Scientific paper

Analysis of Nefopam by TLC-densitometry. A Study of Degradation Mechanism in Solutions Under Stress Conditions

Malgorzata Starek,[1,]* Monika Dąbrowska[1] and Monika Tarsa[2]

[1] *Department of Inorganic and Analytical Chemistry,*

[2] *Department of Organic Chemistry, Faculty of Pharmacy, Collegium Medicum, Jagiellonian University, 9 Medyczna Str, 30-688 Kraków, Poland*

* *Corresponding author: E-mail: mstarek@interia.pl*

Received: 13-10-2010

Abstract

A new, simple, selective, precise and accurate thin-layer chromatographic method for quantification of nefopam hydrochloride in formulations has been established and validated. TLC F_{254} silica gel plates in combination with a mixture of chloroform : methanol : glacial acetic acid (9 : 2 : 0.1, v/v/v) as mobile phase were used. UV detection was performed densitometrically at a wavelength of 222 nm. The method was validated for linearity, precision, accuracy, selectivity and specificity in accordance with ICH guidelines. Nefopam hydrochloride was subjected to acid and alkaline hydrolysis at different temperatures. The degradation products were well resolved from the pure drug with substantially different R_F values and absorption spectra. LC-MS/MS analysis was used to characterize the chemical properties of degradation products. The likely chemical structures of the major degradation products are 2-[(2-benzylbenzyl)(methyl)amino]ethanol, 2-[{2-[hydroxyl(phenyl)methyl]-benzyl}(methyl)-amino] ethanol and diphenylmethanone.

Keywords: TLC-densitometry / Nefopam hydrochloride / Stability studies / Degradation process

1. Introduction

Nefopam hydrochloride (5-methyl-1-phenyl-1,3,4,6-tetrahydro-2,5-benzoxazocine hydrochloride) has a unique structure and is derived from orphenadrine and diphenhydramine by cyclization of the side chain. It is unrelated chemically or pharmacologically to any other analgesic compound. It appears safe and seems to have no depressant action on the central nervous system. Nefopam is a centrally acting analgesic with both supra-spinal and spinal sites of action. It is neither an opiate nor a non-steroidal anti-inflammatory drug. Nefopam does not induce respiratory depression, even in postoperative patients. It reduces the feeling of mild to moderate pain and it is sometimes used as an alternative to morphine. The drug is available in Europe but is not approved to be used in the United States by the Food and Drug Administration.[1,2]

Nefopam, a non-narcotic analgesic used to relieve moderate, acute and chronic pain, has been shown to possess analgesic activity with a profile distinct from that of opioid analgesics and NSAIDs. It has been demonstrated that nefopam is used to relieve postoperative pain, pain associated with cancer and is also used in the prevention of postanaesthetic shivering.[3]

Different techniques exist for the determination of nefopam in dosage forms and biological samples. A spectrophotometric method using alizarins has been used.[4] In the literature some GC methods report on the quantification of nefopam in human plasma.[5–7] Also some HPLC methods with various detectors have been established for the determination of nefopam and its metabolites in biological fluids and aqueous solutions.[8–11] The degradation mechanism of nefopam in buffer solutions at pH 2.0 and 9.0 has been reported. Eight different degradates were proposed by proton nuclear magnetic resonance.[12] Nefopam undergoes nucleophylic attack by a hydroxyl ion to form a some products (in acidic solution three and in alkaline solution five different com-

pounds). A stability-indicating RP-HPLC method was developed for the detection of nefopam and its degradation products in acetate, phosphate and borate buffer.[13] A smaller effect of hydroxyl ion on the degradation of non-protonated than on the degradation of protonated nefopam was observed.

Thin-layer chromatography is a useful method for identifying and testing the purity of drugs, because it is relatively quick and requires small quantities of material. The major advantage of TLC is that several samples can be run simultaneously using a minimal quantity of mobile phase (unlike HPLC), thus reducing analysis time and cost per analysis. Over the past years TLC has been successfully used for analysis of pharmaceuticals, plant and biological materials. As far as we are aware no validated and stability-indicating TLC method for analysis of nefopam hydrochloride in pharmaceutical forms has been reported in the literature.

The aim of our work was to develop a new thin layer chromatographic method with densitometric detection for the determination of nefopam hydrochloride and its degradation products and related impurities. After validation,[14] this TLC-densitometry method was used for identification and quantification of the drug and to study of purity and stability of the drug under different stress conditions. In addition, a LC-MS/MS technique was used to identify nefopam and its main degradation products.

