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ORIGINAL ARTICLE

HPLC-DAD stability indicating determination of nizatidine in bulk and capsules dosage form

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Abstract This work describes the stability-indicating determination of the H_2-receptor antagonist nizatidine in its bulk and capsules dosage form using high performance liquid chromatography coupled with diode array detector (HPLC-DAD). The developed method involved the use of Thermo Hypersil BDS-C8 (4.6 × 250 mm, 5 μm particle size) column and a mobile phase composed of 0.05 M phosphoric acid and acetonitrile (50:50, v/v). The mobile phase was pumped at a flow rate of 1 mL/min. Quantification of nizatidine was based on measuring its peak area at 320 nm. The retention time for nizatidine was about 3.61 min. The reliability and analytical performance of the proposed HPLC procedure were statistically validated with respect to linearity, range, precision, accuracy, specificity, robustness, detection and quantification limits. Calibration curve of nizatidine was linear in the range of 5–50 μg/mL with correlation coefficient >0.9999. The drug was subjected to forced-degradation conditions of acidic and basic hydrolysis, oxidation, dry heat and UV photolysis where it showed considerable degradation in basic and oxidative conditions. The proposed method proved to be specific and stability-indicating by resolution of the drug from its forced-degradation products. The validated HPLC method was applied to the analysis of nizatidine in capsules dosage form where it was quantified with recoveries not less than 98.2%. Assay results were statistically compared to USP 2011 pharmacopeial method where no significant difference was observed between the proposed and reference methods.

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1. Introduction

Nizatidine (NZ) (Fig. 1) chemically known as *N*-[2-[[[2-[(dimethylamino)methyl]-4-thiazolyl]methyl]thio]ethyl]-*N*′-methyl-2-nitro-1,1-ethenediamine, is a histamine H_2-antagonist. It inhibits the actions of histamine mediated by H_2-receptors such as gastric acid secretion and pepsin output. It is used where the inhibition of gastric acid secretion may be beneficial,

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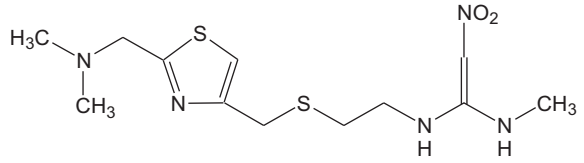

Figure 1 Chemical structure of nizatidine (NZ).

as in peptic ulcer disease including stress ulceration, gastroesophageal reflux, dyspepsia, pathological hypersecretory states such as the Zollinger–Ellison syndrome and in patients at risk of acid aspiration during general anesthesia.[1]

NZ is an official drug in both the British Pharmacopoeia (BP 2010)[2] and the Unites States Pharmacopeia (USP 2011)[3] where HPLC procedures are described for the assay of the bulk powder and dosage forms (capsules and intravenous infusion). The analytical profile[4] of NZ provides a survey for the reported methods of analysis during the eighties of the last century. Moreover, the quantification of NZ in its pharmaceutical formulations and/or biological samples was addressed in several reports. Analytical methodology in these reports involved the use of potentiometric titration with palladium (II) chloride,[5] oxidimetric titration with N-bromosuccinimide,[6] DC and differential-pulse polarography,[7] cathodic stripping voltammetry on hanging mercury drop electrode[8] and several color-producing spectrophotometric methods employing various reactions and reagents.[6,8–12] Recently, a sensitive fluorescence probe for determination of NZ in tablets and biological fluids was presented.[13] Also, the scientific literature showed the use of separation techniques such as capillary zone electrophoresis for separation and simultaneous determination of some H_2 receptor antagonists including NZ,[14,15] HPLC-tandem mass spectrometry (LC–MS–MS) for detection of eight anti-ulcer drugs simultaneously in horse urine[16] and several HPLC-UV detection methods which were directed for NZ determination in commercial products[17] or in human plasma and urine samples.[18–20]

