
10000	269738

3.3 System suitability

System suitability tests were carried out on freshly prepared standard stock solutions of NFD and it was calculated by determining the standard deviation of NFD standards by injecting standards in six replicates at 6 minutes interval and the values were recorded and represented in Table 3.

Table 3: System suitability parameters

Concentration	Injection	Area	R_t
2500 ng/mL	Inj-1	66439	3.78
	Inj-2	66501	3.76
	Inj-3	66582	3.76
	Inj-4	66654	3.79
	Inj-5	66719	3.78
	Inj-6	66786	3.76
Statistical Analysis	Mean	66613	
	SD	131.62485	3.77167
	%RSD	0.1976	0.01329
	Tailing Factor	0.899	0.352
	Plate Count	1771.634	

3.4 Precision

Precision of the method was tested by performing intra-day and inter-day studies. For intra-day studies, a triplicate of prepared samples of tablet preparation was analyzed within the same day. For inter-day variations, samples were analyzed to observe the changes in peak area of drug solution on three different days. The intra-day and inter-day variations were calculated in terms of percentage relative standard deviation (%RSD) and were shown in Table 4.

Table 4: Intra-day, inter-day precision and accuracy (%) of QC standards at 1500, 2500ng/mL

	Intra-day (n=3)			Inter-day(n=3)		
Theoretical Conc. (ng/mL)	Mean Conc.±SD (ng/mL)	% RSD	Accuracy (%)	Mean Conc.±SD (ng/mL)	%RSD	Accuracy (%)
1500	1550 ± 15.42	0.9948	104.20	1592 ± 10.71	0.672	106.13
2500	2564±28.82	1.124	102.57	2533 ± 28.78	1.13	101.33

3.5 Accuracy

In order to examine the accuracy of the method and to check the interference from the excipients used in the tablet dosage form, the recovery studies were performed by using standard addition method. NFD reference standards were accurately weighed and added to a mixture of the tablets excipients, at different concentration levels (500 and 1000 µg/mL). At each level, samples were prepared in triplicate and the recovery percentage was determined and given in Table 5.

Table V Recovery studies

Actual Conc.(µg/mL)	Calculated Conc.(µg/mL) ± SD	%RSD	Recovery (%)
500	504.06±3.06	0.022	100.80
1000	1018±4.90	0.103	108.00

3.6 Specificity and selectivity

Specificity was tested against standard compounds and possible interference peaks in the presence of placebo under optimized test conditions. The comparison of the chromatograms of the placebo mixture and the spiked drug solution revealed that there were no additional peaks co-eluting with the peaks of NFD in sample solution. No interference was observed from the placebo at the retention time of the NFD (Fig. 4 and Fig. 5). Therefore, it can be

concluded that the method is specific and can assess unequivocally the analyte of the interest in the presence of possible interferences. Peak purity indices for proniosomal power and marketed tablets were shown in Fig. 6.

