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Short communication

Photochemical stability of nimesulide

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Abstract

HPLC and TLC methods for monitoring of the photochemical stability of nimesulide are presented. Solution of nimesulide sodium salt was exposed to the light of wavelengths 254 nm. The presence of degradation products (2-phenoxy4-nitroaniline and methanesulfonic acid) was observed. In the exposed sample, 2-phenoxy4-nitroaniline was detected by HPLC analysis and sulfonic acid was detected by TLC analysis. An isocratic HPLC chromatographic condition was described for determination of nimesulide in a presence of its degradation product. The sample was analysed on Separon SGX, C_{18}, 250 × 4.6 i.d. 7 μm analytical column. The mobile phase was consisted of a mixture of acetonitrile and ammonium phosphate (pH 7.9; 0.02 M) (35:65 v/v). UV detector was performed at 245 nm. Propylparaben was employed as an internal standard. Standard area response was linear respect to concentration of nimesulide over range 150–500 μg/ml. As a validation of the method, the accuracy and between-day precision were done. The detection limit of 2-phenoxy4-nitroaniline was 0.12 μg/ml. The solvent system for TLC analysis was consisted of ethylacetate and cyclohexane (45:55), the samples were plotted on silica gel UV-254 nm. UV lamp (254 nm) and the chemical detection were used.

Keywords: Nimesulide; HPLC; TLC; Photochemical stability

1. Introduction

Nimesulide (4-nitro-2-phenoxymethanesulfonanilide) is a modern anti-inflammatory drug belonging to the general class of NSAID (nonsteroidal anti-inflammatory drugs). This compound differs to the others NSAID by the chemical structure and selective inhibition of cyclooxygenazy 2. Thank to this effect, nimesulide seems to cause less several gastrointestinal side effects. Nimesulide is widely used for the treatment of rheumatoid arthritis and another antipyretic properties [1]. Nimesulide is a light yellow crystalline powder, which is practically odourless. The various pK_a values (from 5.9 to 6.56) were printed in Ref. [2]. The value of pK_a clearly indicates the acid natural of the drug. Nimesulide is soluble

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in organic polar solvents; the solubility is diminished in solvents of high polarity such as methanol. The solubility in water is reported to be 0.01mg/ml but the solubility depend on the pH of the aqueous solution [2].

Information about the stability of the drug substance is an integral part of the systematic approach on the stability evaluation. Investigation of influence of light on the stability of drugs has gained more and more importance in recent years. Characterization of the photochemical degradation of drugs using accurate, specific and well-characterized methods is of interest regarding safety, quality and effectiveness of drug formulation. Light exposure (during production, storage and use) can change the stability of drug and toxic decomposition products may be formed. Information about photo stability of drugs can help to determine the storage conditions in order to achieve high quality of pharmaceutical products. As regards the structural similarity to sulfonamides, nimesulide was considered to be photo unstable compound. [3]. Although certain information about the photo stability of some NSAID's has been reported [4,5], no reports about the photo stability of nimesulide as a modern agent with different structure are available. Only one report has been presented in literature about the stability of nimesulide and its main metabolite (hydroxy-nimesulide) in plasma. [2]. The chromatographic methods of analysis for nimesulide include, HPLC [6–14] and HPTLC [15,16].

The aim of this paper was to study the photochemical stability of nimesulide and to describe the development of a sensitive analytical method using HPLC and TLC for determination nimesulide in the presence of its degradation products. The conclusions of this paper could spread unpublished information about the stability profile of nimesulide, which could be helpful in effort to ensure quality, safety and effectiveness of drugs. So that the novelty of this work is based on presentation of two stability indicating analytical methods (HPLC and TLC), which could be used for determination of purity of this substance.

2. Experimental

2.1. Chemical and reagents

Standards of nimesulide and methanesulfonic acid were obtained from Sigma–Aldrich (Gmbh, Germany). HPLC-grade cyclohexane, ethylacetate, methanol and reagent-grade phosphoric acid, sodium hydroxide, ammonium phosphate monobasic, potassium iodide, argentum nitrate, sodium nitrite, potassium hydroxide were provided by Lachema (Brno, Czech Republic). HPLC-grade acetonitrile was Balex (Pardubice, Czech Republic) product.

2.2. Instrumentation and chromatographic conditions

2.2.1. HPLC

All HPLC instruments were obtained from Ecom s.r.o. Czech Republic. System consisted of a pump, a spectrophotometric detector (LCD 2084) and a data station with CW software, version 1.7. The separation was performed on a Separon SGX, C18, 250 × 4.6, i.d. 7 μm analytical column (Tessek, Prague, Czech Republic).