A review of the literature reveals a few number of reported stability indicating assay methods for NZ. Spectrophotometric stability indicating assay methods for the determination of intact NZ in the presence of its degradation products were developed.[21,22] These methods involved the formation of colored products between NZ and bromophenol blue[21] or 3-methyl-2-benzothiazolinone hydrazone (MBTH)[22] followed by measuring peak heights of their first derivative spectra. Stability indicating determination of NZ in the presence of its oxidative degradation product (sulfoxide derivative) was carried out using derivative and derivative ratio spectrophotometry as well as TLC densitometry.[23] Recently, RP-HPLC was adopted for the stability indicating determination of NZ in the presence of its impurities and forced degradation products.[24] Finally, a RP-UPLC method was reported for the stability indicating assay of oral liquid pharmaceutical formulation containing NZ, methylparaben and propylparaben.[25]

The aim of this work is the development, validation and application of a simple, rapid, selective and reliable HPLC-DAD method for the analysis of NZ in bulk powder and in capsules dosage form. The method was thoroughly tested for its specificity and stability-indicating properties by resolution of the parent drug from its forced hydrolytic, oxidative, dry heat and photolytic degradation products.

2. Experimental

2.1. Instrumentation

The HPLC-DAD system consisted of Agilent 1200 series (Agilent Technologies, Santa Clara, CA, USA) (quaternary pump, vacuum degasser and diode array, autosampler and thermostated column compartment) connected to a computer loaded with Agilent ChemStation software. Columns used in the study were Thermo Hypersil BDS-C8 (4.6×250 mm, 5 μm particle size), Thermo Hypersil BDS-C18 (4.6×250 mm, 5 μm particle size) and Zorbax Eclipse XDB–C18 (4.6×150 mm, 5 μm particle size). Filtration was done using cellulose nitrate membrane filters (0.45 μm pore size) (Sartorius Stedim Biotech GmbH, Goettingen, Germany) with the aid of VALUE VG215 2-stage vaccum pump (Zhejiang, China).

2.2. Materials and chemicals

Authentic sample of Nizatidine (NZ) was kindly provided by the Alexandria Company for Pharmaceuticals and Chemical Industries, Alexandria, Egypt, and was certified to contain 99.5% NZ. Analytical grade of orthophosphoric acid, sodium hydroxide, hydrochloric acid, 30% hydrogen peroxide and high purity distilled water were used. HPLC grade acetonitrile and methanol (LAB-SCAN Analytical Sciences, Poland) were used. Pharmaceutical formulation assayed in the study was Ulcfree® capsules (EVA Pharma for Pharmaceuticals & Medical Appliances, Giza, Egypt, BN. 905656) labeled to contain 150 mg of NZ per capsule, and it was purchased from the local market.

2.3. General procedure

The optimal composition of the mobile phase was determined to be acetonitrile and 0.05 M phosphoric acid (50:50, v/v). The mobile phase was pumped isocratically at a flow rate of 1 mL/min. The injection volume was 20 μL. The eluant was monitored by the diode array detector from 190 to 400 nm, and chromatograms were recorded at 210, 254 and 320 nm. All determinations were performed at 25 °C.

NZ stock standard solution (1000 μg/mL) was prepared in HPLC-grade methanol. The prepared stock solution was stored and refrigerated at 4 °C. The working solutions were prepared by the dilution of NZ stock standard solution with the mobile phase to reach the concentration range of 5–50 μg/mL. Triplicate 20 μL injections were made for each concentration and chromatographed under the previously described LC conditions. The peak areas at 320 nm were plotted against the corresponding concentrations to construct the calibration graph.

2.4. Assay of capsules

The contents of 10 Ulcfree® capsules were accurately weighed, mixed, finely powdered and the average weight per capsule was determined. An accurate weight of the finely powdered sample equivalent to 50 mg of NZ was extracted into 25 mL methanol (HPLC grade) with the aid of sonication for 30 min then filtered into a 50 mL-volumetric flask. The residue was washed

with 2 × 10 mL portions of methanol and washings were added to the filtrate. The filtrate was diluted to volume with methanol to reach a final concentration of 1000 μg/mL for NZ (stock sample solution). For the prepared stock sample solution, further dilutions in the mobile phase were made to obtain sample solutions of final concentrations within the linearity range of 5–50 μg/mL, and the general procedure was then followed. Recovery values were calculated from similarly treated standard solutions. For standard addition assay, sample solutions were spiked with aliquots of stock standard NZ to obtain total concentrations within the previously specified range then treated as under general procedure. Recovered concentrations were calculated by comparing the analyte response with the increment response attained after the addition of the standard.