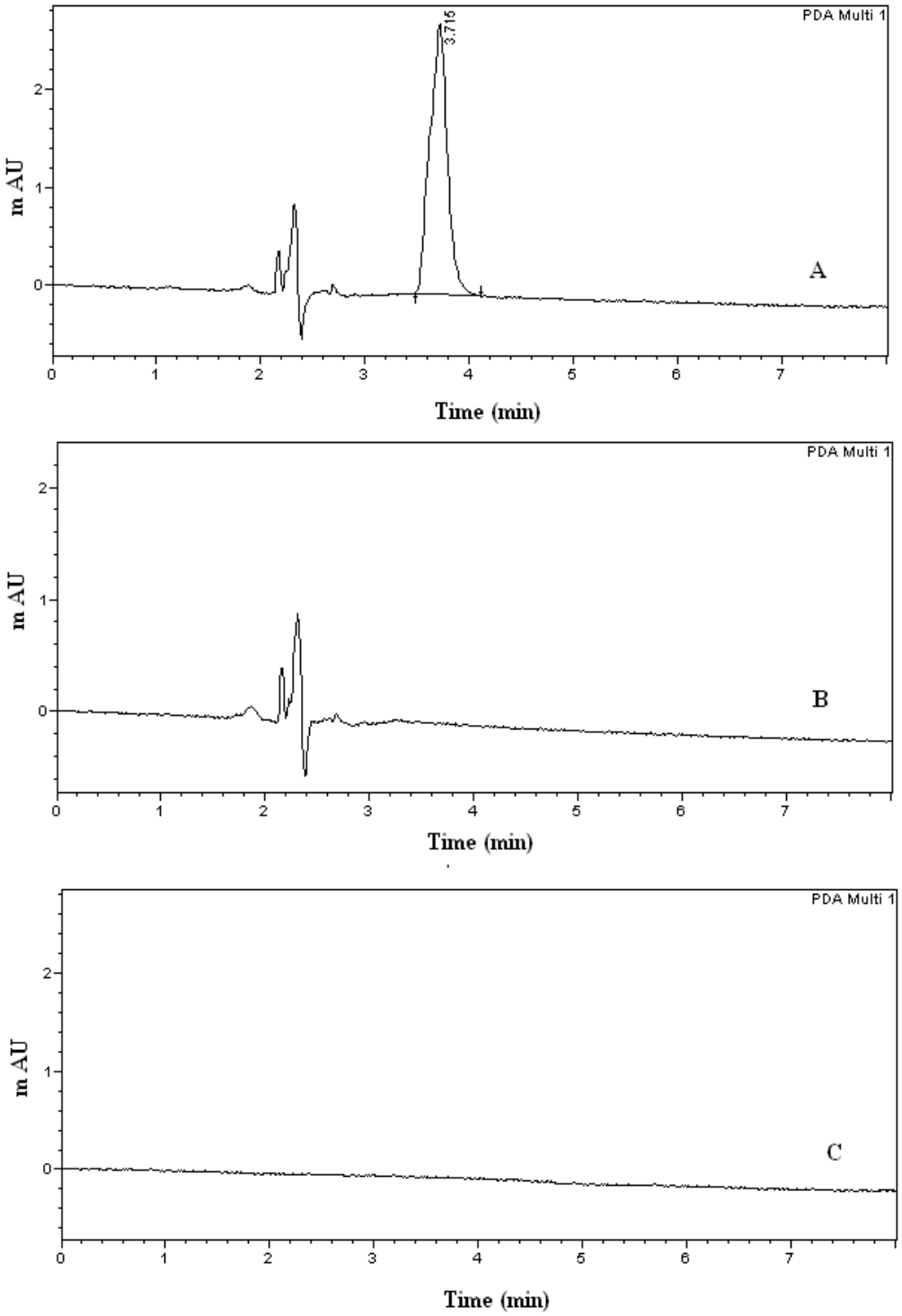


Figure 4: Specificity chromatograms of