

Various analytical estimation techniques for quantification of Nifedipine: A review

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Abstract

Nifedipine, sold under the brand name Adalat and Procardia, among others, is a calcium channel blocker medication used to manage angina, high blood pressure, Raynaud's phenomenon, and premature labor. The review presented here systematizes various analytical and bio-analytical methods developed for Nifedipine alone and in combination with other drugs. It represents a comprehensive data of UV-Visible spectroscopy, HPTLC, HPLC and LC-MS methods reported by various scientists on Nifedipine.

Keywords: Nifedipine; Analytical methods; Bioanalytical methods; Review article

1. Introduction

Nifedipine is chemically 3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [citation needed], sold under the brand name Adalat and Procardia, among others, is a calcium channel blocker medication used to manage angina, high blood pressure. It is one of the treatments of choice for Prinzmetal angina. It may be used to treat severe high blood pressure in pregnancy. Its use in preterm labor may allow more time for steroids to improve the baby's lung function and provide time for transfer of the mother to a well qualified medical facility before delivery. It is a calcium channel blocker of the dihydropyridine type. Nifedipine is taken by mouth and comes in fast- and slow-release formulations. Its molecular Formula $C_{17}H_{18}N_2O_6$. Molecular weight 346.33 g/mol. Nifedipine is a weak acid (pKa = 3.93). The structure of Nifedipine is in fig.1.

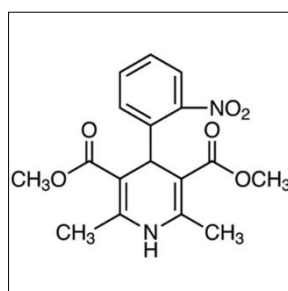


Figure 1 Structure of Nifedipine

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2. Various Analytical Methods

2.1. Colorimetric estimation methods

Two rapid, simple, sensitive and selective spectrophotometric methods have been developed for the quantitative estimation of nifedipine in pharmaceutical formulations and different human body fluids (serum and urine). The proposed methods are based on the reduction of the nitro group to amino group of the drug. The resulting amine was then subjected to proposed methods. Method A is based on the oxidation followed by coupling of nifedipine with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in presence of ferric chloride (FeCl_3) to form green colored chromogen at 685 nm. Method B is based on the formation of oxidative coupling reaction between the corresponding drug and brucine – NaIO_4 to form violet colored chromogen at 546 nm. The procedures described were applied successfully to the determination of the compound in their dosage forms and body fluids. The results showed that the proposed procedures compared favorably with reference method are satisfactory sensitive, accurate and precise. The optical characteristics such as Beer's law limits, molar absorptivity, Sandell's sensitivity and various statistical data are reported. The results of the analysis for the two methods have been validated statistically and by recovery studies¹.

Raynaud's Phenomenon is a vascular affliction resulting in pain and blanching of the skin caused by excessive and prolonged constriction of arterioles, usually due to cold exposure. Nifedipine is a vasodilatory calcium channel antagonist, which is used orally as the first-line pharmacological treatment to reduce the incidence and severity of attacks when other interventions fail to alleviate the condition and there is danger of tissue injury. Oral administration of nifedipine, however, is associated with systemic adverse effects, and thus topical administration with nifedipine locally to the extremities would be advantageous. However, nifedipine is subject to rapid photodegradation, which is problematic for exposed skin such as the hands. The goal of this project was to analyze the photostability of a novel topical nifedipine cream to UVA light. The effect of incorporating the photoprotectants rutin, quercetin, and/or avobenzone (BMDDBM) into the nifedipine cream on the stability of nifedipine to UVA light exposure and the appearance of degradation products of nifedipine was determined. Rutin and quercetin are flavonoids with antioxidant activity. Both have the potential to improve the photostability of nifedipine by a number of mechanisms that either quench the intermolecular electron transfer of the singlet excited dihydropyridine to the nitrobenzene group or by preventing photoexcitation of nifedipine. Rutin at either 0.1% or 0.5% (*w/w*) did not improve the stability of nifedipine 2% (*w/w*) in the cream after UVA exposure up to 3 h. Incorporation of quercetin at 0.5% (*w/w*) did improve nifedipine stability from 40% (no quercetin) to 77% (with quercetin) of original drug concentration after 3 h UVA exposure. A combination of BMDDBM and quercetin was the most effective photoprotectant for maintaining nifedipine concentration following up to 8 h UVA exposure².