The mobile phase consisted of acetonitrile and ammonium phosphate (pH 7.9; 0.02 M) (35:65 v/v). The pump was operated in an isocratic mode with a flow-rate of 0.6 ml/min. The UV-detection was performed at 245 nm. Injections were carried out using a 20-μl loop.

2.2.2. TLC

As the stationary phase for TLC analysis was used silica gel plates UV-254 (Kavalier, Czech Republic). The solvent system consisted of ethylacetate and cyclohexane (45:55). The saturation time was 15 min. The samples were applied to the plate using a 5-μl pipette.

The length of chromatogram run was 12 cm. Nimesulide and 2-phenoxy4-nitroaniline were detected using the spray solution consisted of the copulation reagents (1% solution of $NaNO_2$ in 1 M HCl) and the diazotation reagents (0.2% solution of 1-naphtol in 1 M KOH). Methane sulphonic acid was found using solution of fluorescein in methanol (1:5).

2.3. Nimesulide decomposition

UV lamp (Camag, Switzerland) was used as a source of UV radiation; radiation intensity 1200 μW/cm^2 the wavelength at 254 nm.

A solution of nimesulide sodium salt (3 mg/ml) was exposed to continuous light of wavelengths 254 nm for 80 h. The UV lamp was located 40 cm far from this solution. After the exposition the sample was evaporated to dryness, dissolved in methanol and analysed employing HPLC and TLC. The other solution of nimesulide sodium salt was prepared using the same way and it was stored in the cooler. After 80 h it was analysed to compare degradation with and without UV exposition.

2.4. Preparation of degradation product

The degradation product was obtained by warming of a solution of nimesulide in the sodium hydroxide solution (10 mg/ml) on water bath for 40 h up. Crystals, which were gained in this process, were recrystallized in methanol. Identification was made using NMR analysis.

2.5. Preparation of standard solutions

The stock solution of nimesulide with a concentration of 1 mg/ml was prepared by dissolving 100 mg of nimesulide in methanol. The internal standard stock solution of propylparaben (1 mg/ml) was prepared in methanol.

2.6. Calibration curves

The calibration curves were prepared using concentration of 150, 200, 300, 450 and 500 μg/ml for nimesulide and the concentration of 300 μg/ml for propylparaben (internal standard). Diluting of the stock solution with the appropriate volumes of methanol made the standard solutions for the calibration curves. The standard solutions of nimesulide in methanol containing propylparaben were injected in triplicate into chromatograph. The peak area ratios (nimesulide to internal standard) were plotted against the corresponding analyte concentration.

2.7. The limit of detection

The limit of detection was calculated to be three times the standard deviation of noise ratio from the analysis of 2-phenoxy4-nitroanilide (80 μg/ml).

2.8. Accuracy

Accuracy of the method is calculated as a percentage of recovery by the assay of the known added amount of analyte in the sample [17]. The samples were gained by further dilutions of the standard stock solution to obtain concentration of 170, 250, 400 μg/ml of nimesulide. All samples were analysed in triplicate.

2.9. Precision

The precision of a method is determined by assaying a sufficient of aliquots of a homogenous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation [17]. The precision of the method was tested as between-day reproducibility of the assay. The independent samples of nimesulide (170, 250, 400 μg/ml) were tested in 2 days to determine inter-day reproducibility.

3. Results and discussion

3.1. Optimization of chromatographic conditions

The preliminary objective of the work was to develop optimal chromatographic conditions for assessment 2-phenoxy4-nitroaniline (notional degradation product) in the sample of nimesulide. Available analytical RP-HPLC methods reported for the determination of nimesulide employ acid mobile phases (pH 3.5–5). According to the previously reported chromatographic conditions and pK_a of the substance, nimesulide was at first analysed using a mixed acid buffer (NaH_2PO_4 0.01 M, KH_2PO_4 0.01 M, NaCOOH 0.1 M, NH_4HPO_4 0.02 M) and organic solvents (methanol, acetonitrile) in a different ratios. The initial experiments with the acid mobile phases did not provide adequate separation of nimesulide and its degra-

dation product, so that the alkaline buffer (NH_4HPO_4 0.02 M, pH 7.9) was employed later. The ratio between aqueous and the organic phases was optimised too. The analytical columns with modified silica gel (C_8, C_{18}, CN) as the stationary phases were tried. Finally the Tessek C_{18} chromatographic column was provided in order to increase number of theoretical plates. The capacity factors (K) were 1.418 for nimesulide and 1.958 for degradation product Fig. 1.