2. Experimental

2. 1. Equipment

Densitometer: TLC Scanner 3 with WinCats4 software (Camag, Switzerland); Sample applicator: Linomat V (Camag, Switzerland); Silica gel (200 µm per thickness) aluminium TLC F_{254} plates, art. No 1.05554.001 (Merck, Germany); TLC vertical chambers 18 × 9 × 18 cm and 29 × 9 × 28 in side (Sigma-Aldrich); Incubator ECOCELL55 – BMT (Brno, Czech Republic); Spectrometer MS API 2000 (Applied Biosystems MDX Sciex, Concord, Ontario, Canada); HPLC system with an XBridge C18 analytical column (30 mm × 2.1 mm, 3.5 µm; Waters, Ireland) and DAD detector (Agilent Technologies, Waldbronn, Germany).

2. 2. Reagents and Chemicals

A standard substance of nefopam hydrochloride was purchased from Biocodex (Montrouge, France). Reagents, such as methanol and diethyl ether were purchased from Merck (Germany) and chloroform and glacial acetic acid from Chempur (Piekary Sląskie, Poland). All reagents were of analytical grade. Nefopam tablets containing 30 mg of nefopam hydrochloride, s. 010406, was produced by Jelfa (Jelenia Góra, Poland).

2. 3. Solutions

A standard solution of nefopam (0.1 mg/mL) was prepared in methanol. Samples were prepared by finely grounding ten tablets with a pestle and mortar; a sample amount corresponding to approximately 10 mg of the active substance was weighed, and the drug was extracted from the powder with methanol. To ensure extraction of the drug the mixture was sonicated for 30 min. The volume was diluted to 10.0 mL. Supernatant (1 mL) was collected and further dilutions were made with methanol to prepare a solution containing 0.1 mg/mL.

2. 4. TLC Procedure

Chromatography was performed on 7 × 10 cm silica gel 60 TLC F_{254} aluminium plates (20 × 20 cm, 0.2 mm thickness, 5–6 mm particle size). Standard and sample solutions of different concentrations were applied to the plates as 10 mm bands, 10 mm apart and 10 mm from the bottom of the plates using a Camag Linomat 5 sample applicator equipped with a 100 µL syringe; the rate of application was constant at 200 nL/s. Plates were developed with a mixture of chloroform : methanol : glacial acetic acid (9 : 2 : 0.1, v/v/v) as a mobile phase in a glass chamber, previously saturated with mobile phase vapour for 15 min at room temperature. The development distance was 90 mm. After development the plates were dried in the air. Densitometric scanning was performed at 222 nm using a Camag TLC Scanner 3 in absorption remission mode. The slit dimensions were 8 × 0.45 mm, the scanning speed 20 mm/s and the data resolution 100 µm per step. Concentrations of the analytes were determined from the intensity of diffused reflected light. Evaluation was by peak area with linear regression.

2. 5. Validation of the Method

2. 5. 1. Linearity

Standard solutions of nefopam hydrochloride were prepared in the concentration range 0.05–1.00 mg/mL and 15 µL of each solution was applied to a TLC plate. Each concentration was spotted two times on the TLC plate. The plates were developed as described above and peak areas were plotted against the corresponding amounts to furnish calibration plots.

2. 5. 2. Precision

The precision of the method was verified by measurement of repeatability and intermediate precision. Repeatability (intra-day precision) was assessed by analysis of three different concentrations of the drug (1.0, 1.5, 2.0 µg/band), in six replicates each, on the same day. Intermediate precision (inter-day precision) was assessed by repeating studies on three different days, using on freshly prepared solutions for each assessment.

2. 5 .3. Sensitivity

The sensitivity of the method was determined with respect to limit of detection (LOD), limit of determination (LOQ), linearity range and correlation coefficient. LOD and LOQ were determined on the basis of the linear relationship of peak area via concentration and obtained from the equations LOD = 3.3 · S_e/a and LOQ = 10 · S_e/a, where S_e is the standard error of the estimate and *a* is the slope of the curve.

2. 5. 4. Accuracy

Recoveries were calculated to study the accuracy of the method by analysis of a drug solution to which a known amount of nefopam hydrochloride, corresponding to 80, 100 and 120 % of the label claim, has been added. At each concentration six analyses were performed.

