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HPLC–DAD Stability Indicating Determination of Nitrofurazone and Lidocaine Hydrochloride in Their Combined Topical Dosage Form

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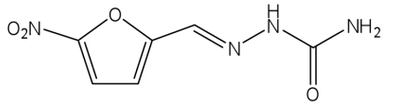
Abstract

In this work, a simple, rapid, and selective high-performance liquid chromatography (HPLC) method with diode array detection was developed for the simultaneous determination of nitrofurazone (NZ) and lidocaine hydrochloride (LD). The chromatographic separation was achieved by using Zorbax Eclipse XDB–C_{18} (4.6 × 150 mm, 5 µm p.s.) analytical column and a mobile phase composed of 0.025 M disodium hydrogen phosphate–methanol–triethylamine (70:30:0.1, v/v/v) (pH 4.0) at a flow rate of 1 mL/min. The detector was set at wavelengths 374 and 220 nm for NZ and LD, respectively, and quantification of the analytes was based on measuring their peak areas. The retention times for NZ and LD were ~ 4.5 and 5.7 min, respectively. The reliability and analytical performance of the proposed HPLC procedure were statistically validated with respect to system suitability, linearity, ranges, precision, accuracy, selectivity, robustness, and detection and quantification limits. The linear dynamic ranges were 0.5–25 and 2.5–100 µg/mL for NZ and LD, respectively, with correlation coefficients > 0.999. The stability-indicating aspects of the proposed method were demonstrated by the resolution of the two analytes from the related substance and potential impurity (2,6-dimethylaniline) as well as from forced-degradation products. The validated HPLC method was successfully extended to the analysis of the combined topical dosage form (soluble dressing) where no interfering peaks were encountered from the dosage form matrix or the inactive ingredients.

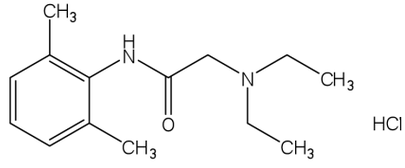
Introduction

Nitrofurazone (NZ) (Figure 1), chemically known as 2-[(5-nitrofuran-2-yl)methylene] diazanecarboxamide (1), is a nitrofuran derivative with antibacterial activity. It is used as a local application for wounds, burns, ulcers, skin infections, and for the preparation of surfaces before skin grafting (2). NZ is an official drug in both the BP (1) and the USP (3) where the bulk powder and pharmaceuticals are assayed by Amax spectrophotometric and HPLC methods. Several analytical methods were reported in the scientific literature for the determination of NZ in pharmaceutical formulations. Examples of these reports are spectrophotometry (4), cathodic stripping voltammetry (5), flow injection chemiluminescence (6,) and spectrofluorimetry (7). High-performance liquid chromatography (HPLC)–UV (8), LC–diode array detection (DAD) (9), and LC–mass spectrometry (MS) (9,10) were applied for the determination of residues of several antimicrobials, including NZ in several food products and feeds. Few methods were published dealing with the stability-indicating assay of NZ, mainly in presence of photodecomposition related substances. These include HPLC–UV (11) and paper chromatographic (12) methods.

Lidocaine hydrochloride (LD) (Figure 1), chemically known as 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide hydrochloride (1), is a local anesthetic with several therapeutic uses, including infiltration anesthesia, regional nerve blocks, surface anesthesia, and in the treatment of ventricular arrhythmias (2). The BP (1) and the USP (3) suggest several procedures for the assay of LD powder and dosage forms. Most BP procedures depend on titrimetry. HPLC was described for the assay of the ointment form. On the other hand, HPLC is predominant in the USP monographs of LD and its dosage forms, while titrimetric procedures are used for the semi-solid topical preparations (ointment and jelly). The quantification of LD in its various drug formulations and/or biological samples was the subject for numerous reports. Analytical methodology in these reports involved separation techniques such as HPLC–UV (13,14), HPLC–DAD (15–17), HPLC–MS (17), high-performance thin-layer chromatography (18), capillary electrophoresis (CE) with electro-chemiluminescence detection (19,20), CE with mass



NZ



LD

Figure 1. Structures of nitrofurazone (NZ) and lidocaine hydrochloride (LD).