A) NIFEDINE® 5 B) Placebo C) Mobile phase

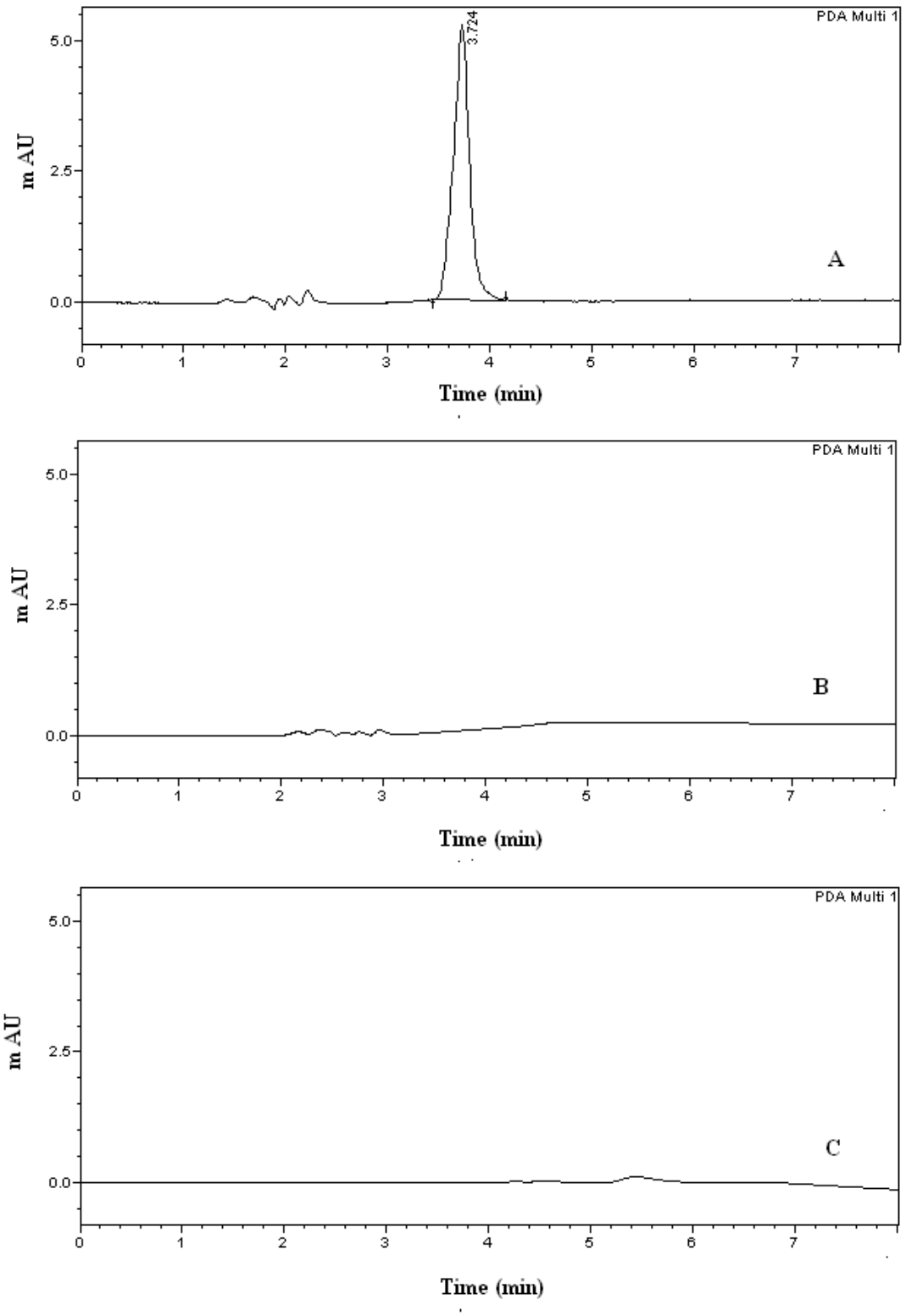