2. 5. 5. Specificity

The specificity of the method was determined by analysis of standard and sample solutions. The identity of the nefopam band in the sample was confirmed by comparison of its R_F values and absorption spectra with those of a standard. The peak purity of nefopam was determined by comparing three different spectra acquired for three regions of the band, peak start, peak apex and peak end.

2. 6. Analysis of Nefopam in Tablets

Quantitative analysis of nefopam in commercial tablets was provided by using the TLC method developed above. The final drug solutions were applied (in triplicate) to a TLC plate in volume of 15 μL. The possibility of interferences by excipients on the analytical procedure was examined.

2. 7. Degradation Studies

The effect of pH, temperature and incubation time on the stability of nefopam hydrochloride in solutions was investigated. Studies of acid- and base-inducted decomposition were performed exposing the solutions of the drug in 0.5, 1.0 and 3.0 M hydrochloric acid and sodium hydroxide solutions. These solutions were incubated for specified lengths of time (in the range from 1 h to 67 days) at 60 °C and 120 °C. After heating, the solutions were diluted with methanol (1 + 1, v/v) and the resulting solutions were applied to TLC plates. Chromatograms were obtained as described above. The measurements were made under conditions established for the determination of the percentage constituent concentrations. The process of nefopam hydrochloride degradation was rated by means of kinetic and thermodynamic parameters, such as the reaction rate constants k, half-life $t_{0.5}$ and the time $t_{0.1}$ in which the concentration of nefopam is reduced by 10% and the activation energy E_a.[15]

2. 8. Analysis of Degradation Products

To identify the nefopam degradation products, beside densitometric analysis (R_F values and absorption spectra), LC-MS/MS analysis was carried out. The nefopam solutions after hydrolysis in 1 M HCl or 1 M NaOH at 120 °C were separated on a chromatotron plate (model 8924, Harrison research, USA). A solution of hydrolyzed substance in methanol was applied to the plate (prepared with Silica Gel PF_{254} and water). Subsequently, appropriate solvents of increasing polarity and different proportions were applied to the plate and a preferred mixture consisting of chloroform : methanol : glacial acetic acid (9:2:0.1 v/v/v) was obtained. The separation obtained on the plate was observed under a UV lamp. After fraction separation, the solvents were evaporated and the products identified by LC-MS/MS. The analysis was carried out by using a mobile phase of acetonitrile – water (50 + 50 v/v) with addition of formic acid 10 mL/L at a fixed flow rate of 600 mL/min using positive ionization and electrospray as an ion source.

3. Results and Discussion

The mobile phase was optimized by testing different mixtures of different polarity, and a mixture of chloroform : methanol : glacial acetic acid (9 : 2 : 0.1, v/v/v) was found to enable satisfactory separation of the components in samples from the pharmaceutical preparations and during the stress testing of nefopam hydrochloride under different conditions. The R_F of nefopam hydrochloride was 0.53 (Figure 1).

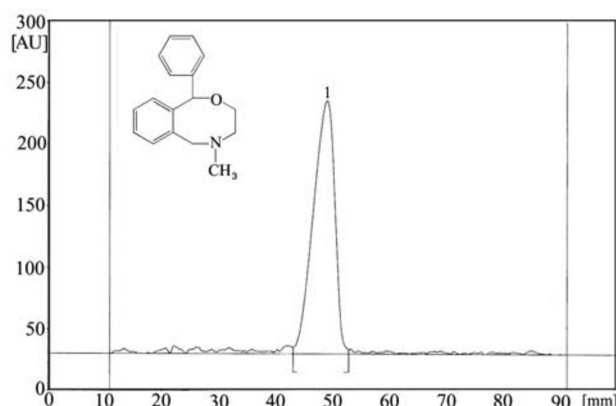


Figure 1. An example of a densitogram of nefopam hydrochloride obtained directly from the chromatogram.

The intensity of the nefopam peaks in different chromatograms was confirmed by comparing the UV spectra of the peak from the standard with the corresponding peak from the sample.

The method was validated in accordance with ICH guidelines for linearity range, precision, sensitivity and accuracy.[14]

Table 1: The results from the precision study.