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spectrometric detection (21), CE with DAD (22), gas chromatography (GC) with nitrogen phosphorus detector (23,24), and GC–MS (25). Non-separation techniques were also adopted, such as derivative spectrophotometry (26,27), chemometrics assisted spectrophotometry (28), cyclic and square-wave voltammetry (29), and selective electrode potentiometry (30). Several procedures were reported describing the stability-indicating analysis of LD, particularly in presence of its potential impurity 2,6-dimethylaniline. These reports include HPLC (31–33), HPLC with amperometric detection (34), GC with FID (35), TLC-densitometry (36), and derivative spectrophotometry (36).

NZ and LD are formulated in a topical dosage form directed to be used in the treatment and relief from local pain in many conditions, such as all degrees of burns, infected wounds including dressing after surgical operations, skin ulcers, bed sores, and as an antibacterial and anaesthetic during urethral catheterization (37). The fact that up till now the analysis of this drug combination has not been reported in the literature has encouraged us to develop a reliable method for the simultaneous determination of NZ and LD for quality control purposes. This work describes a simple, rapid, and selective HPLC–DAD method for the analysis of this drug combination. The method was evaluated for its stability indicating properties where the decomposition products and related substances were resolved from the intact drugs.

Table I. Analytical Parameters for the Determination of NZ–LD Mixture using the Proposed HPLC–DAD Method

Parameter	NZ (220 nm)	NZ (262 nm)	LD (374 nm)
Conc. range (µg/mL)	1–25	0.5–25	2.5–100
Intercept (a)	10.00	8.93	18.02
S_a*	4.64	4.97	15.49
Slope (b)	94.01	119.06	36.29
S_b†	0.91	0.98	0.31
RSD% of the slope	0.97	0.82	0.85
Correlation coefficient (*r*)	0.99981	0.99987	0.99986
Sy/x‡	6.51	6.98	21.72
LOD§ (µg/mL)	0.15	0.04	0.40
LOQ** (µg/mL)	0.49	0.13	1.33

* Standard deviation of the intercept.
† Standard deviation of the slope.
‡ Standard deviation of residuals.
§ Limit of detection.
** Limit of quantification.

Table II. Precision and Accuracy for the Determination of NZ and LD in Bulk Form Using the Proposed HPLC–DAD Method

Analyte	Nominal value (µg/mL)	Within-day Found ± SD*	Within-day RSD(%)‡	Within-day Er(%)d	Between-day Found ± SD*	Between-day RSD(%)‡	Between-day Er(%)§
NZ†	2.0	1.984 ± 0.023	1.16	–0.80	2.009 ± 0.024	1.20	0.45
	5.0	5.043 ± 0.036	0.71	0.86	4.984 ± 0.055	1.10	–0.32
	10.0	9.894 ± 0.103	1.04	-1.06	9.945 ± 0.140	1.41	–0.55
LD†	10.0	9.857 ± 0.139	1.41	-1.43	9.946 ± 0.148	1.49	–0.54
	30.0	30.174 ± 0.357	1.18	0.58	30.253 ± 0.399	1.32	0.84
	50.0	50.390 ± 0.500	0.99	0.78	50.460 ± 0.784	1.55	0.92

* Mean ± standard deviation for three determinations, measured in (µg/mL).
† Quantification of NZ and LD was carried out at the wavelengths 374 and 220 nm, respectively.
‡ % Relative standard deviation.
§ % Relative error.

Experimental

Instrumentation

The HPLC–DAD system consisted of Agilent 1200 series (quaternary pump, vacuum degasser and diode array and multiple wavelength detector G1315 C/D and G1365 C/D) connected to a computer loaded with Agilent ChemStation Software. A Rheodyne manual injector with 20-µL loop was used. The column used was Zorbax Eclipse XDB–C_{18} (4.6 × 150 mm, 5 µm particle size) (Agilent, Palo Alto, CA).