The ultraviolet spectra of nimesulide and 2-phenoxy4-nitroaniline were recorded using a UV-Shimanzu 2401PC spectrophotometer. The spectrum of nimesulide exhibits maxima at 323, 298 and 201 nm. The absorption spectrum of 2-phenoxy4-nitroaniline was almost the same as the spectrum of nimesulide. According to the absorption maximum of nimesulide and its degradation product the detector was employed at 245 nm.

3.2. Assay of nimesulide

Assay of nimesulide was performed employing propylparaben as the internal standard. Propylparaben was acceptable as the internal standard because it exhibits similar chromatographic properties to nimesulide and 2-phenoxy4-nitroaniline. Under the chromatographic conditions used, nimesulide and propylparaben have retention times of 8.10 and 17.01 min. The calibration cure was linear in the studied range. The mean equation (curve coefficient $\pm$ standard deviation) of the calibration curve ($n = 5$) obtained for five points was $y = (1.139 \pm 0.064)x + (0.016 \pm 0.022)$ with correlation coefficient $r = 0.9969$. The value of y represents the concentration of nimesulide μg/ml and the value of x represents the peak area ratio between analyte and internal standard. The solution of nimesulide was stable during this chromatographic analysis.

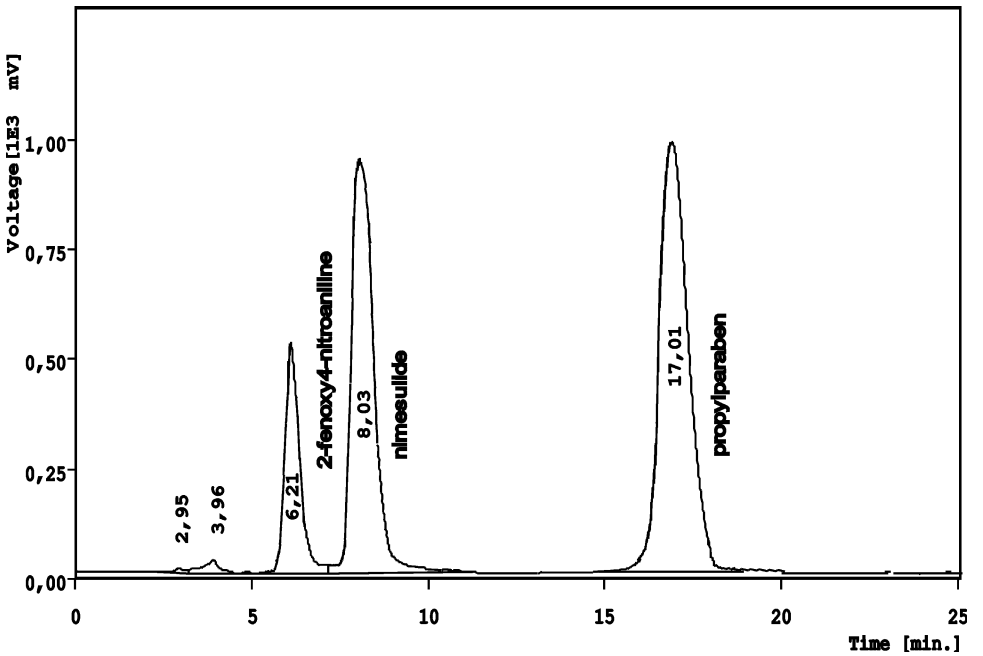

Fig. 1. Chromatogram shows separation of 2-phenoxy4-nitroanilide, nimesulide and internal standard-propylparaben. Chromatographic conditions were described in Section 2.2.1.

3.3. Accuracy and inter-day precision

Obtained accuracy values were within an acceptable limit Table 1. The results for inter-day precision are presented in Table 2.

3.4. The limit of detection

The limit of detection was calculated to be 0.12 μg/ml of 2-phenoxy4-nitroanilide.

3.5. Stability study

At first the solution of nimesulide sodium salt was exposed to UV radiation of the maximum at 350 nm. (UV lamp with wavelength range from 300 to 400 nm). After 100 h of exposition to this radiation the solution was stable. So that's why we decided to use another UV lamp (Camag UV lamp; maximum at 254 nm). Employing HPLC method the presence of 2-fenoxy4-nitroanilide in the solution was detected after 80 h of UV irradiation. The presence of 2-fenoxy4-nitroanilide in the exposed sample of nimesulide was confirmed by agreement of t_R value of the standard solution of 2-phenoxy4-nitroanilide and t_R value of the solution of nimesulide after exposition to radiation. This agreement was also proved by application of additional amount of standard solution (2-phenoxy4-nitroanilide) to the sample and the significant growth of the peak area was determined. Fig. 2 shows the chromatogram of the sample after 80 h of exposition to UV radiation. In addition, the presence of the other unknown degradation product was discovered on this chromatogram.