Concentration		Intra-day			Inter-day	
[μg per band]	Mean area	S	% RSD	Mean area	S	% RSD
1.0	2030.4	33.08	1.63	1976.9	37.76	1.91
1.5	2605.6	26.33	1.01	2648.9	48.54	1.83
2.0	4101.4	49.07	1.20	3998.7	74.78	1.87

S – standard deviation; RSD – relative standard deviation [%]

Table 2: The results from the recovery studies.

Label claim [mg/tablet]	Amount added [mg]	Total amount [mg]	Amount recovered [mg]	Reco-very [%]	% RSD
30	24 (80 %)	54	53.25	98.61	0.98
	30 (100 %)	60	59.45	99.09	0.24
	36 (120 %)	66	65.19	98.78	1.21

The calibration plot for the analyte was a linear function of the drug concentration over the range between 0.05 – 1.00 mg/mL and was defined by the linear equation $y = 14170.0 \cdot x + 1217.1$, where y is peak area and x the

Table 3: The results of nefopam hydrochloride determination in pharmaceutical preparation with statistical analysis.

Formu-lation	Declared concentration	Determined concentration		Statistical analysis
Nefopam	30 mg / tablet	29.64	29.51	$x_m = 29.96$
		30.52	29.80	$S = 0.35$
		30.31	29.88	$S_{x\,m} = 0.12$
		29.82	30.23	$\mu = 29.96 \pm 0.28$
				RSD = 1.17

x_m – arithhmetic mean; S – stanndard deviation; $S_{x\,m}$ – stanndard deviation for arithmetic mean; μ – connfidence interval at 95 % probability; RSD – reelative standard deviation [%]

Table 4: The results for determination of nefopam and its degradation products after incubation at a temperature of 60 °C.

Environment	Time		Concentration [%]	
	[day]	RF ZA ≈ 0.40	RF NEF ≈ 0.53	RF ZB ≈ 0.88
3 M HCl	0	5.22	92.19	2.59
	1	24.42	73.03	2.55
	4	25.77	71.57	2.66
	25	50.55	36.58	12.87
	67	49.68	27.52	22.80
1 M HCl	0	2.83	95.34	1.93
	1	41.36	56.97	1.67
	4	57.88	39.26	2.86
	25	57.05	33.63	9.32
	67	54.20	30.17	15.63
0.5 M HCl	0	5.73	94.27	–
	1	59.04	40.96	–
	4	56.21	39.50	4.29
	25	52.73	37.40	9.87
	67	50.10	35.75	14.15
0.5 M NaOH	0	–	100	–
	4	–	96.41	3.59
	11	–	95.06	4.94
	39	–	88.06	11.94
	67	2.22	81.50	16.28
1 M NaOH	0	–	100	–
	4	–	100	–
	11	–	98.05	1.95
	39	–	91.43	8.57
	67	–	87.24	12.76
3 M NaOH	0	–	100	–
	4	–	100	–
	11	–	99.38	0.62
	39	–	92.32	7.68
	67	–	91.25	8.75

concentration of nefopam. The values of the standard deviation of the slope (S_a), the standard deviation of the intercept (S_b) and the standard error of estimate (S_e) were 520.9, 298.8 and 403.1, respectively. The *r* value was 0.9973, which indicates a good linear relationship between peak area and amount.

The results from testing of repeatability and intermediate precision experiments are shown in Table 1. The method was found to be precise; RSD values obtained in this study were < 2 %. All peaks on each plate obtained in stress conditions are identical with respect to position and spectra. R_f values for each of the peaks don't vary more than 0.05.

The method is characterized by a very high sensitivity (LOD = 0.95 μg per band; LOQ = 3.16 μg per band) and a high recovery as shown by the data in Table 2 (% RSD < 1.5).

Measured amounts of active compound in the Nefopam tablets were in good agreement with the label claim of 30 mg and are shown in Table 3. The drug content was found to be 99.87 %. The developed method is specific for the studied component occurring in the tablets with coexisting compounds. There are no interferences in the recorded chromatograms for placebo solutions where the studied component is present.

TLC studies of the samples obtained during the stress testing of nefopam hydrochloride under different conditions suggested the following degradation behavior (Tables 4 and 5).

