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Short communication

Determination of nelfinavir mesylate as bulk drug and in pharmaceutical dosage form by stability indicating HPLC

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Abstract

A isocratic, selective, accurate and stability indicating HPLC method of analysis of nelfinavir mesylate both as a bulk drug and in formulations was developed and validated. A CN chromatographic column (250 mm × 4.6 mm, 5 μm) was used for the separation at 40 °C. The mobile phase consisted of a mixture of acetonitrile (MeCN) and 25 mM monobasic ammonium phosphate (containing 25 mM triethylamine, pH 3.4 with phosphate acid) (40:60, v/v) was delivered at a flow rate of 1.0 ml/min with detection at 210 nm. The developed method was validated in terms of selectivity, linearity, limit of quantitation, precision, accuracy and solution stability. As the proposed LC method achieved satisfactory resolution between nelfinavir mesylate, its degradation products, intermediate product possibly present in nelfinavir drug substance and other impurities in the end product before refining in the final step of synthetic process, it can be employed as a stability indicating one, used for the synthetic process control and determination of nelfinavir mesylate in pharmaceutical preparations.

Keywords: Nelfinavir mesylate; Reversed-phase HPLC; Pharmaceutical preparations; Validation; Isocratic elution

1. Introduction

Nelfinavir mesylate (Fig. 1) is the mesylate salt of a basic amine and has demonstrated a potent and selective human immunodeficiency virus 1 (HIV-1) protease inhibitor. It has been shown to significantly reduce viral load and to increase $CD4^+$ T cells in adults and children infected with HIV infection especially when administered in association with other anti-HIV agents, nucleoside analogues and non-nucleoside reverse transcriptase inhibitor [1]. Many analytical methods were available for the simultaneous determination of nelfinavir with other HIV protease inhibitors or non-nucleoside reverse transcriptase inhibitor, which involved HPLC [2–8], ion-pair HPLC [9], HPLC–MS [10], LC–MS–MS [11]. However, all of these methods were developed either for determination of nelfinavir in biological samples or for simultaneous determination of nelfinavir with other drugs applied altogether to HIV patients. A gradient HPLC for preformulation [12] and a HPTLC method [13] were reported about individual determination of nelfinavir. Due to baseline drift, not easy to balance, and low robustness of gradient elution, a simple LC method with ultraviolet detection is often more preferred in ordinary laboratories for quality control of pharmaceuticals. To our knowledge, thus far, no simple, rapid LC method for single determination of nelfinavir mesylate in pharmaceutical dosage forms and related substances has ever been mentioned and was available in literatures. The aim of the present work was to develop a simple, rapid, selective, accurate, and stability indicating method for the determination of nelfinavir mesylate in presence of its degradation products and related impurities as per ICH guidelines [14,15]. The three possible known impurities originating from the manufacture of nelfinavir mesylate included No. 1 ($C_{14}H_{26}N_2O$), No. 2 ($C_{18}H_{17}NO_3SCl$), and No. 3 ($C_9H_7O_3$) (structures as Fig. 1) which may be contained in the final product due to the incomplete reaction. Therefore, it was necessary to develop a simple and selective LC method for the simultaneous determination of nelfinavir mesylate, No. 1, No. 2, No. 3 and its pharmaceutical preparations.

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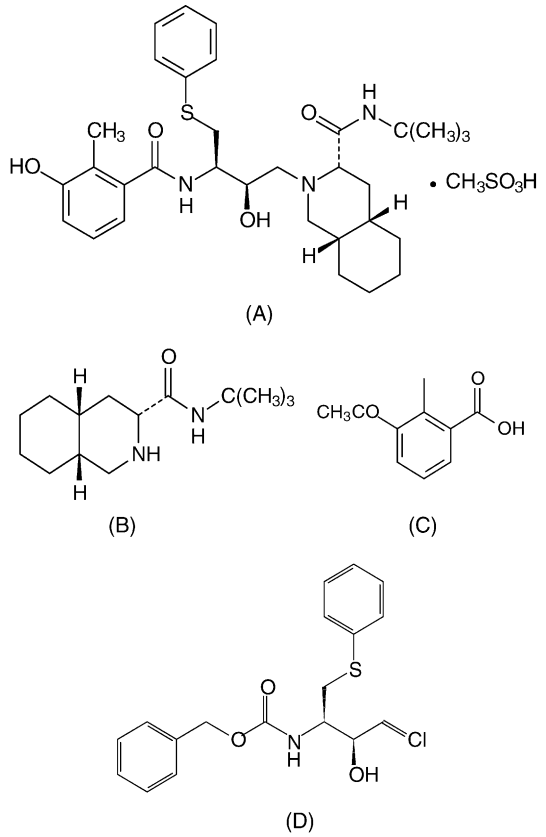

Fig. 1. Chemical structures of nelfinavir mesylate (A), No. 1 (B), No. 2 (C), and No. 3 (D).