A sensitive and selective spectrophotometric method was developed for determination of nifedipine (NIF) and nicardipine (NIC) in their pharmaceutical preparations. The method based on a rapid reduction of the nitro to primary amino groups using zinc dust and hydrochloric acid. The resulting primary aromatic amine was subjected to a condensation reaction with p-dimethyl amino benzaldehyde to produce a yellowish-green color of Schiff's bases which quantified spectrophotometrically at the absorption maxima of 434 and 441 nm for NIF and NIC, respectively. Beer's law was obeyed in the concentration ranges 2.0 to 12.0 $\mu\text{g/mL}$ with a limit of quantitation 1.4 and 1.9 $\mu\text{g/mL}$ and the mean percentage recoveries 98.2 \pm 0.3 to 99.5 \pm 0.3% NIF and NIC, respectively. The proposed methods were successfully applied to assay NIF and NIC in their capsules and tablets³.

Two simple, sensitive and economical spectrophotometric methods were developed for the determination of nifedipine in pharmaceutical formulations. Method A is based on the reaction of the nitro group of the drug with potassium hydroxide in dimethyl sulphoxide (DMSO) medium to form a coloured product, which absorbs maximally at 430 nm. Method B uses oxidation of the drug with ammonium molybdate and subsequently reduced molybdenum blue is measured at 830 nm. Beer's law is obeyed in the concentration range of 5.0–50.0 and 2.5–45.0 $\mu\text{g mL}^{-1}$ with methods A and B, respectively. Both methods have been successfully applied for the assay of the drug in pharmaceutical formulations. No interference was observed from common pharmaceutical adjuvants. The reliability and the performance of the proposed methods are established by point and interval hypothesis tests and through recovery studies⁴.

2.2. UV estimation

A simple, precise, accurate and reproducible spectrophotometric method has been developed for Simultaneous estimation of Atenolol and Nifedipine by employing second order UV derivative Spectrophotometric method in Water: ACN (60:40). The Second order derivative absorption at 245.63 nm (zero cross point of Nifedipine) was used for quantification of Atenolol and 218.7 nm (zero cross point of Atenolol) for quantification of Nifedipine. The linearity was

established over the concentration range of 50-150 µg/ml and 20-60 µg/ml for Atenolol and Nifedipine with correlation coefficient (r^2) of 0.999 and 0.998 respectively. Interday and intraday studies showed repeatability of the method. The mean % recoveries were found to be in the range of 99.25% – 101.15% and 98.56% – 101.85% for Atenolol and Nifedipine, respectively. The method is successfully applied to pharmaceutical formulation, with no interference from excipients as indicated by the recovery study. The proposed method has been validated as per ICH guideline and successfully applied to the simultaneous estimation of Atenolol (ATN) and Nifedipine (NIF) in their combined Tablet dosage form. So it was recommended for routine analysis. The use of 60% Distilled water as a solvent makes method Cost effective and economical⁵.

A new simple, economical, precise and accurate method are described for the simultaneous determination of Nifedipine (NIF) and Metoprolol Succinate (MET) in combined tablet dosage form. The proposed method was applied for the determination of Nifedipine and Metoprolol Succinate in synthetic mixture, for determination of sampling wavelength, 10µg/ml of each of NIF and MET were scanned in 200-400 nm range and sampling wavelengths were 313nm for NIF and 275.40nm for MET are selected for development and validation of absorption correction method. For this method linearity observed in the range of 5-25µg/ml for NIF and 25- 125µg/ml for MET, and in their pharmaceutical formulation with mean percentage recoveries 100.68 and 100.33, respectively. The method was validated according to ICH guidelines and can be applied for routine quality control testing ⁶.