In addition to the nefopam peak, some additional peaks were observed in the chromatograms, depending on the test conditions. When the drug solution was exposed to HCl at 60 °C for 60 days or at 120 °C for 24 h, approximately 70 % degradation was observed. From the R_F values is follows that in the recorded absorption spectra two (at 60 °C) or three (at 120 °C) impurities ($R_{F\ ZA}$ ≈ 0.40, $R_{F\ ZB}$ ≈ 0.88 and $R_{F\ ZC}$ ≈ 0.93) are present under acidic conditions

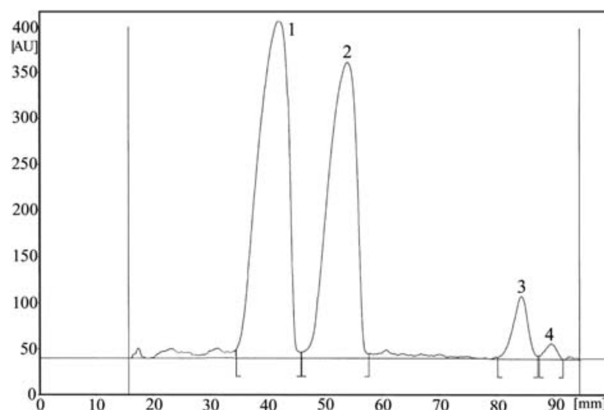


Figure 2. An example of a densitogram obtained after nefopam decomposition under acidic conditions (1-ZA; 2-nefopam; 3-ZB; 4-ZC).

Table 5: The results for determination of nefopam and its degradation products after incubation at a temperature of 120 °C.

Environment	Time [h]	Concentration [%] RF ZA ≈ 0.40	RF NEF ≈ 0.53	RF ZB ≈ 0.88	RF ZC ≈ 0.93
1 M HCl	0	2.83	95.34	1.83	–
	1	42.64	52.50	4.48	0.38
	2	42.79	51.82	4.73	0.66
	4	51.99	42.10	5.12	0.79
	6	54.19	38.41	6.01	1.39
	24	61.10	29.86	7.54	1.50
0.5 M HCl	0	5.73	94.27	–	–
	1	38.58	48.09	6.23	7.10
	2	40.23	44.09	7.21	8.47
	4	44.56	40.17	7.15	8.12
	6	44.90	37.76	7.88	9.46
	24	52.46	32.52	8.59	6.43
0.5 M NaOH	0	–	100.00	–	–
	1	–	96.13	3.87	–
	2	–	96.42	3.58	–
	4	–	96.20	3.80	–
	6	–	95.88	4.12	–
	24	3.44	95.22	1.34	–
1 M NaOH	0	–	100.00	–	–
	1	–	98.99	1.01	–
	2	–	97.72	2.28	–
	4	–	97.20	2.80	–
	6	–	96.38	3.62	–
	24	–	95.76	4.24	–

(Figures 2 and 3). The peaks obtained are well separated; the resolution factor values (R_s) are in the range from 0.4 to 1.4. The spectra obtained for nefopam indicated that none of the degradation products affects its quantification.

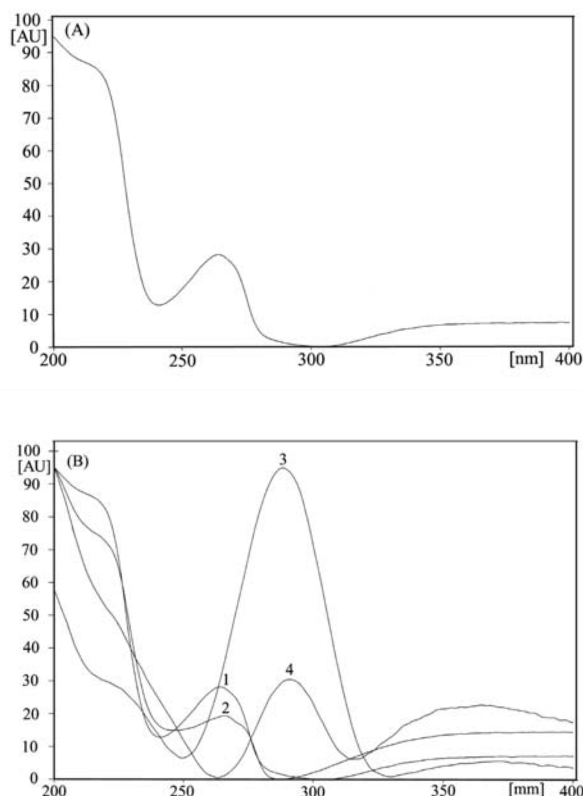


Figure 3. UV spectra for individual constituents obtained directly from the chromatograms ((A) nefopam; (B) 1-nefopam, 2-ZA, 3-ZB, 4-ZC).