Materials

NZ was kindly supplied by Medical Union Pharmaceuticals (Abu-Sultan, Ismailia, Egypt). LD was kindly provided by Alexandria Company for Pharmaceuticals (Alexandria, Egypt). 2,6-Dimethylaniline (Sigma-Aldrich, St. Louis, MO), HPLC-grade methanol (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland), triethylamine (Sisco Research Laboratories Ltd, Mumbai, India, minimum 98.0%), analytical-grade anhydrous disodium hydrogen phosphate, ortho-phosphoric acid, hydrochloric acid, sodium hydroxide, 30% hydrogen peroxide, and high purity distilled water were used. Pharmaceutical preparation assayed in the study is Furaseen-L® soluble dressing (Chemipharm Pharmaceutical Industries, 6 th October City, Egypt, BN. 70309A and 90064A) labeled to contain 0.2 gm NZ and 2.0 gm LD per 100 gm.

General procedure

The mobile phase was prepared by mixing 0.025 M disodium hydrogen phosphate, methanol, and triethylamine (TEA) in a ratio of 70:30:0.1 (v/v/v), and the pH was adjusted to 4.0 using phosphoric acid. The mobile phase was filtered by passing through a 0.45-µm pore size membrane filter prior to use. The flow rate was 1.0 mL/min. The injection volume was 20 µL. The eluant was monitored by the diode array detector (DAD) from 190 to 400 nm, and chromatograms were recorded at the wavelengths 220, 262, and 374 nm. All determinations were performed at 25°C. NZ and LD stock solution (50 and 500 µg/mL, respectively) were prepared in methanol. NZ stock solution and its working solutions are stored protected from light. The working solutions were prepared by dilution of the stock solutions with the LC mobile phase to reach concentration ranges mentioned in Table I. Triplicate injections were made for each concentration and chromatographed under the previously described LC conditions. The peak areas for NZ and LD at 374 and 220 nm, respectively, were plotted against the corresponding concentrations to construct the calibration graphs. The accuracy and precision

study was performed by preparing three replicates for three selected concentrations of each compound (Table II). Aliquots of the methanolic stock solutions of NZ and LD were diluted with the mobile phase to reach the selected concentrations. Solutions were injected into the column, assayed using the previous procedure, and the recovered concentrations were calculated from the previously constructed calibration graphs. Laboratory-prepared mixtures of the two compounds were prepared by mixing aliquots of their methanol stocks and then dilution was made with the mobile phase to reach the concentrations mentioned in Table III. Mixture solutions were injected into the column, assayed using the previous procedure, and the recovered concentrations were calculated from standard calibration graphs of NZ and LD.

Forced degradation and stability-indicating study

A stock solution of 2,6-dimethylaniline (1000 µg/mL) was prepared in methanol. An aliquot of this stock solution was added to the two drugs under analysis, and the solution was diluted to volume with the mobile phase. This mixture was chromatographed under the previously described LC conditions. Forced degradation studies under different conditions were carried out on NZ standard according to the following conditions:

(*i*) Photo-degradation: NZ solution was subjected to near fluorescent illumination for 1 hr at room temp;

(*ii*) Acidic and basic conditions: NZ solutions were treated with 2 mL of 1 M HCl or 1 M NaOH. A set of solutions was kept at room temperature for 24 h and another set was placed in a water bath at 100°C for 2 h. Both sets were stored protected from light. After the specified time intervals, all solutions were neutralized by adjusting the pH to 7.0 and then diluted with the mobile phase to reach a final concentration of 10 µg/mL NZ.

(*iii*) Oxidation with H_2O_2: NZ solutions were treated with 0.4 mL of hydrogen peroxide 30%. One solution was kept at room temperature for 24 h and another was placed in a water bath at 80°C for 2 h. Both solutions were kept protected from light. After the specified time intervals, the solutions were diluted with the mobile phase to reach a final concentration of 10 µg/mL NZ.