2.5. Preparation of forced-degradation solutions

For the acid and base forced degradation solutions, volumes of 1 mL of NZ stock standard solution were transferred into 50-mL volumetric flasks. Volumes of 2-mL of 1 M HCl or 1 M NaOH were added and the mixtures were kept at room temperature for 24 h. Similar reaction mixtures were prepared in test tubes and were placed in a water-bath at 80 °C for 2 h (for the acid degradation solution) and 30 min (for the base degradation solution). During heating, volume loss was compensated with methanol. After the specified time intervals, the mixtures in the test tubes were quantitatively transferred into 50-mL volumetric flasks. All solutions were neutralized with appropriate volumes of 1 M NaOH or 1 M HCl and diluted to volume with mobile phase to reach final concentrations of 20 μg/mL NZ.

For the oxidative degradation solution, a volume of 1 mL of NZ stock standard solution was transferred into a 50-mL volumetric flask. A volume of ½ mL of H_2O_2 6% (prepared by dilution of hydrogen peroxide 30% with water) was added and the mixture was kept at room temperature for 24 h. Another similar reaction mixture was prepared in a test tube and was placed in a water-bath at 80 °C for 30 min. After the specified time interval, the mixture in the test tube was quantitatively transferred into a 50-mL volumetric flask, and then both solutions were diluted to volume with mobile phase to reach final concentrations of 20 μg/mL NZ.

For the UV photolytic and dry heat degradations, amounts of NZ powder (50 mg) were subjected to UV irradiation at 254 nm for 3 h or kept in an oven at 100 °C for 24 h. After the specified time intervals, each powder was dissolved in methanol, and aliquots of these methanolic stocks were diluted with the mobile phase to reach final concentrations of 20 μg/mL NZ.

3. Results and discussion

3.1. Optimization of chromatographic conditions

A stability-indicating HPLC-DAD method was developed to provide a simple, rapid and reliable quality control analysis of NZ in capsules. The most important aspect in LC method development is the achievement of sufficient resolution with acceptable peak symmetry in a reasonable analysis time. To achieve this goal, several experiments were carried out in order to optimize both the stationary and mobile phases. For the stationary phase, several reversed phase C8 and C18 columns were tested. The best resolution of NZ from its degradation products and best NZ peak shape were attained by using Thermo Hypersil BDS-C8 (4.6 × 250 mm, 5 μm) column, and hence it was used in this study. Several mobile phases were tried using various proportions of different aqueous phases and organic modifiers. Decreasing the acetonitrile content in the mobile phase led to longer retention times and excessive peak tailing. While increasing acetonitrile content yielded a NZ peak that was very close to the solvent peak, in addition to insufficient resolution of the parent drug peak from some of its degradation products peaks. Methanol was tried as an organic modifier and different aqueous phases (water, acetate buffer) were examined. In these trials, chromatograms showed broad asymmetric NZ peaks and/or increased retention times and, consequently, fewer theoretical plates for NZ. The best chromatogram (Fig. 2) was obtained using a mobile phase consisting of acetonitrile and 0.05 M phosphoric acid (50:50, v/v) pumped isocratically at a flow rate of 1.0 mL/min. The proposed method has the advantage of using such simple mobile phase where there is no need for the preparation of buffer or adjustment of pH.

Diode array detection enhances the power of HPLC and is an elegant option for assessing method specificity by monitoring the recorded spectra during peak elution. Quantification was achieved using diode array detection based on peak area measurement. NZ exhibits considerable absorption over the range of 200–350 nm with a prominent maximum at 320 nm, therefore it was selected for NZ quantification. In addition, other wavelengths such as 210 and 254 nm were found suitable for recording chromatograms of the degradation solutions because some of the degradation products did not show enough absorption at 320 nm. Table 1 assembles the optimized chromatographic conditions for this study. The previously described chromatographic conditions showed well defined NZ peak at 3.608 ± 0.033 min. Column performance (apparent efficiency) can be expressed by the number of theoretical plates (N) which equals 4380.