2. Experimental

2.1. Chemicals and reagents

Nelfinavir mesylate, reference standard (purity of 99.8%), the impurities (No. 1, No. 2, No. 3) and capsules were prepared in our laboratory. Each capsule contains 292.3 mg nelfinavir mesylate (250 mg nelfinavir base). LC-grade acetonitrile was used. All other chemicals and reagents used were of analytical grade unless indicated otherwise.

2.2. Apparatus and chromatographic conditions

Chromatographic separation was performed on an Agilent 1100 liquid chromatographic system equipped with a G1310A pump, a G1314A variable UV/vis detector, a G1328A manual injector, and Agilent ChemStation chromatography workstation (Agilent, USA). A TU1800PC UV/vis spectrophotometer (China) was used for scanning and selecting the detection wavelength. A Kromasil-CN column (250 mm × 4.6 mm, 5 μm, Sweden) was used for the separation. The mobile phase consisted of a mixture of acetonitrile (MeCN) and 25 mM monobasic ammonium phosphate (containing 25 mM triethylamine, pH 3.4 with phosphate acid) (40:60, v/v) was delivered at a flow rate of 1.0 ml/min with detection at 210 nm. The injection volume was 10 μl. Analysis was performed at 40 °C.

2.3. Standard solution

A stock solution containing 500 μg/ml nelfinavir mesylate was prepared by dissolving reference standard in mobile phase. Standard solutions were prepared by dilution of the stock solution with mobile phase to give solutions containing nelfinavir mesylate in concentration range of 5.0–150.0 μg/ml.

2.4. Method validation

The LC method was validated with respect to the following parameters: linearity, accuracy, precision, selectivity, stability indicating capability, and stability of reference standard solutions and capsule sample solutions.

2.4.1. Precision

The system precision of the assay was investigated by performing five replicate analyses of three standard samples at different concentrations of nelfinavir mesylate (10, 50, and 100 μg/ml, $n = 5$ at each concentration) on the same day and on three separate days and evaluated by relative standard deviation (R.S.D.) of the peak area of the analyte. The method precision of the developed LC method was determined by preparing the capsule samples of the same batch in nine replicate determinations. The R.S.D. value of the assay results, expressed as a percentage of the label claim, was used to evaluate the method precision.

2.4.2. Accuracy

The accuracy studies was carried out by applying the developed method to mixtures of excipients to which known amount of nelfinavir mesylate corresponding to 80, 100, and 120% of label claim had been added. At each level of the amount, three determinations were performed.

2.4.3. Limit of detection and limit of quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. The signal-to-noise ratio was determined.

2.5. Selectivity

For detection of the related impurities, a mixed solution containing No. 1, No. 2, No. 3, and the end product before refining in the final step of synthetic process was determined under the proposed chromatographic conditions.

2.6. Forced degradation of nelfinavir mesylate

Forcedly degraded capsule samples under different stress conditions (heat, light, hydrogen peroxide acid, and base) were prepared for further evaluation of the selectivity of the proposed LC method.

For preparing acid and base induced degradation product, 5 ml of 1 M HCl and 5 ml of 1 M NaOH were separately added to 80 mg nelfinavir mesylate capsule content sample which were individually placed into two test tubes. These mixtures were refluxed and heated, respectively, for 2 h at 80 °C and then cooled to room temperature. The degraded samples were then neutralized and transferred into 100 ml volumetric flasks and brought to volume with mobile phase. The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible effect of light.

For preparing hydrogen peroxide induced degradation product, 0.5 ml of hydrogen peroxide 3.0% (v/v) was added to 80 mg nelfinavir mesylate capsule content sample. The degraded samples were transferred into 100 ml volumetric flasks and brought to volume with mobile phase.

For preparing dry heat degradation product, 80 mg nelfinavir mesylate capsule content sample was powdered, stored at 80 °C for 6 h under dry heat condition in the dark and then cooled to room temperature. The degraded sample was dissolved and transferred into a 100 ml volumetric flask and brought to volume with mobile phase.

The photochemical stability of the drug was also studied by exposing the capsule content sample to direct sunlight for 24 h and then proceeded the same as indicated for dry heat degradation. The resulting solutions were used as the degraded sample solutions and determined under the described chromatographic.