After the previous treatments, solutions were filtered with a 0.45-µm filtration disk prior to injection to the column.

Table III. Determination of NZ–LD Laboratory-Prepared Mixtures Using the Proposed HPLC–DAD Method

Nominal value (µg/mL)		Found ± SD† (µg/mL)		RSD(%)‡		Er(%)§	
NZ*	LD*	NZ	LD	NZ	LD	NZ	LD
1.0	20.0	0.986 ± 0.011	19.894 ± 0.143	1.12	0.72	–1.40	–0.53
2.0	20.0	2.016 ± 0.021	20.154 ± 0.154	1.04	0.76	0.80	0.77
4.0	20.0	3.990 ± 0.052	20.180 ± 0.240	1.30	1.19	–0.25	0.90
10.0	10.0	9.887 ± 0.093	9.920 ± 0.086	0.94	0.87	–1.13	–0.80
20.0	10.0	19.896 ± 0.246	9.976 ± 0.137	1.24	1.37	–0.52	–0.24
25.0	5.0	25.228 ± 0.183	5.051 ± 0.053	0.73	1.05	0.91	1.02

* Quantification of NZ and LD was carried out at the wavelengths 374 and 220 nm, respectively.

† Mean ± standard deviation for five determinations.

‡ % Relative standard deviation.

§ % Relative error.

Assay of commercial dosage form

A portion of the topical preparation (1.25 gm) equivalent to 2.5 mg NZ and 25.0 mg LD was weighed, transferred into a 50-mL volumetric flask, dissolved in methanol with the aid of shaking, and diluted to volume with methanol. Aliquots of the dosage form solution were diluted with the mobile phase to obtain final concentrations within the specified ranges, then treated as under "General Procedure".

Results and Discussion

Optimization of chromatographic conditions

An isocratic LC method coupled with DAD was developed to provide a suitable procedure for the rapid and reliable quality control analysis of NZ and LD in their combined pharmaceutical preparation. The most important aspect in LC method development is the achievement of sufficient resolution with acceptable peak symmetry in reasonable analysis time. For optimization of the stationary phase, several reversed phase columns [Zorbax SB-C_8 (4.6 × 250 mm), Zorbax SB-C_{18} (4.6 × 250 mm), and Zorbax Eclipse XDB-C_{18} (4.6 × 150 mm)] were tested. The last was found optimum; hence, it became the column of choice for this study. Several mobile phases were tried using various proportions of the aqueous phase and the organic modifier (methanol) at different pH values. The best resolution within acceptable analysis time was obtained through isocratic elution using a mobile phase consisting of phosphate solution (0.025 M disodium hydrogen phosphate) and methanol in the ratio 70:30 (v/v). Increasing the proportion of the aqueous phase led to prolonged retention times and peak tailing particularly for LD, whereas increasing the organic proportion led to inadequate separation of the peaks. Triethylamine (TEA) was added at a concentration level of 0.1 mL per 100 mL mobile phase in order to improve the symmetry and sharpness of the peaks, especially that of LD, thus improving performance and system suitability parameters of the chromatographic separation. The effect of mobile phase pH was examined within the range from 2.5 to 5.0 in 0.5 pH unit increments. No significant difference in resolution and retention times was noticed up to pH 4.0, above which slight peak broadening and increase in retention time of LD was observed; therefore, pH 4.0 was selected for the subsequent studies. Another reason for choosing pH 4.0 is to avoid more acidic pH, which may attack the easily degradable NZ.

The multiple wavelength detector offers the advantage of measuring each analyte at its maximum wavelength, thus improving sensitivity. This is important in case of analytes with completely different absorption characteristics, such as the current mixture NZ–LD. In addition, DAD enhances the power of HPLC and is an elegant option for assessing method specificity by comparison of recorded spectra during peak elution. Quantification was achieved using DAD based on peak-area

measurement. NZ is a yellow colored compound with broad absorption spectrum and two peaks at 262 and 374 nm of which the latter is stronger, while LD exhibits UV absorption mainly below 250 nm, and the suitable wavelength for measuring LD was selected at 220 nm. The wavelengths 220, 262, and 374 nm were chosen for recording the chromatograms of the NZ–LD separation.