The aim of present work was to develop an accurate, simple, sensitive and Cost effective UV Spectrophotometric method for simultaneous estimation of Atenolol and Nifedipine. The method has followed linearity in the range of 5-25µg/ml and 1.25-6.25µg/ml for Atenolol and Nifedipine respectively. The value of correlation coefficient was 0.9987 & 0.9980 for Atenolol and Nifedipine at 274.5 nm and 0.9979 & 0.9970 for Atenolol and Nifedipine at 235 nm. Satisfactory values of Percent relative standard deviation for the intra-day and inter-day precision indicated that method is precise. Results of the recovery studies (97.77 % to 99.39% for Atenolol & 98.40 % to 100.37 % for Nifedipine) showed accuracy of the method. LOD calculated as 0.97µg/ml and 0.097µg/ml for Atenolol and Nifedipine at 274.5 nm & 0.46µg/ml and 0.07325µg/ml for Atenolol and Nifedipine at 235 nm. LOQ calculated as 2.94µg/ml and 0.322µg/ml for Atenolol and Nifedipine at 274.5 nm & 1.41µg/ml and 0.222µg/ml for Atenolol and Nifedipine at 235 nm .The developed method was extended to the formulation for routine estimation of Atenolol and Nifedipine⁷.

Simple, sensitive and specific spectrophotometric method was developed for the validation of nifedipine in tablet dosage form in two different brands. Active ingredient showed the absorption maximum in ethanol and chloroform at 235.5 nm and 235 nm respectively. The linearity was established in the concentration range of 2-10 µg/ml for nifedipine in different solvent with correlation coefficient (r^2) of 0.997 - 0.999 respectively. The mean % recoveries were found to be in the range of 99.57 - 99.81 % for nifedipine in different brands. Statistically potency of two marketed brands were determined that there were no significant difference between the two brands where ANOVA at $f(5,3) = 196.0143$ and significant level of p-value observed at 0.004. Hence the proposed method can be applied for the routine analysis of active molecule from the formulations⁸.

2.3. HPLC Estimation

A method has been devised for the sensitive, specific and reproducible estimation of nifedipine in human serum. The assay is based on reverse-phase HPLC of an ether extract of serum at pH 9 using nisoldipine as internal standard. The two compounds are detected and quantitated by spectrophotometry at 350 nm. The mean recovery of nifedipine added to blank serum (60-250 ng/ml) was 70%, with a minimum detectable level of 10 ng/ml. Replicate determinations of patients' serum nifedipine (90-150 ng/ml) yielded coefficients of variation of 3.3%. Twelve drugs and non-prescription medicaments which could be ingested at the same time as nifedipine were assessed for interference with the assay but none were found to do so. The rates of absorption and peak serum levels of nifedipine were compared in normo- and hypertensive subjects who received sublingually a single 10 mg dose of the drug. Half the subjects displayed mean maximum serum levels of 148 +/- 34 ng/ml while the other half achieved only 48 +/- 15 ng/ml. The times required to reach those peak levels varied from 30 min to greater than 60 min. These results suggest that the rate of absorption of this drug varies widely among individuals. No significant differences were observed between the normo- and hypertensive groups⁹.

To assess the bioequivalence of two oral formulations containing 10 mg of nifedipine. The test preparation were Macorel tablets, the reference preparation were Adalat tablets. The study was designed as a single-dose, three-period crossover randomized design to 18 non-smoker, healthy male volunteers under fasting conditions. Seventeen volunteers completed the study. Plasma samples were analyzed for nifedipine by HPLC after solid-phase extraction. The pharmacokinetic parameters used to assess the bioequivalence of the two formulations were AUC(0-infinite) and AUC(0-t) for the extent of absorption and C_{max} and T_{max} for the rate of absorption. Statistical comparisons of AUC(0-

infinite) AUC(0-t), and C_{max} data were evaluated after logarithmic transformation by two-way analysis of variance (ANOVA), and differences of T_{max} were tested non-parametrically. Point estimates (90% confidence intervals) of the test/reference ratios were 97.4% (87.6%-108.3%) for AUC(0-infinite) 97.0% (85.6%-110.1%) for AUC0-t, and 107.7% (89.1%-130.7%) for C_{max}. No statistically significant difference was found for T_{max} and elimination half-life values. Therefore, in accordance with the European Union bioequivalence requirements, the test and reference nifedipine preparations are bioequivalent for both the extent and the rate of absorption¹⁰.