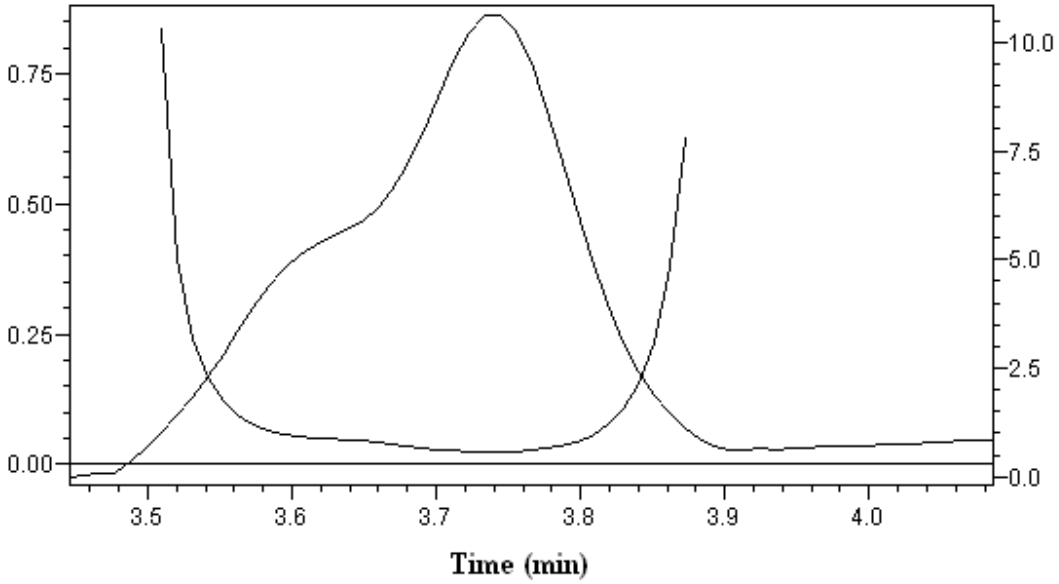
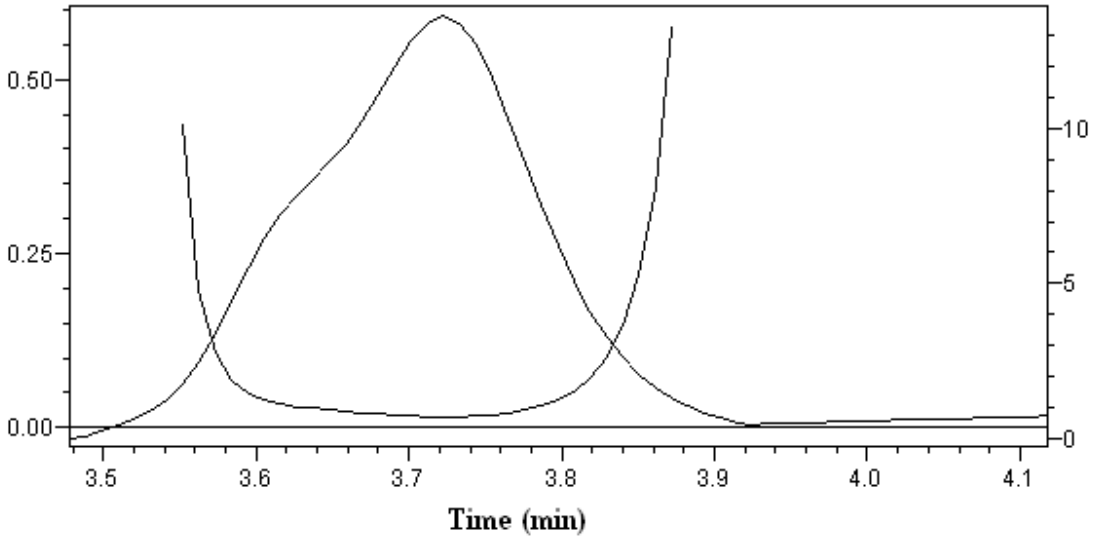