The drug was found to undergo alkaline degradation more slowly than acid degradation. Treatment with sodium hydroxide at 60 °C for 3 days resulted in no degradation. When the drug solution was treated with 1 M NaOH at 60 °C for 60 days approximately 15% degradation was observed. Densitograms obtained from base-degraded nefopam contained two degradation product peaks at $R_{F\,ZA} \approx 0.40$ and $R_{F\,ZB} \approx 0.88$. When the drug solution was exposed to a NaOH solution at 120 °C for 24 h approximately 5% degradation was observed (Tables 4 and 5).

The kinetic and thermodynamic parameters describing the degradation process of nefopam under consideration were determined and compliance with first-order kinetic reaction was found, for which the rate constants k, $t_{0.1}$ and $t_{0.5}$ and the energy of activation E_a were determined (Table 6). The results of kinetic and thermodynamic investigations indicate that nefopam hydrochloride is a relatively stable product. The values of the reaction rate constant k increase with increasing temperature. The drug demonstrates a higher susceptibility to degradation in an acidic than a basic environment.

Table 6: Kinetic and thermodynamic parameters describing degradation process of nefopam hydrochloride.

Temperature	HCl or NaOH concentration / Kinetic parameters	
60 °C	**3 M HCl**	**3 M NaOH**
	$k = 7.52 \cdot 10^{-4}$	$k = 5.70 \cdot 10^{-5}$
	t0,1 = 140.0	t0,1 = 1847.4
	t0,5 = 921.5	t0,5 = 12157.9
	1 M HCl	**1 M NaOH**
	$k = 7.16 \cdot 10^{-4}$	$k = 8.49 \cdot 10^{-5}$
	t0,1 = 147.1	t0,1 = 1240.3
	t0,5 = 967.9	t0,5 = 8162.5
	0.5 M HCl	**0.5 M NaOH**
	$k = 6.03 \cdot 10^{-4}$	$k = 1.27 \cdot 10^{-4}$
	t0,1 = 174.6	t0,1 = 829.1
	t0,5 = 1149.3	t0,5 = 5456.7
120 °C	**0.5 M HCl**	**0.5 M NaOH**
	k = 0.0444	$k = 2.04 \cdot 10^{-3}$
	t0,1 = 2.4	t0,1 = 52.4
	t0,5 = 15.6	t0,5 = 339.7
	1 M HCl	**1 M NaOH**
	k = 0.0484	$k = 1.81 \cdot 10^{-3}$
	t0,1 = 2.2	t0,1 = 58.2
	t0,5 = 14.3	t0,5 = 382.9
	$E_a = 7.64 \cdot 10^4$ (1 M HCl)	
	$E_a = 5.55 \cdot 10^4$ (1 M NaOH)	

k – the staability constants [h^{-1}]; $t_{0,1}$ – the tiime, concentration will decrease about 10 % [h]; $t_{0,5}$ – the time, concentration will decrease about 50% [h]; E_a – the energy of acctivation [J/mol K]

The chemical structures of the degradation products were obtained by LC-MS-MS analysis (Figure 4) leading to nefopam possible decomposition reactions as presented in Scheme 1.