The previously described chromatographic conditions showed symmetrical peaks and adequate resolution between NZ and LD.

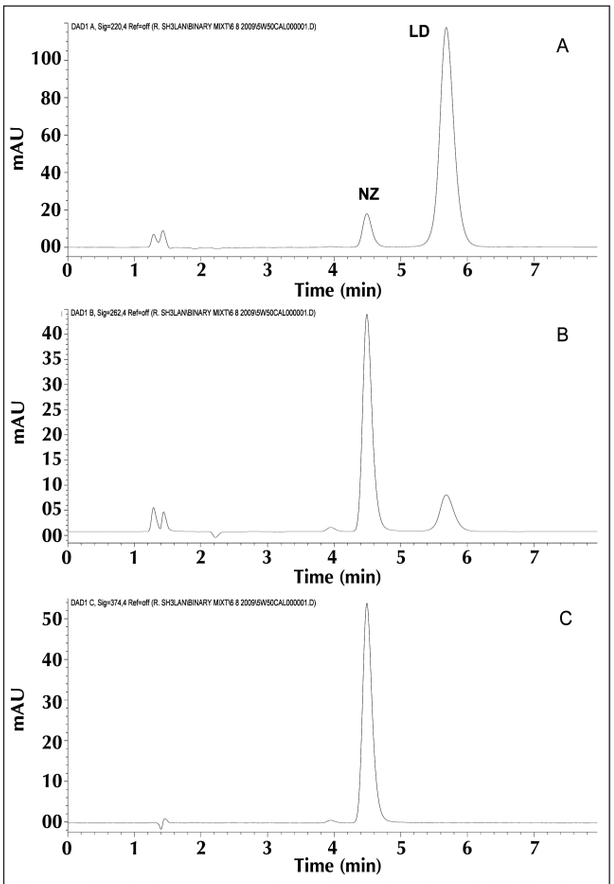

Figure 2. HPLC chromatograms of 20 mL injection of 5 mg/mL NZ and 50 mg/mL LD mixture at 220 nm (A), 262 nm (B), and 374 nm (C).

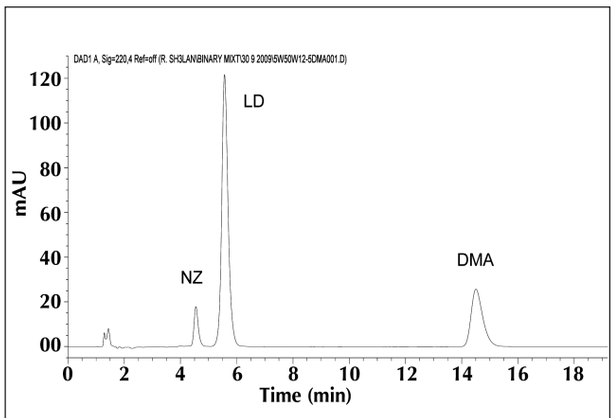

Figure 3. HPLC chromatogram of 5 mg/mL NZ, 50 mg/mL LD, and 12.5 mg/mL DMA mixture at 220 nm.

Figure 2 shows a typical chromatogram for the separation of the two analytes. NZ and LD eluted at retention times 4.48 ± 0.050 and 5.67 ± 0.063 min, respectively. Retention factors (k') are 1.65 and 2.35 for NZ and LD, respectively. A value of 1.5 for resolution implies a complete separation between two consecutive peaks (1). Resolution (Rs) and selectivity (α) for the mixture under analysis are 3.74 and 1.42, respectively. Finally, column performance (apparent efficiency) can be expressed by the number of theoretical plates (N), which equals 6950 and 4730 for NZ and LD, respectively.