2.7. Analysis of the nelfinavir mesylate capsule

To determine the content of nelfinavir mesylate in capsules (label claim: 292.3 mg per capsule), the contents of 20 capsules were accurately weighed, their mean weight determined and they were finely powdered. A quantity of powder equivalent to 25 mg nelfinavir mesylate was weighted and transferred to a 50 ml volumetric flask. After about 20 ml of mobile phase was added, the mixture was shaken well and brought to volume with mobile phase, and filtered. The first 10 ml of the filtrate was rejected, and 5 ml of the subsequent filtrate was quantitatively transferred to a 50 ml volumetric flask and made to volume with mobile phase. The resulting solution was used as the sample solution for assay. Then 10 μl of this solution was injected in to column and chromatogram was recorded. The analysis was repeated in triplicate. The possibility of excipients interference in the analysis was studied.

3. Results and discussion

3.1. LC–UV method development

Besides quantification of nelfinavir mesylate, determination of possible degradation products and impurities is of importance during the development of a pharmaceutical dosage form. To analyze nelfinavir mesylate together with its impurities and possible degradation products, reversed phase LC in combination with ultraviolet (UV) detection was developed and optimized. UV spectrums of nelfinavir mesylate, No. 1, No. 2, and No. 3 in the mobile phase were recorded and showed in Fig. 2. The

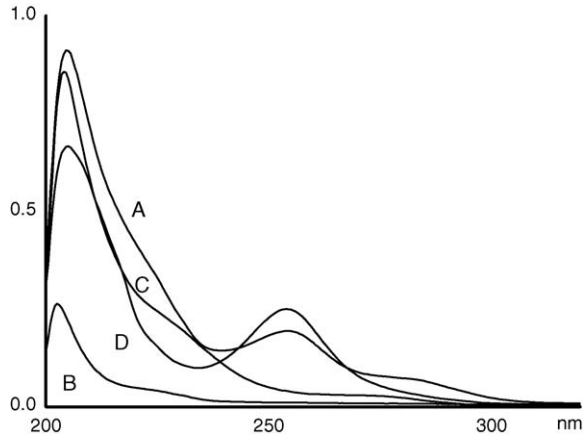

Fig. 2. UV spectrums of nelfinavir mesylate(A), No. 1 (B), No. 2 (C), and No. 3 (D) in the mobile phase.

absorption maximum presents in the UV-spectrum of nelfinavir mesylate at the wavelength of 210 and 254 nm. Not all intermediates displayed absorption maximum at 254 nm. Because all analytes demonstrated significant end absorbance at 210 nm, we can but select 210 nm as detection wavelength for nelfinavir mesylate, No. 1, No. 2, and No. 3 in this chromatographic system. It should be noted that excipients in pharmaceutical dosage form showed negligible absorption at this wavelength. Our attention was mainly focused on the optimization of the rest chromatographic conditions such as mobile phase, the polarity and sorts of chromatographic column in order to detect with isocratic elution. CN chromatographic column with polarity bonded-phase was selected to produce the interaction between nitrile group and the analytes, resulting in change of relative retention time. It was suggested that a mobile phase containing triethylamine at acidic pH value might favor the peak shape of nelfinavir mesylate on the column to achieve a reasonable retention and resolution. After several trials, the mobile phase consisted of a mixture of acetonitrile (MeCN) and 25 mM monobasic ammonium phosphate (containing 25 mM triethylamine, pH 3.4 with phosphate acid) (40:60, v/v) was finally adopted at a flow rate of 1.0 ml/min. The described chromatographic conditions achieved satisfactory

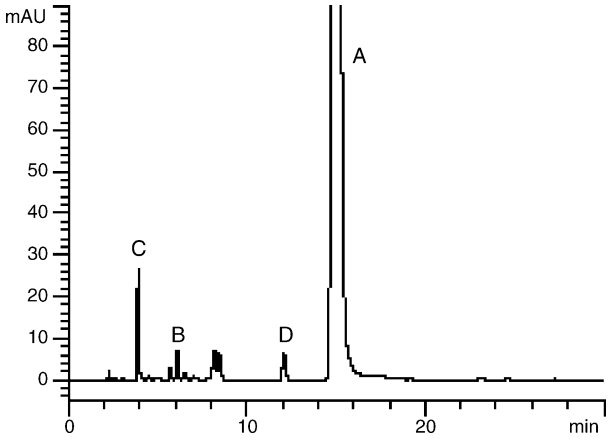

Fig. 3. LC chromatogram for the separation of nelfinavir mesylate (A), No. 1 (B), No. 2 (C), and No. 3 (D).

resolution, reasonable retention and symmetric peak shapes for nelfinavir mesylate and its related substances, under which the retention time was 14.8 min for nelfinavir mesylate, 5.7 min for No. 1, 3.9 min for No. 2 and 12.1 min for No. 3, respectively (Fig. 3).