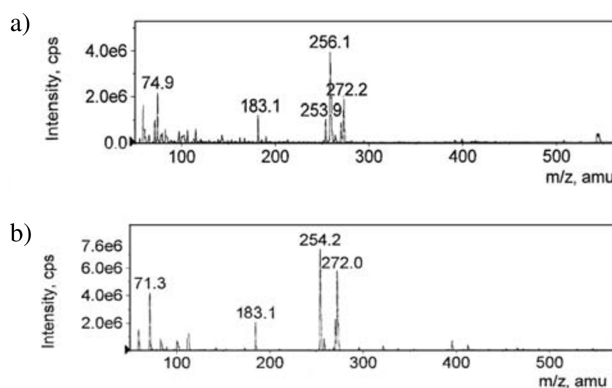
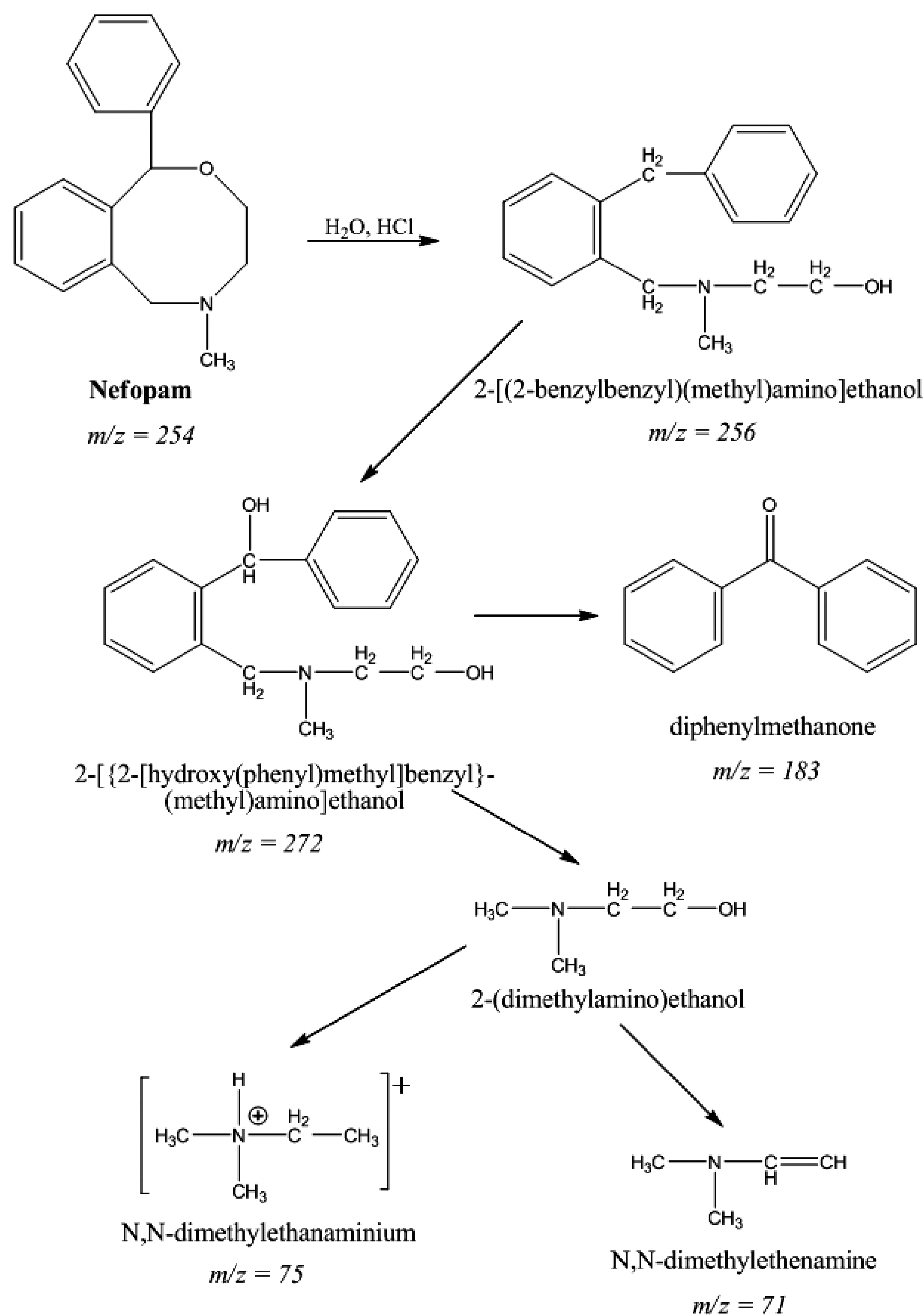


Figure 4. Mass spectra of tested sample done ESI mode ((A) $R_{F\,ZA} \approx 0.40$; (B) $R_{F\,ZB} \approx 0.88$).

Irrespective of the complexity of the tracks by which the degradation of nefopam proceeds, the initial ring-opening process at the site of ether linkage appears to be the rate-determining step of degradation in both acidic and



Scheme 1. A diagram of the proposed degradation mechanism of nefopam in solutions under stress conditions.

basic solutions. The main likely products of decomposition of nefopam are 2-[(2-benzylbenzyl)(methyl)amino]ethanol (ZA), 2-[{2-[hydroxyl(phenyl)methyl]benzyl}(methyl)amino]ethanol (ZB) and diphenylmethanone (ZC).