The pharmacokinetic parameters (AUC, C_{max}, T_{max}, and t_{1/2}) of nifedipine following single oral administration of a 10 mg capsule of test product were compared to those of the same amount of a reference product. The two products in capsule form were administered according to a randomized two-way crossover design in 22 healthy male volunteers. Nifedipine plasma concentrations were determined using a rapid, sensitive and precise high performance liquid chromatography (HPLC) method with ultraviolet (UV) detection at 235 nm. The parametric 90% confidence intervals of the mean value of the ratio [Myogard (test product) /Adalat (reference product)] for pharmacokinetic parameters were 0.90-1.08, 0.80-1.07, and 0.93-1.12 for AUC0-∞, C_{max} and t_{1/2}, respectively. In each case, values were within the acceptable bioequivalence range of 0.8-1.25. Distribution free point estimate for the difference in expected medians of T_{max} of the two products (Myogard-Adalat) was 0.00 h with a 90% confidence interval of 0.00-0.13 which is greater than the accepted bioequivalence of +/- 0.12. The kinetic parameters were comparable to those reported for nifedipine, and no statistically significant differences were found in any of them when comparing the two products by analysis of variance (ANOVA) on log-transformed data. Thus, the two products could be considered bioequivalent regarding absorption rate (C_{max} and T_{max}), extent of absorption (C_{max} and AUC) and elimination (t_{1/2})¹¹.

We report an isocratic, HPLC procedure for assay of the orally administered hypertension drugs [atenolol, amlodipine, nifedipine, nitrendipine, nimodipine and felodipine given in retention order] of which atenolol, an aryloxypropanolamine beta-blocker is administered with anyone of the other five dihydropyridine calcium channel blockers in combined hypertension therapy. The drugs were dissolved in methanol and 20 microliters of a mix of the drugs was injected onto a reverse phase JASCO-metaphase ODS (250 x 4.0 mm) 5 μm column. Any one of the six drugs could be used as the internal standard. The drugs were resolved by elution with a pH 4.5 equivolume mobile phase of acetonitrile-0.01 M KH₂PO₄, with pH adjustments done with H₃PO₄ (flow-rate 1.5 ml min⁻¹). The column effluent was monitored at 250 nm. The detector response (peak height ratio) was linear in the dynamic range of 25-3200 ng ml⁻¹ of these drugs, with the detection limits at approximately 15 ng ml⁻¹. Full statistical evaluation of the data including linear regression (least-square fit) analysis was performed. The suggested procedure has the advantage that all the five dihydropyridine derivatives can be quantified alone or in formulation with atenolol¹².

A reversed-phase HPLC method is described for the simultaneous determination of nifedipine and its primary pyridine metabolite dehydronifedipine in blood and plasma, that involves UV detection and neutral (blood) or alkaline (plasma) extraction. The limit of reliable determination is found to be 3 ng ml⁻¹ with an inter-assay RSD of below 11%. In the presence of haemoglobin, nifedipine is unstable at pH greater than 10, necessitating neutral extraction for the measurement of nifedipine in haemolysed blood. Published methods for analysis of nifedipine are reviewed, emphasizing the lack of specificity and sensitivity which render many of them unsuitable for the investigation of nifedipine disposition in man¹³.