3.2. Stability indicating aspects

Forced degradation experiments were carried out on standard NZ in order to produce the possible relevant degradation products and test their chromatographic behavior using the developed method. Hydrolytic, using strong acidic (1 M HCl) and strong basic (1 M NaOH) media, oxidative (6% H_2O_2), photolytic and dry heat degradation experiments were

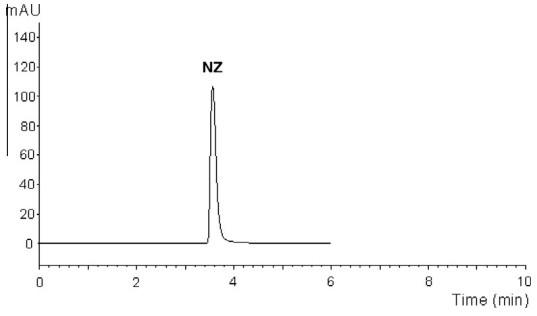

Figure 2 Typical HPLC chromatogram of a 20-μL injection of 20 μg/mL NZ at 320 nm.

Table 1 Optimized chromatographic conditions.

Column	Thermo Hypersil reversed phase BDS-C8 (4.6 × 250 mm, 5 μm particle size)
Mobile phase	Isocratic elution of 0.05 M ortho-phosphoric acid and acetonitrile (50:50, v/v)
Wavelength (nm)	320 nm (for quantification of NZ) 210, 254 and 320 nm (for recording of degradation chromatograms)
Flow rate (mL/min)	1.0 mL/min
Temperature	25 °C

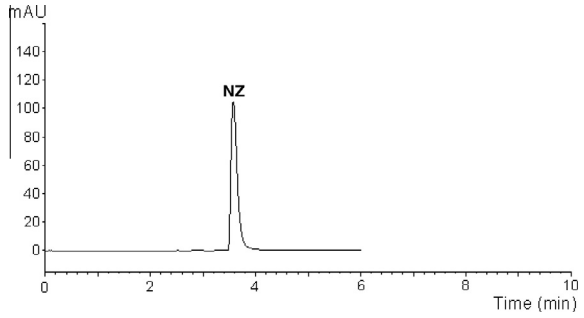

Figure 3 HPLC chromatogram of 20 μg/mL NZ after exposure to acid degradation with 1 M HCl/80 °C for 2 h.

conducted, and the resulting chromatograms were compared with that obtained from standard untreated solution of the drug (Fig. 2).

Hydrolytic and oxidative degradation studies on NZ were conducted either at room temperature or with the aid of heating. In strong acidic medium, no degradation of NZ was noticed. The drug peak appeared at its specific retention time with area identical to that of standard of the same concentration, additionally, the chromatograms of NZ after exposure to forced acidic conditions did not show any extra peaks. Fig. 3 shows the intact NZ peak after heating at 80 °C for 2 hrs with 1 M HCl. On the other hand, alkaline degradation with 1 M NaOH at room temperature caused about 6% reduction in the peak area of NZ, while about 26% decrease in NZ peak area was observed after heating at 80 °C for 30 min. A well-resolved major degradation peak can be seen in the chromatogram at a retention time of 2.34 min (R_s = 7.69 between NZ peak and the alkaline degradation peak) (Fig. 4).

Oxidative H_2O_2 degradation at room temperature revealed quite an intact NZ peak as indicated from its peak area compared to standard of the same concentration. The situation was much different upon heating at 80 °C for 30 min where a remaining NZ peak eluted with about 40% of the expected area, and a degradation product peak appeared at 3.20 min (R_s = 2.43). The sulfur atom in the side chain of NZ is susceptible to oxidation, accordingly, the oxidative degradation product is most probably the S-oxide derivative of NZ (nizatidine sulfoxide).[23] Fig. 5 illustrates the chromatogram of NZ after heating with 6% hydrogen peroxide at 80 °C for 30 min. No degradation was observed after exposure of NZ powder to UV photolytic or dry heat forced degradation