Stability-indicating aspects

The optimized HPLC method was applied to test the chromatographic behavior of LD related substance: 2,6-dimethylaniline (DMA) as well as the forced degradation products of NZ. Under the previously described optimized conditions, DMA eluted after the two analytes at retention time 14.51 min as shown in Figure 3.

Forced degradation studies should be considered during development of chromatographic procedures, particularly when degraded products are unknown or not available (38). Forced degradation experiments were carried out on NZ in order to produce the possible relevant degradants and test their chromatographic behavior using the developed method. Nitrofurans, including NZ, are well known as photo-sensitive drugs (11,39). Illumination of NZ solutions with fluorescent or tungsten light for at least 1 h caused the appearance of a related substance peak at 3.95 min. Resolution was calculated between the two adjacent peaks of NZ and its photo-decomposition product, and it was found at 2.55, which revealed an adequate baseline separation (Figure 4). The peak shows two absorption maxima near 260 and 370 nm like NZ but with higher absorbance at the shorter wavelength maximum. These results are in agreement with previous work, which suggested that this photo-decomposition product is actually the cis-isomer of NZ (39).

Hydrolytic (using both strong acidic and basic media) and oxidative degradation studies on NZ were conducted either at room temperature or with the aid of heating. In strong acidic medium, degradation of NZ was noticed from the reduction of its peak area which reached 40% of the expected area at room temperature, while heating at 100°C for 2 h led to increased degra-

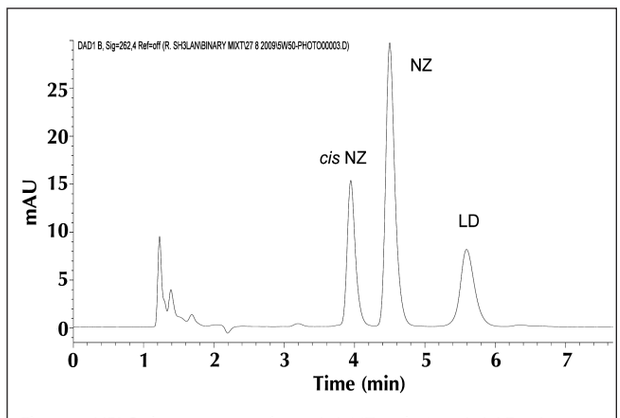

Figure 4. HPLC chromatogram of 5 mg/mL NZ and 50 mg/mL LD mixture at 262 nm after being exposed to fluorescent light for at least 1 h.

dation and only ~ 24% of the expected area remained. Degradation products co-eluted with the solvent peak at ~ 1.30 and 1.39 min. Figure 5A shows the chromatogram of NZ after treatment with 1 M HCl–100°C for 2 h. On the other hand, sodium hydroxide strongly attacked NZ, causing its solution turn colorless, and the chromatograms showed almost no peak for NZ. Both pathways (room temperature for 24 hr and 100°C for 2 h) resulted in practically complete degradation of NZ. Several degradation products peaks eluted in the time range between 1.20 and 2.63 min of which the major peak was at 1.73 min (Figure 5B). Oxidative H_2O_2 degradation at room temperature revealed quite intact NZ as indicated from its peak area compared to standard of the same concentration. The situation was different upon heating at 80°C for 2 h, where a remaining NZ peak eluted with ~ 50% of the expected area. Only minor extra peaks can be detected at retention times of 1.84, 2.05, 3.58, and 3.96 min. Figure 5C illustrates the chromatogram of NZ after treatment with 30% H_2O_2–80°C for 2 h. In all these forced degradation experiments, NZ was successfully separated from all the degradation products. Moreover, none of these degradation peaks eluted at the retention time of LD (~ 5.67 min). Also, NZ peak purity was examined, and no signs of co-elution from any of the degradation products were detected.