4. Conclusion

A novel TLC – densitometric method for the separation and quantitative determination of nefopam hydrochloride and its degradation products (2-[(2-benzylbenzyl)(methyl)amino]ethanol, 2-[{2-[hydroxyl(phenyl)methyl]benzyl}(methyl)-amino]-ethanol and diphenylmethanone) was developed. The presented technique is precise, specific, accurate and stability-indicating. Statistical analysis proves the method is suitable for analysis of nefopam in pharmaceutical formulations without interference from excipients. The method can be used to determine the purity of the drug available from formulations by detecting the related impurities. It may be extended to the study of the degradation kinetics of nefopam.

6. References

1. R. C. Heel, R. N. Brogden, G. E. Pakes, T. M. Speight, G. S. Avery, *Drugs* **1980**, *19*, 249–267.
2. A. M. Gray, M. J. Nevinson, R. D. Sewell, *Eur J. Pharmacol.* **1999**, *365*, 149–157.
3. S. N. Piper, K. D. Rohm, S. W. Suttner, W. H. Maleck, P. Kranke, J. Boldt, *Anaesthesia* **2004**, *59*, 559–564.
4. S. A. Shama, A. S. Amin, *Spectrochim. Acta A*, **2004**, *60*, 1769–1774.
5. H. Ehrsson, S. Eksborg, *J. Chromatogr. A* **1977**, *136*, 147–153.
6. S. F. Chang, C. S. Hansen, J. M. Fox, R. E. Ober, *J. Chromatogr*. **1981**, *13*, 79–89.
7. D. Schuppan, C.S. Hansen, R. E. Ober, *J. Pharm. Sci*. 1978 on-line: **2006**, *67*, 1720–1723.
8. D. T. Y. Lin, J. M. Savage, D. Donnal, *Br. J. Clin. Pharmacol.* **1987**, *23*, 99–101.
9. L. C. Burton, N. J. Loftus, D. W. Vere, R. Whelpton, *J. Chromatogr*. **1990**, *526*, 159–168.
10. G. Aymard, D. Warot, P. Demolis, I. Laville, B. Diquet, *J. Pharm. Biomed. Anal*. **2002**, *30*, 1013–1021.
11. G. Hoizey, A. Goglin, J.-M. Malinovsky, A. Robinet, L. Binet, M. L. Kaltenbach, H. Millart, D. Lamiable, *J. Pharm. Biomed. Anal*. **2006**, *42*, 593–600.
12. D.-P. Wang, Y.-H. Tu, L. V. Allen Jr., F.-Ch. Cheng, *Acta Pharm. Nord*. **1990**, *2*, 73–82.
13. Y.-H. Tu, D.-P. Wang, L. V. Allen Jr., *J. Pharm. Sci* 1989 online: **2006**, *79*, 48–52.
14. ICH Guideline Q2B on "Validation of Analytical Procedures: Text and Methodology", International Conference on Harmonization, 6 Nov 1996, incorp. Nov **2005**, Geneva.
15. A. Morski, *Wprowadzenie do kinetyki chemicznej*, WNT, Warszawa, Poland, **2000**.

Povzetek

Razvita in validirana je bila nova, enostavna, selektivna, in natančna metoda za kvantitativno določanje nefopam hidroklorida s tenkoslojno kromatografijo. Uporabljene so bile TLC F_{254} silikagelske plošče, z mobilno fazo kloroform: metanol: ledocet v razmerju 9:2:0,1, v/v/v. UV detekcija je bila izvedena densitometrično pri valovni dolžini 222 nm. Metoda je bila validirana na linearnost, pravilnost, natančnost in specifičnost v skladu z ICH navodili. Nefopam hidroklorid je bil hidroliziran v kislem in bazičnem pri različnih temperaturah. Razgradni produkti so se dobro ločili od učinkovine, saj so se bistveno razlikovale R_F vrednosti in absorpcijski spektri. Za karakterizacijo kemijskih lastnosti razgradnih produktov je bila uporabljena LC-MS/MS analiza. Predlagane kemijske structure glavnih razgradnih produktov so 2-[(2-benzylbenzyl)(methyl)amino]ethanol, 2-[{2-[hydroxyl(phenyl)methyl]-benzyl}(methyl)-amino] etanol in difenilmetanon.