A simple stability-indicating analytical method was developed and validated to quantify nifedipine in polymeric nanocapsule suspensions; an in vitro drug release study was then carried out. The analysis was performed using an RP C18 column, UV-Vis detection at 262 nm, and methanol-water (70 + 30, v/v) mobile phase at a flow rate of 1.2 mL/min. The method was validated in terms of specificity, linearity and range, LOQ, accuracy, precision, and robustness. The results obtained were within the acceptable ranges. The nanocapsules, made of poly(epsilon-caprolactone), were prepared by the solvent displacement technique and showed high entrapment efficiency. The entrapment efficiency was 97.6 and 98.2% for the nifedipine-loaded polymeric nanocapsules prepared from polyvinyl alcohol (PVA) and Pluronic F68 (PF68), respectively. The particle size and zeta potential of nanocapsules were found to be influenced by the nature of the stabilizer used. The mean diameter and zeta potential for nanocapsules with PVA and PF68 were 290.9 and 179.9 nm, and -17.7 mV and -32.7 mV, respectively. The two formulations prepared showed a drug release of up to 70% over 4 days. This behavior indicates the viability of this drug delivery system for use as a controlled-release system¹⁴.

Nifedipine, a dihydropyridine calcium channel antagonist, is widely used in the treatment of hypertension and other cardiovascular disorders. A simple, rapid, sensitive, precise and accurate HPLC method, using solid-phase extraction, for the quantitation of nifedipine in human plasma was developed and validated. The calibration graphs were linear in the 5-400 ng/ml concentration range ($r > 0.999$). Recovery for nifedipine was greater than 93.9% and for internal standard nitrendipine was 96.1%. Intra-day and inter-day precision ranged from 1.4 to 4.2 and 3.9 to 5.6%, respectively.

Intra-day and inter-day accuracy was ranged from 94.5 to 98.0 and 93.1 to 98.0%, respectively. The method was not interfered with by other plasma components and was applied for the determination of nifedipine in pharmacokinetic study after single oral administration of 10 mg nifedipine to 18 healthy male subjects¹⁵.

A simple, selective, rapid, precise, sensitive and accurate HPLC method has been developed for the estimation of nifedipine using methanol and water (70:30 v/v) as mobile phase; pH was adjusted to 3.0 with orthophosphoric acid. Detection was carried out using UV detector at 238 nm. The method was carried out on a Shim-Pack CLC, ODS (C18), 5 μ column and dimension of column was 250 \times 4.6mm. The retention time of nifedipine was 3.401 min. The developed method was validated in terms of linearity, precision, accuracy, precision, limit of detection and limit of quantitation. The method was found to be linear in the range of 5-40 μ g/ml. In the linearity study, regression equation and coefficient of correlation was found to be $y = 361627x + 338075$, $r^2 = 0.9996$ ¹⁶.

A simple, specific, precise and accurate Stability indicating RP-HPLC method for simultaneous estimation of Nifedipine and Lignocaine HCl In their Combined Dosage Form has been developed. A RP-HPLC method was developed for the simultaneous estimation of Nifedipine and Lignocaine HCl in their Combined Dosage Form has been developed. The separation was achieved by LC- 20 AT C18 (250 mm \times 4.6 mm i.d, 5 μ m) hypersil BDS column and Buffer (0.05 KH₂PO₄ pH 3.0): Methanol (50:50) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 234 nm. Retention time of Lignocaine HCl and Nifedipine were found to be 4.170 min and 6.530 min respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Nifedipine 1.5-4.5 μ g/ml and for Lignocaine HCl 7.5-22.5 μ g/ml. Developed method was found to be accurate, precise and simple, specific for simultaneous estimation of Nifedipine and Lignocaine HCl in their Combine Dosage Form. The drug was subjected to stress condition of hydrolysis, Oxidation, Photolysis and Thermal degradation. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial combined dosage form¹⁷.

The process related impurity of Nifedipine diethyl 1, 4-dihydro-2, 6-dimethyl pyridine 3, 5 dicarboxylate in bulk and formulations was synthesized. The characterization of synthesized impurities by using FTIR, NMR and MS. The RP-HPLC method was developed according to ICH Q2B guidelines for quantitation of impurity in bulk and formulations. The method was validated as per ICH guidelines. The method was found to be linear, precise, accurate, robust and rugged. The diethyl 1,4-dihydro-2,6- dimethyl pyridine 3,5 dicarboxylate impurity was quantified from bulk Nifedipine and its marketed tablet formulations. It was revealed that the amount of impurity present in tablet batch I and II was found to be 0.26% and 0.32% respectively and the bulk was found to be negligible¹⁸.