Analytical performance of the proposed method

Linearity and concentration ranges

The linearity of the proposed HPLC procedure was evaluated by analyzing a series of different concentrations for each compound. The linear regression equations were generated by least squares treatment of the calibration data. Under the optimized conditions described earlier, the measured peak areas were found to be proportional to the analytes' concentrations. Table I presents the performance data and statistical parameters including linear regression equations, concentration ranges, correlation coefficients, standard deviations of the intercept (S_a), slope (S_b), and standard deviations of residuals ($S_{y/x}$). Regression analysis shows good linearity as indicated from the correlation coefficient values (> 0.999). In addition, linearity can be evaluated by calculation of the RSD % of the slope (Sb%) values, which did not exceed 1%. It is noteworthy to mention that LD was detected at 220 nm while both wavelengths 262 and 374 nm were adopted for the estimation of NZ with better sensitivity at 374 nm as indicated from the higher slope and lower limit of detection (LOD) and quantification (LOQ) values. Accordingly, quantification of NZ in the following parts of this study was carried out using 374 nm.

Detection and LOD and LOQ

According to the pharmacopoeial recommendations (1,3), the LOD is defined as the concentration that has a signal-to-noise ratio of 3:1, while for LOQ is the ratio considered to be 10:1. The LOD and LOQ values for the studied analytes were calculated and presented in Table I.

Precision and accuracy

The within-day precision and accuracy for the proposed method were studied at three concentration levels for each compound using three replicate determinations for each concentration within one day. Similarly, the between-day precision and accuracy were tested by analyzing the same three concentration levels for each compound using three replicate determinations on three days. Recoveries were calculated using the corresponding regression equations and they were satisfactory. The percentage relative standard deviation (RSD %) and percentage relative error (Er %) did not exceed 2%, proving the high repeatability and accuracy of the developed method for the estimation of the analytes in their bulk form (Table II).

Selectivity

Method selectivity was examined by preparing several laboratory-prepared mixtures of the two compounds at various concentrations within the linearity ranges mentioned in Table I. These mixtures were of different ratios both above and below the normal ratio expected in the dosage form. The laboratory-prepared mixtures were analyzed according to the previously described procedure. The recovery values, RSD %, and the Er % shown in Table III were satisfactory thus validating the selectivity, precision, and accuracy of the developed method and demonstrating its capability to resolve and quantify the analytes in different ratios.

Robustness

Robustness was examined by evaluating the influence of small

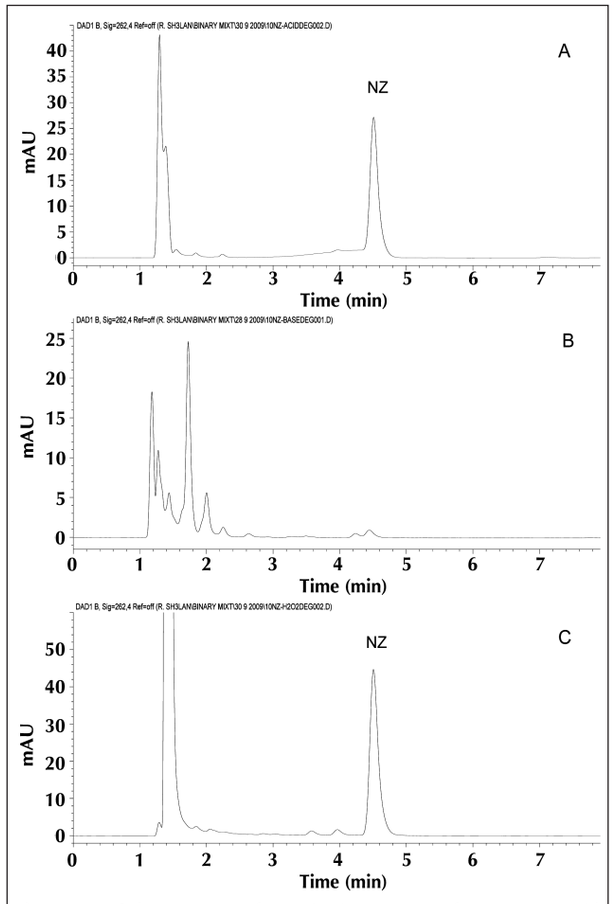

Figure 5. HPLC chromatograms of 10 mg/mL NZ at 262 nm after exposure to acid degradation (A), base degradation (B), and oxidative degradation with H_2O_2 (C).