Figure 5: Specificity chromatograms of A) Proniosomal powder B) Placebo C) Mobile phase



Impurity :Not Detected
Peak purity index : 0.999981
Single point threshold : 0.976103

A



Impurity :Not Detected
Peak purity index : 0.999998
Single point threshold : 0.984235

B

Figure 6: Peak purity index A) Proniosomal powder B) NIFEDINE® 5

3.7 Robustness

To determine the robustness of the method, three parameters (flow rate, composition of mobile phase, and pH of the mobile phase) from the optimized chromatographic conditions were varied. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust the results were presented in Table 6.

Table 6: Robustness study

System suitability parameters (variations)	%RSD Peak area (n=6)		Mean Tailing factor (n=6)	Mean R_t (min) (n=6)
Varied pH (± 0.2%)	7.2	0.1946	0.875	0.321
	7.6	0.1994	0.858	0.336
Varied flow rate (±0.2%) (mL/min)	0.9	0.1998	0.891	0.338
	1.1	0.1952	0.915	0.346
Varied mobile phase Composition (±5%)	73	0.1989	0.912	0.328
	83	0.1966	0.945	0.336

3.8 Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ were determined using the signal-to-noise (S/N) method by comparing results of the test of samples with known concentrations of analyte to blank samples. The LOD & LOQ were generated by LCsolutions Version 1.25 (Shimadzu Corporation, Japan). A signal-to-noise ratio of 3:1 is used for LOD whereas a signal-to-noise ratio of 10:1 is used for LOQ. The LOD & LOQ values for NFD were found to be 232.03 ng/mL and 703.12 ng/mL respectively.

3.9 Dissolution study

Dissolution studies were performed on pure drug, proniosomal powder and marketed tablets as described in Section 2.5.2. The percentage cumulative drug release (%CDR) for pure drug was not more than 25.8 ± 3.42 whereas from novel maltodextrin based proniosomal powder and marketed tablets were found to be 96.33±3.88 and 72.36±4.44 respectively at 15th min. The % CDR profiles of pure drug, proniosomal powder and marketed tablet were shown in Fig. 7

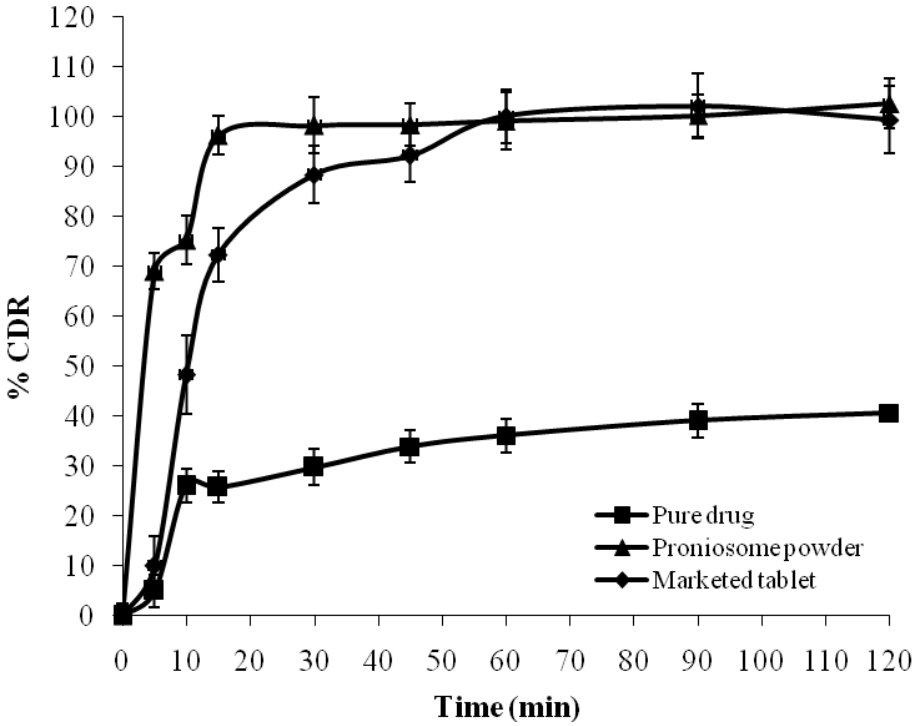


Figure 7: In-vitro drug release profile of pure drug, proniosomal powder and marketed tablet

CONCLUSION

A new RP-HPLC method has been developed and validated according to ICH guidelines for the estimation of NFD in novel maltodextrin based proniosomes, bulk and marketed tablets. The proposed method is simple, rapid, accurate, precise and specific. Therefore, it can be concluded that the developed method is suitable for routine analysis of NFD in pharmaceutical dosage forms.

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