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. 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. 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 %). 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 form¹⁹.

Atenolol and nifedipine were determined by HPLC in compound atenolol tablets. Methods: Phenomenex-ODS 3 column(250 mm \times 4.60 mm5 μ m)the mobile phase consisted of a mixture of methanol-water-phosphate buffer(dissolved 6.8 g KH₂PO₄in 1000 mL water,adjusted pH to 3.0 with H₃PO₄)weigh a suitable quantity of sodium heptanesulfonate at a flow rate of 1.0 mL \cdot min⁻¹(65:35:3:0.13 g=V:V:V:W)detection was done at 240 nm, the injection volume was 10 μ L.Results:The method offered good linearity 10-250 μ g \cdot mL⁻¹ for atenolol($r=0.9999$)and 4-100 μ g \cdot mL⁻¹ for nifedipine($r=1.0000$).The average recovery rates were 99.45% and 99.51% respectively. Conclusion: This method is accurate and reliable for the quality control of this compound preparation²⁰.

A simple precise and economical reverse phase high performance liquid chromatographic method has been developed and validated for the estimation of atenolol and nifedipine simultaneously in combined dosage form. The method was developed using agilent ODS C18 column with a mobile phase constituting of methanol: acetonitrile: phosphate buffer(60:20:20) final adjusted to pH 3.0 with o-phosphoric acid at a flow rate of 1.0ml/min and detection was carried

out at 235nm. The selected chromatographic conditions were found to effectively separate atenolol (Rt: 2.80 min) and Nifedipine (Rt: 4.40 min) having a resolution of 12.307. The developed method was validated for linearity, accuracy, precision, LOD, LOQ, robustness and for system suitability parameters as per ICH guidelines. Linearity for atenolol and nifedipine were found in the range of 5-25 μ g/ml and 2-10 μ g/ml, respectively. The percentage recoveries for atenolol and nifedipine ranged from 99.38-100.56% and 99.16-99.71%, respectively. The proposed method could be used for routine analysis of atenolol and nifedipine in their combined dosage forms²¹.

A simple, sensitive, highly specific, stability-indicating, reversed-phase HPLC method for the quantitation of nifedipine and its related compounds such as nitrophenyl pyridine, nitrosophenyl pyridine analogs with average recoveries greater than 100% were obtained using a mobile phase methanol-water (55:45, v/v) at 265 nm²².

A rapid and sensitive RP-HPLC method has been developed for the purpose of analysis of antihypertensive: nifedipine (NF), antidiabetic: nateglinide (NG) and hypolipidemic: lovastatin (LT) drugs simultaneously in cardiovascular polypill based synthetic ternary mixture. Analysis was performed on C18 (125 \times 4.6 mm id, 5- μ m particle) column with acetonitrile-10 mM phosphate buffer (pH 3.5) 60:40 (v/v) as mobile phase, started at a flow rate of 1 mL min⁻¹ continued for 4 min and further 6 min at a flow rate of 2 mL min⁻¹. UV detection was performed at 208 nm for NF, NG and at 236 nm for LT. The run time under these chromatographic conditions was less than 10 min. The method was linear in the range of 0.125-8.0 μ g mL⁻¹ for NF and 0.25-16.0 μ g mL⁻¹ for NG and LT. Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges. The sensitivity of the method, as the limits of detection (LOD) and quantification (LOQ) for each active ingredient was also determined. The validated method was successfully applied to the analysis of synthetic mixture of tablets of three drugs; the percentage recoveries obtained were 100.23% for NF, 100.35% for NG and 100.93% for LT²³.