variations in the experimental conditions such as source of methanol (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland or LabScan Analytical Sciences, Dublin, Ireland), working wavelengths (± 2 nm), mobile phase pH (± 0.2 pH units), percentage of the organic modifier (± 2%), and flow rate (± 0.1 mL/min). These variations did not have any significant effect on the measured responses or the chromatographic resolution. RSD% for the measured peak areas after the studied variations did not exceed 2%.

Stability of solutions

The stability of standard working solutions as well as sample solutions in the mobile phase was examined, and no chromatographic changes were observed within 24 h at room temperature when they were kept protected from light. Also, the stock solutions were stable for at least a week when refrigerated at 4°C. Retention times and peak areas of the drugs remained unchanged, and no significant degradation was observed during these periods.

Analysis of topical dosage form

The developed HPLC–DAD procedure was applied for the assay of this drug combination in two different batches of the pharmaceutical formulation available in the local market (Furaseen-L® soluble dressing). Sample preparation included only dissolving an amount of the dosage form in methanol without any extraction or prior to separation of the active drugs from the matrix, and then aliquots were diluted with the mobile phase before injection into the column. The two active ingredients eluted at their specific retention times. No extra peaks were observed from any of the inactive ingredients or the dosage form matrix. Also, the DAD enables peak purity verification, where no signs of co-elution from any of the inactive components were detected. Recoveries were calculated using both regression equations and standard addition methods. The assay results revealed satisfactory accuracy and precision as indicated from % recovery, SD, and RSD % values (Table IV). It is evident from these results that the proposed method is applicable for the analysis of NZ and LD in their combined commercial formulation with minimum sample preparation and satisfactory level of selectivity, accuracy, and precision.

Table IV. Application of HPLC–DAD for the Analysis of NZ–LD Mixture in Commercial Topical Dosage Form (Furaseen-L®)*

Batch	Regression Equation		Standard Addition	
Number	NZ	LD	NZ	LD
70309A				
%Recovery ± SD†	96.84 ± 0.90	96.41 ± 0.83	97.02 ± 1.09	97.23 ± 1.21
RSD%‡	0.93	0.86	1.12	1.25
90064A				
%Recovery ± SD†	97.29 ± 0.98	97.55 ± 1.13	97.51 ± 1.39	97.66 ± 0.94
RSD%‡	1.01	1.16	1.43	0.96

* Quantification was carried out at the following wavelengths: 374 nm, NZ; 220 nm LD.
† Mean ± standard deviation for five determinations.
‡ % Relative standard deviation.

Conclusion

In this work, a simple, selective, and reliable stability-indicating HPLC–DAD procedure was developed for the assay of NZ and LD HCl in their pharmaceutical combination. The proposed method made use of DAD as a tool for peak identity and purity confirmation. Also, the multiple wavelength detection was useful in measuring each drug at its maximum wavelength, thus improving sensitivity. To the best of our knowledge, there are no analytical reports describing the simultaneous determination of the two analytes in their combined dosage form. Selectivity was demonstrated by separation of the two analytes from the potential related substance 2,6-dimethylaniline as well as from cis-nitrofurazone isomer obtained by photolytic degradation of the drug, in addition to nitrofurazone hydrolytic and oxidative degradation products. Obviously, the selectivity and stability-indicating properties render the developed method superior to all non-separation methods. Reliability was guaranteed by testing various validation parameters of the method and successful application to the commercial formulation. Regarding sensitivity and reliability, the proposed method is superior to several previously published HPLC methods (11,13,31–33). Therefore, the proposed method can be recommended for routine analysis and for checking quality during stability studies of the two drugs.

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