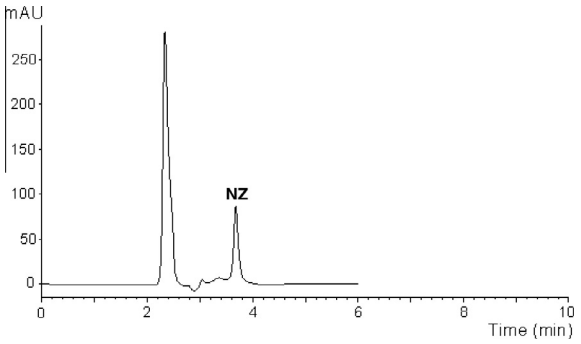

Figure 4 HPLC chromatogram of 20 μg/mL NZ after exposure to alkaline degradation with 1 M NaOH/80 °C for 30 min.

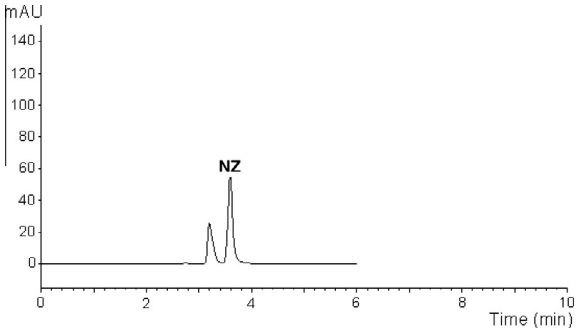

Figure 5 HPLC chromatogram of 20 μg/mL NZ after exposure to oxidative degradation with 6% H_2O_2/80 °C for 30 min.

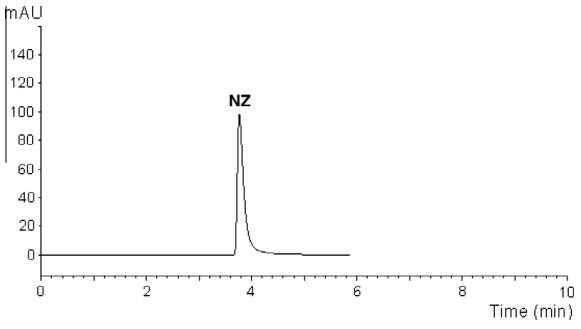

Figure 6 HPLC chromatogram of 20 μg/mL NZ after exposure to UV irradiation at 254 nm for 3 h.

conditions. After the specified time intervals, solutions were prepared from the stressed powder samples. The NZ peak appeared at its specific retention time with area identical to that of a standard of the same concentration, additionally, no extra peaks were observed in the chromatograms (Figs 6 and 7).

In all these forced degradation experiments, NZ was successfully separated from all the degradation products as confirmed by the resolution values calculated for each chromatogram (R_s > 1.5). Also the identity and purity of NZ were confirmed by the diode array detector (DAD), and no signs of co-elution from any of the degradation products were detected.

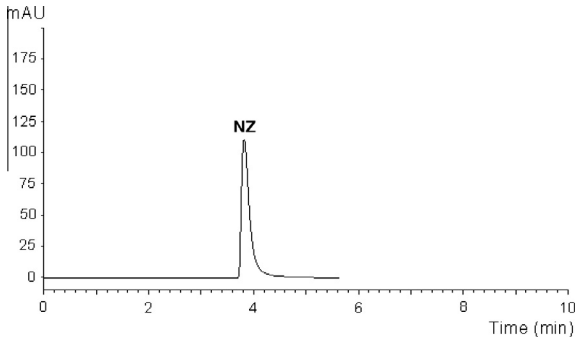

Figure 7 HPLC chromatogram of 20 μg/mL NZ after exposure to dry heat degradation at 100 °C for 24 h.