3.2. Selectivity

A mixed solution containing No. 1, No. 2, No. 3 and the end product before refining in the final step of synthetic process was determined under the proposed chromatographic conditions. The representative chromatogram was shown in Fig. 3, indicating the satisfactory resolution between nelfinavir mesylate and synthetic impurities.

For the further evaluation of the selectivity of the LC method, the forcedly degraded capsules sample solutions prepared by subjecting the capsule samples to such stress conditions as heat, light, hydrogen peroxide, acid and base were determined under the proposed chromatographic conditions. The obtained LC chromatograms for the separation of nelfinavir mesylate from its degraded products in forcedly degradation capsule samples were shown in Fig. 4. Nelfinavir mesylate exhibited a symmetric peak shape and could be well resolved from the degradation products. The capsule excipients were also determined and found no interferences with the determination. The above results showed that the developed LC method was selective for the determination of nelfinavir mesylate in drug substance and pharmaceutical preparations.

3.3. Linearity

The linearity of the method was determined at six concentration levels ranging from 5.0 to 150.0 μg/ml for nelfinavir mesylate. This range corresponded to 10–300% of the intended test concentration of 50 μg/ml for the pharmaceutical quality control of nelfinavir mesylate and the drug in its formulation.

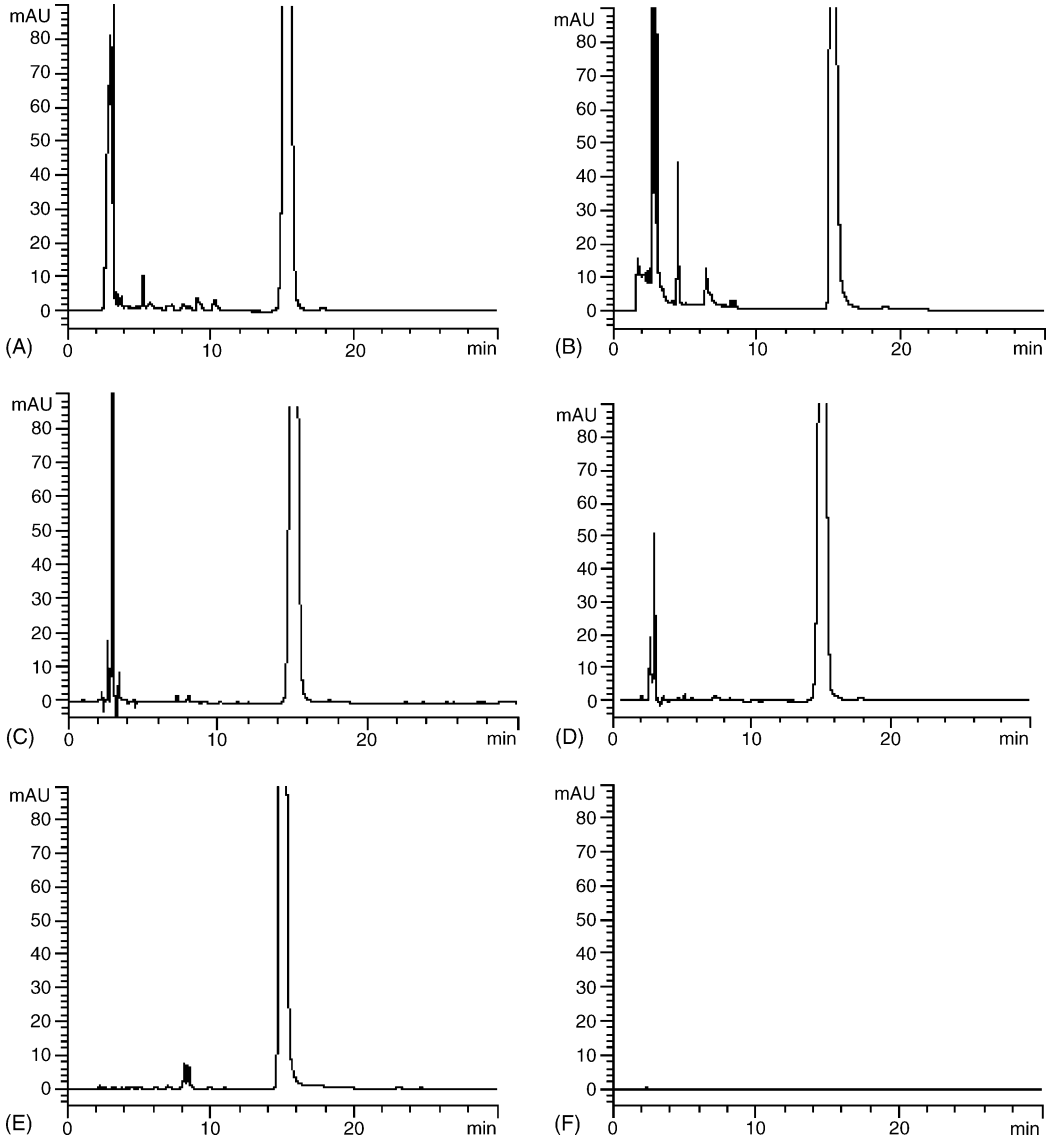

Fig. 4. Chromatograms for the separation of nelfinavir mesylate from its degraded products in forcedly degradation capsule samples: (A) acid condition; (B) base condition; (C) oxidation condition; (D) thermal condition; (E) photo condition; (F) blank excipients.

Table 1
System precision of the developed LC method

	Concentration		
	10 μg/ml	50 μg/ml	100 μg/ml
Within-day ($n=5$) R.S.D. (%)	0.6	0.3	0.4
Between-day ($n=5$) R.S.D. (%)	1.1	0.5	0.7

The calibration curve was constructed by plotting mean area response (A) against concentration (C) of the drug. The equation for calibration curve was $A = 30.37C - 2.2107$ ($r = 0.9999$). The results showed that an excellent correlation existed between peak area and concentration of the drug within the concentration range indicated above.

3.4. Limit of quantitation

The signal-to-base line ratio of 10:1 was considered for LOQ. LOQ was determined by diluting a solution of nelfinavir mesylate, No. 1, No. 2, and No. 3 with mobile phase. LOQ of nelfinavir mesylate, No. 1, No. 2, and No. 3 were experimentally verified to be 0.10, 0.40, 0.20, 0.20 μg/ml, respectively. Actually, the developed LC method was successfully used for the limit test of No. 1, No. 2, and No. 3 as an impurity in nelfinavir mesylate.

3.5. Precision