2.4. LC-MS/MS Methods

Nifedipine is a dihydropyridine calcium channel blocker used for the treatment of hypertension in pregnant women. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for analysis of nifedipine in human plasma and amniotic fluid. Separation of nifedipine and nitrendipine (IS) was performed using a LiChroCART RP-Select B column and a mixture of water: acetonitrile: glacial acetic acid (30:70:0.5 v/v) as the mobile phase. Aliquots of 500 μ L of biological samples were extracted at pH 13 using dichloromethane:n-pentane (3:7 v/v). The validated method was applied to a study of the pharmacokinetics of nifedipine in human plasma and amniotic fluid samples collected up to 12h after administration of the last slow-release nifedipine (20mg/12h) dose to 12 hypertensive pregnant women. The estimated pharmacokinetic parameters of nifedipine showed a mean AUC(0-12) of 250.2ng/h/mL, ClT/F of 89.2L/h, Vd/F of 600.0L and t_{1/2} 5.1h. The mean amniotic fluid/plasma concentration ratio was 0.05. The methods proved to be highly sensitive by showing a lower quantification limit of 0.1ng/mL for both matrices. And this study reports for the first time the complete development and validation of the method to quantify nifedipine in amniotic fluid using LC-MS-MS²⁴.

The method for simultaneous determination of nifedipine (NIF) and lidocaine (LID) in human plasma by one-step sample preparation has been developed for the first time. Due to the photosensitivity of nifedipine and its low plasma concentrations a precise and reliable method was required. The method involved liquid-liquid extraction (methyl tert-butyl ether, MTBE), and 10 μ L of the resulting sample was analyzed by HPLC-MS/MS. Chromatographic separation was achieved on an YMC-Triart C18 HPLC column (100 \times 2.0mm; S-5 μ m 12nm). The mobile phase was methanol:water, 60:40 (v/v) and contained 0.15% acetic acid. The linearity of the method was established in the concentration ranges of 0.5-50ng/mL for NIF and 1.0-500ng/mL for LID. Photodestruction of NIF under ambient light was evaluated. The validated method was successfully applied to analyze human plasma samples after rectal application of the drug (1g) containing 2.0% LID and 0.3% NIF²⁵.

A more rapid, sensitive and specific high-performance liquid chromatography coupled to -tandem mass spectrometry (HPLC-MS/MS) was developed and validated for the quantification of nifedipine in human plasma, and applied to the pharmacokinetic study of nifedipine in Chinese healthy volunteers. Nifedipine and internal standard (IS) acetaminophen in plasma were extracted with ethyl acetate, separated on a C18 (150 mm \times 4.6 mm, 5 μ m) reversed-phase column, eluted with acetonitrile mixed with 5 mM ammonium acetate solution (pH=6.62) (60:40, v/v), ionized by negative ion pneumatically assisted electrospray and detected in the multi-reaction monitoring mode using precursor \rightarrow product ions of m/z 354.1 \rightarrow 222.2 for nifedipine and 150.1 \rightarrow 107.1 for the IS. A single oral dose of 20 mg nifedipine sustained release tablets and blood samples (4 mL) was collected before and 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 24, and 36 h after administration. The main pharmacokinetic parameters of nifedipine, as T_{max}, t_{1/2 α} , t_{1/2 β} , t_{1/2z}, C_{max}, AUC₀₋₃₆, AUC_{0- ∞} were 2.80 \pm 0.50 h, 6.78 \pm 2.52 h, 6.82 \pm 2.53 h, 6.69 \pm 2.22 h, 76.69 \pm 19.51 (ng/mL), 546.49 \pm 162.28 (ng \cdot h/mL) and 564.05 \pm 176.74 (ng \cdot h/mL), respectively. The calibration curve was linear over the

concentration range of 0.17-102 ng/mL ($r^2 > 0.99$, $n=5$) with a lower limit of quantification (LLOQ) of 0.17 ng/mL. The intra- and inter-day precision was less than 15% for all quality control samples at concentrations of 0.42, 6.53 and 81.60 ng/mL and the accuracy (relative error, RE) was - 3.92% to 7.31% at 3 quality control levels. The specificity, matrix effect, recovery, sensitivity, linearity, accuracy, precision and stabilities were validated, and can fulfill the requirement of pharmacokinetic study of nifedipine sustained release tablets in Chinese volunteers²⁶.