3.3. Validation of the proposed method

3.3.1. Linearity and concentration range

Under the optimal experimental chromatographic conditions, linear relationship exists between the integrated peak area and the corresponding concentration of NZ. The performance data and statistical parameters including linear regression equation, concentration range, correlation coefficient (r) and other statistical parameters such as the standard deviation of the intercept (S_a), the slope (S_b) and standard deviation of residuals ($S_{y/x}$) are listed in Table 2. Regression analysis for the calibration curve showed good linear relationship over the concentration range of 5–50 μg/mL as judged by the correlation coefficient value (r = 0.99993), the RSD% of the slope which did not exceed 1% and the y-intercept, a, which was less than 2% of the response for the target value of the analyte.[26]

3.3.2. Detection and quantification limits

According to the pharmacopeial recommendations[3] and the ICH guidelines on validation of analytical procedures,[27] the limit of detection (LOD) is defined as the concentration of the analyte which has a signal-to-noise ratio of 3:1. For the limit of quantification (LOQ), the ratio considered is 10:1. The LOD and LOQ values of NZ were calculated using the signal-to-noise ratio method and are given in Table 2. Both LOD and LOQ values confirm the sensitivity of the proposed HPLC procedure.

Table 2 Analytical parameters for the determination of NZ using the proposed HPLC-DAD method.

Parameter	Value
Linearity range (μg/mL)	5–50
Intercept (a)	−7.61
% y-intercept[a] (Peak area at 100% target concentration)	0.87 (874.6)
Slope (b)	44.11
RSD% of slope	0.61
Correlation coefficient (r)	0.999925
S_a[b]	8.17
S_b[c]	0.27
$S_{y/x}$[d]	10.50
LOD (μg/mL)	0.31
LOQ (μg/mL)	1.03

[a] % y-intercept $= \frac{y-\text{intercept}}{\text{peak area at 100\% target concentration}} \times 100$

[b] S_a: standard deviation of intercept.

[c] S_b: standard deviation of slope.

[d] $S_{y/x}$: standard deviation of residuals (standard error of estimate).

3.3.3. Accuracy and precision

The within-day (intra-day) precision and accuracy for the proposed method were studied at three concentration levels (10, 20 and 40 μg/mL) using three replicate determinations for each concentration within one day. Similarly, the between-day (inter-day) precision and accuracy were tested by analyzing the same three concentrations using three replicate determinations repeated for three days. Recovered concentrations were calculated using the corresponding regression equation and they were satisfactory. The percentage relative standard deviation (RSD%) and percentage relative error (E_r%) were less than 1.5% proving the high repeatability and accuracy of the developed method for the estimation of NZ in bulk form (Table 3).

3.3.4. Robustness

The robustness of an analytical procedure is a measure of its capability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.[3,27] Robustness was examined by making small changes in acetonitrile content in the mobile phase (±2%), flow rate (±0.05 mL/min), column temperature (±2 °C) or working wavelength (±2 nm) and examining the results. These variations did not have any significant effect on the measured response (peak area) or retention time of NZ. Table 4 shows the effects of the studied variations on the retention time and peak area of NZ. Additionally, these minor experimental changes did not affect the separation of NZ from its degradation products.

3.3.5. Specificity and selectivity

Specificity of the method was assessed by comparing the chromatograms obtained from standard solution with the chromatograms obtained from capsules sample solution. As the retention time of the standard drug and the retention time of the drug in capsule test solution were the same, so the method was specific. On the other hand, the use of photodiode array detector allowed confirming the selectivity of the method by comparison with the reference drug spectrum, hence the method proved to be selective in separation of the investigated drug. Selectivity was also demonstrated by the separation of NZ from forced degradation products and formulation additives.

3.3.6. Stability of solutions

The stability of the analyte's working solutions in the mobile phase was examined, and no chromatographic changes were observed within 5 h at room temperature. Also, the stock solutions prepared in HPLC-grade methanol were stable for at least one week when stored and refrigerated at 4 °C.

3.4. Assay of capsules

The developed stability-indicating HPLC procedure was applied to the assay of NZ in the pharmaceutical formulation available in the local market (Ulcfree® capsules). The active ingredient eluted at its specific retention time, and no interfering peaks were observed in the chromatograms of NZ capsules. The diode-array detection enables peak purity verification where no signs of co-elution from any of the inactive components were

Table 3 Accuracy and precision for the analysis of NZ in bulk form using the proposed HPLC-DAD method.