About the system precision of the developed LC method, the obtained R.S.D. value was shown in Table 1 for the determination of three standard samples of nelfinavir mesylate. Within-day precision ranged from 0.3 to 0.6%, between-day precision ranged from 0.5 to 1.1%, respectively. About the method precision, the obtained R.S.D. value for the determination of nelfinavir mesylate in capsules was 0.46% ($n = 9$). The results for the system precision and method precision indicated the good precision of the developed method.

3.6. Accuracy

The accuracy was then calculated as the percentage of the drug recovered from the formulation matrix. Mean recovery (mean ± S.D.) for nelfinavir mesylate from the formulation was 100.3 ± 0.6% ($n = 9$), indicating the good accuracy of the developed method for the determination of nelfinavir mesylate in the capsules.

3.7. Solution stability

In order to demonstrate the stability of both standard solutions and capsule sample solutions during analysis, both solutions were analyzed over a period of 12 h at room temperature. The results showed that for both solutions, the retention time and peak area of nelfinavir mesylate remained almost unchanged (R.S.D. less than 0.11 and 0.47%) and no significant degradation was observed within the indicated period, suggesting that both solutions were stable for at least 12 h, which was sufficient for the whole analytical process.

3.8. Method application

The validated LC method was successfully applied for the assay of nelfinavir mesylate in drug substance and capsule formulation for three batches. Assay results for three batches of nelfinavir mesylate capsules, expressed as the percentage of the label claim, were found to be 99.4, 100.3 and 100.1% ($n = 3$), respectively, showing that the content of nelfinavir mesylate in the capsule formulation conformed to the content requirements (90–110% of the label claim). The above results demonstrated that the developed LC method achieved rapid and accurate determination of nelfinavir mesylate and can be used for the determination of nelfinavir mesylate in drug substance and pharmaceutical formulations.

4. Conclusions

The developed LC method is simple and selective for simultaneous determination of nelfinavir mesylate and possibly impurities (No. 1, No. 2, No. 3) present in synthesis process and pharmaceutical formulations. The response of the method was found to be linear in the range of 5–150 μg/ml, and it proved to be precise and accurate. The stress testing showed that all degradation products were well separated from the nelfinavir mesylate, confirming its stability indicating capability. This stability indicating LC–UV method was found suitable for the pharmaceutical quality control of nelfinavir mesylate and its formulated products in ordinary laboratories.

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