Table 1
Accuracy of assay-analysis of known amount of nimesulide

Concentration added (μg/ml)	Concentration found (μg/ml) ± S.D.	Recovery %
170	169.5 ± 0.49	99.70
250	249.0 ± 0.57	99.60
400	397.4 ± 1.67	99.35

Table 2
Between-day precision of assay of nimesulide

Concentration added (μg/ml)	Concentration found (μg/ml) ± S.D.	RSD %
170	169.3 ± 0.37	0.22
250	248.8 ± 0.71	0.28
450	397.5 ± 1.71	0.43

3.6. TLC

As regard that nimesulide had been degradated into 2-phenoxy4-nitroanilide, the presence of the other degradation product (methane sulphonic acid) was supposed. Because of the fact that methane sulphonic acid cannot be indicated by HPLC analysis, a TLC method was developed. The standard solution of nimesulide, 2-phenoxy4-nitroanilide and methane sulphonic acid was spotted on TLC plates and run in different solvent systems. The following solvent system were tried in different portions:

Ethylacetate–methanol–25% ammonium
Hexane–acetone
Chloroform–methanol
Ethylacetate–cyclohexane.

The solvent system consisted of ethylacetate and cyclohexane (45:55 v/v) gave a compact spots of all components (nimesulide and its degradation products) with a significant Rf values. Nimesulide had Rf value of 0.29 2-phenoxy4-nitroanilide had Rf value of 0.43.

4. Conclusions

The propose stability study shows nimesulide to be relative photostable compound. Nimesulide must be exposed to UV radiation for a relative long time to obtain its decomposition products. As the main photochemical decomposition product 2-phenoxy4-nitroanilide was found. The chromatogram of the exposed sample of nimesulide shows that the presence of the other unknown decomposition product is expected. Further study has to be done for identification of unknown decomposi-

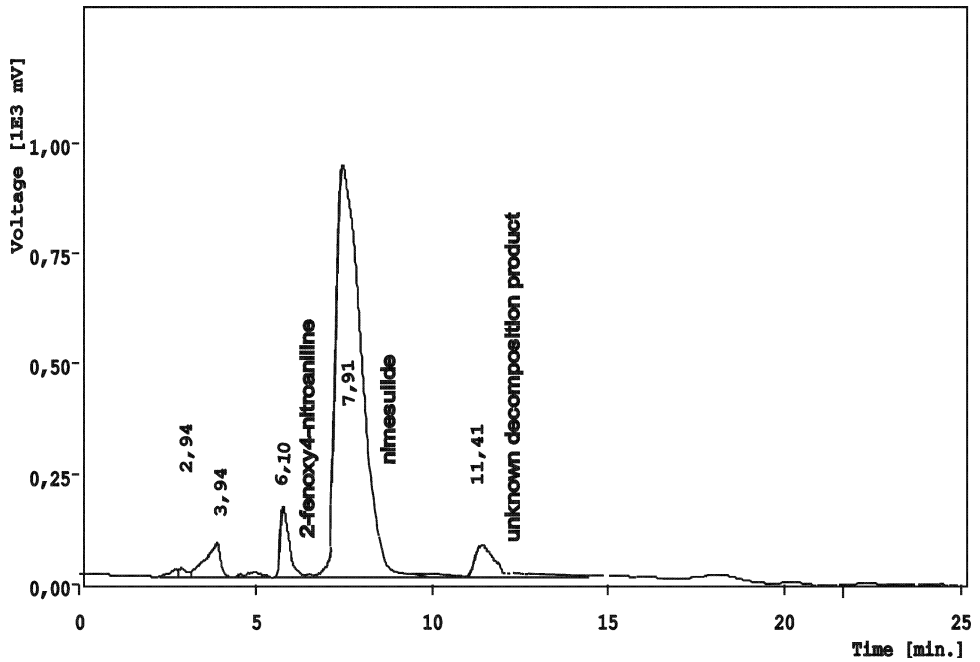

Fig. 2. Chromatogram of degradation of nimesulide after 80 h exposition to UV radiation. Chromatographic conditions were described in Section 2.2.1.

tion product of nimesulide. This analytical method allows determining the amount of nimesulide in the presence of its decomposition products.

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