	Nominal value (μg/mL)	Found ± SD[a] (μg/mL)	RSD(%)[b]	E_r (%)[c]
Within-day	10	9.93 ± 0.07	0.71	−0.70
	20	19.74 ± 0.16	0.81	−1.30
	40	40.01 ± 0.23	0.58	0.03
Between-day	10	9.96 ± 0.11	1.10	−0.40
	20	19.98 ± 0.27	1.35	−0.10
	40	40.19 ± 0.34	0.85	0.48

[a] Mean ± standard deviation for three determinations.
[b] % Relative standard deviation.
[c] % Relative error.

Table 4 Robustness of the proposed HPLC-DAD method.

Chromatographic parameter	NZ peak area	NZ retention time (min)
Acetonitrile percentage in the mobile phase		
48	843	3.81
50	831	3.74
52	820	3.70
RSD%	1.38	1.49
Flow rate (mL/min)		
0.95	868	3.95
1.00	831	3.74
1.05	803	3.58
RSD%	3.91	4.94
Column temperature (°C)		
23	835	3.76
25	831	3.74
27	823	3.71
RSD%	0.74	0.67
Working wavelength (nm)		
318	817	
320	831	
322	828	
RSD%	0.89	

detected. Recoveries were calculated using both external standard and standard addition methods. The assay results revealed satisfactory accuracy and precision as indicated from % recovery, SD and RSD% values (Table 5).

Furthermore, the USP reference HPLC method[3] was applied for the estimation of NZ in its commercial product. The pharmacopeial method is based on the analysis of NZ using a RP-C18 column (4.6 mm × 15 cm, 5 μm particle size), the mobile phase consisted of methanol and 0.1 M ammonium acetate adjusted to pH 7.5 with acetic acid (24:76, v/v) and UV detection at 230 nm. According to these conditions, the internal standard (phenol) and NZ eluted at retention times 8.74 and 11.17 min respectively. Recovery data obtained from the proposed HPLC method were statistically compared with those of the reference method using the Student's *t*- and the variance ratio *F*-tests. In both tests, the calculated values did not exceed the theoretical ones at the 95% confidence level which indicated that there were no significant differences between the recoveries obtained from the developed method and those of the reference method (Table 5). It is evident from these results that the proposed method is applicable to the assay of NZ capsules with satisfactory level of selectivity, accuracy and precision.

4. Conclusion

A simple, rapid and selective HPLC-DAD procedure was developed for the assay of NZ in bulk form and in capsules. The analyte was quantified using a RP-C8 column in a short run time; consequently, the developed method can be considered cost and time-effective. The proposed method is sensitive enough to determine a concentration down to 5 μg/mL NZ. Moreover, the method was extended to study the degradation behavior of NZ under different forced degradation conditions. Few reports were published concerning the forced degradation of NZ and/or its stability indicating determination. Obviously, the described HPLC method offers selectivity advantage over the spectrophotometric non-separation methods describing the stability indicating estimation of NZ.[21–23] Also, the proposed HPLC method is more reliable than the stability

Table 5 Analysis of NZ in its pharmaceutical preparation (Ulcfree® capsules) using the proposed HPLC-DAD method and the reference method.

Method results	External standard	Reference method[3]	Standard addition
%Recovery ± SD[a]	99.24 ± 0.552	99.78 ± 0.406	99.08 ± 0.791
RSD%[b]	0.556	0.407	0.798
t	1.78		
F	1.85		

Theoretical values for *t* and *F* at $P = 0.05$ are 2.31 and 6.39, respectively.
[a] Mean ± standard deviation for five determinations.
[b] % Relative standard deviation.

indicating TLC-densitometry procedure which was only applied for the assay of NZ in the presence of its oxidative degradation product.[23] The method holds a challenge and is advantageously compared with the other reported liquid chromatographic methods,[2,3,18,25,26] briefly mentioning the following points: short run time (~5 min), simple since no internal standard is required and the mobile phase is pumped isocratically while multi-step gradient elutions are applied in other procedures[24,25] and finally the developed method made use of the diode array detector as a tool for peak identity and purity confirmation; however, it can be adapted to conventional HPLC with UV detection which is the most popular in quality control laboratories.

5. Conflict of interest

None.

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