2.5. HPTLC Estimation

An experimental combination of analytical quality by design and green analytical chemistry approaches is introduced to develop an high-performance thin-layer chromatography (HPTLC) approach to quantify barnidipine hydrochloride in the pharmaceutical matrix. The analytical quality by design approach was introduced to green analytical chemistry to enhance protocol knowledge while ensuring efficiency and reducing environmental impacts, energy consumption and analyst visibility. This analytical approach was systematically addressed by exploring failure mode effect analysis, risk assessment and optimization design. Subsequently, a screening of primary variables was performed to select the aptest proportion of solvents in the mobile phase resulting from the principles of green analytical chemistry. Failure mode effect analysis and a risk assessment study were attempted to estimate the critical method parameters (CMPs). The influence of the CMPs on critical analytical attributes, i.e. retention factor and peak area, was assessed through a screening design. A response surface methodology was then executed for the critical analytical attributes as a concern of the determined CMPs, and the conditions for excellent resolution were determined using a desirability procedure. The established protocol was validated in compliance with the International Conference on Harmonization guideline Q2(R1) and showed excellent specificity and sensitivity²⁷.

Nifedipine is almost completely absorbed from the gastrointestinal tract as shown by plasma levels after sublingual, oral, and rectal administration. Because of presystemic metabolism, the bioavailability is about 56% to 77%. After oral administration of 10 mg, the mean plasma concentration of nifedipine reaches maximum values of 160 +/- 49 micrograms/liter after 30 to 60 minutes. After 8 hours, the mean concentration drops to 3.4 +/- 1.2 micrograms/liter. After intravenous administration (0.015 mg/kg) biphasic elimination occurs, the half-life of the alpha-phase being about 13 minutes and of the beta-phase 1.26 +/- 0.55 hours in healthy volunteers. After oral administration of higher doses (40 mg) and after continuous infusion over 24 hours, a third phase with a half-life of about 8 hours can be seen. The apparent volume of distribution of the central compartment (V_{ce}) is 0.294 +/- 0.1 l/kg, and the total body clearance amounts to 0.45 +/- 0.1 liter/hr .kg. Nifedipine is eliminated from the body by hepatic metabolism to the major metabolites 2,6-dimethyl-4-(2-nitrophenyl)-5-methoxycarbonyl-pyridine-3-carboxylic acid (M I) and the corresponding 2-hydroxymethyl-pyridinecarboxylic acid (M II). Methods for the quantitative detection of unchanged nifedipine in the presence of the pyridine analog in plasma (HPLC) and of the main metabolites in plasma and urine (GLC) have been developed. A simple semiquantitative method for detecting metabolites in urine (HPTLC) can be used to monitor patient compliance²⁸.

A stability indicating high performance thin layer chromatographic (HPTLC) method for quantification of nifedipine, as bulk drug and from solid oral dosage forms has been developed. The extraction solvent was methanol and the mobile phase was chloroform: ethyl acetate: cyclohexane (19:2:2, v/v/v). The calibration curve of nifedipine in methanol was linear in the concentration range of 180-720 ng. The mean values of correlation coefficient, slope and intercept were 0.995 +/- 1.02, 1.467 +/- 0.56 and 184.16 +/- 2.15. The limit of detection for nifedipine was 20 ng and limit of quantification was 40 ng. No interference was found from photodecomposition products. The percent recovery of nifedipine using the described procedure was 99.08 +/- 1.51. The coefficient of variation for within day and between day analysis was 0.60 and 0.84% for 480 ng and 0.47 and 0.25% for 720 ng nifedipine concentration. The method was utilized to monitor concentration of nifedipine from sustained release marketed solid oral dosage forms as also from the developed sustained release liquid filled and multi unit hard gelatin capsules²⁹.

3. Conclusion

The collected methods are various analytical methods for the estimation of nifedipine. A few colorimetric methods, HPLC, HPTLC and LC-MS/MS methods have been reported for estimation nifedipine.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest

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