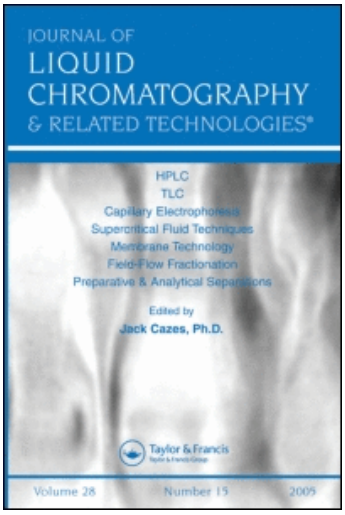


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of the Stabilities of New Quinazoline Derivatives by HPLC

K. Błaszczak-Świątkiewicz[a]; E. Mikiciuk-Olasik[a]
[a] Department of Pharmaceutical Chemistry and Drug Analysis, Medical University, Łódź, Poland

To cite this Article Błaszczak-Świątkiewicz, K. and Mikiciuk-Olasik, E.(2006) 'Determination of the Stabilities of New Quinazoline Derivatives by HPLC', Journal of Liquid Chromatography & Related Technologies, 29: 16, 2367 — 2385
To link to this Article: DOI: 10.1080/10826070600864817
URL: http://dx.doi.org/10.1080/10826070600864817

Journal of Liquid Chromatography & Related Technologies®, 29: 2367–2385, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600864817

Determination of the Stabilities of New Quinazoline Derivatives by HPLC

K. Błaszczak-Świątkiewicz and E. Mikiciuk-Olasik
Department of Pharmaceutical Chemistry and Drug Analysis,
Medical University, Łódź, Poland

Abstract: The quinazoline derivatives reported in this work belong to a new group of compounds with a type of chemical bonding that has potential anticancer features. A biochemical study determining anticancer activity of quinazoline derivatives and N^3-quinazoline oxide will be conducted in the medium of 0.2% DMSO. The HPLC method was used to determine stabilities of these compounds in a biochemical study environment. Optimisation of the chromatographic system and validation of the established analytical method was performed. Analysis of the stabilities of the derivatives examined was performed in the reversed phase RP-18 system, in which pure acetonitrile was used as an eluent for the analysis of compounds **1** and **3**, and a mixture of acetonitrile/methanol in the ratio of 95/5% for compounds **2** and **4**. The HPLC analysis revealed that the compounds studied are homogeneous in the 0.2% DMSO medium, up to 96 hours.

Keywords: HPLC, RP-18, Quinazoline, Quinazoline N^3-oxide

INTRODUCTION

Cancer cell hypoxia seems to be one of the new approaches in anticancer therapy, based on drugs affecting the bioreduction activity mechanism. The characteristic feature of these agents is their selective cytotoxicity towards the cells in hypoxia.[1–5] The prognostic compounds to be used in chemotherapy, particularly in the case of solid tumours, are those containing N-oxide groups, such as tirapazamine. Tirapazamine has become

Address correspondence to E. Mikiciuk-Olasik, Department of Pharmaceutical Chemistry and Drug Analysis, Medical University, ul. Muszyńskiego 1, Łódź 90-151, Poland. E-mail: eolasik@pharm.am.lodz.pl

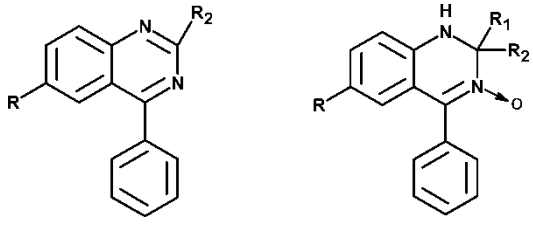
the leading structure in our search for anticancer compounds among quinazoline derivatives.[6] The present study is a continuation of our previous research.[1,7,8] Working towards the study's primary objective to receive potential anticancer compounds of high specificity of action in hypoxia, we have started our analytical experiments to establish stability of the obtained quinazoline derivatives in the medium used for the biochemical experiments (0.2% DMSO), and in a time adequate to perform the experiments. Determination of cytotoxicity of the studied connections in relation to selected cell lines, i.e., the cells of human myeloid leukemia (HL-60) and lymphoblastic leukemia (NALM-6) was performed.[1] A solution of DMSO in water is a common solvent for assay of cytotoxicity of new compounds. The structures of the compounds analysed are presented in Figure 1.

The experiments conducted by the use of high performance liquid chromatography method (HPLC) were concerned with optimisation of the chromatographic system for the compounds analysed, validation of the established analytical method, and stability examination of quinazoline derivatives in the environment of 0.2% DMSO with the validated HPLC method.

EXPERIMENTAL

Equipment and Reagents

The HPLC system consisted of a Waters 600 LC system. Chromatographic separation was achieved by analysis on a Supelco RP-18 column



	1	2	3	4
R	Cl	Cl	Cl	Cl
R1	—	—	H	H
R2	PIP	NAPH	PIP	NAPH

PIP - piperonyl, NAPH - 2-naftyl

Figure 1. Formula structural of quinazoline derivatives and N^3-oxide quinazoline.

(15 cm × 4 mm × 5 μm plus symmetry C18 quard, Waters) held at 20°C. Chromatographic peaks were identified with a UV detector (Waters). Compound **1** was monitored at a wavelength of 221 nm, compound **2**–240 nm, compound **3**–235 nm and compound **4**–254 nm, respectively. Compound samples were weighed on the analytical scales with the accuracy of 0.01 mg. They were dissolved in a mobile phase consisting of solvents of chromatographic purity (acetonitrile, acetonitrile/methanol in proper ratios) (J.T. Baker). The mobile phase consisting of acetonitryle and methanol was prepared by mixing acetonitryle and methanol (98 : 2% v/v or 95 : 5% v/v). All the samples were prepared in 5 mL, 10 mL, 25 mL volumetric flasks. Solution injections of 6 μL and 10 μL were performed with the use of an autosampler (Waters). Each sample was analysed three times. The final result was presented as an arithmetical mean. A mobile phase flow rate through the column was 1 mL/min. The time for chromatograph development was maximum 10 min, being specific for a particular compound. A computer programme Millenium 32 version 4.0 (Waters) was used for the processing of the chromatograms.

Optimisation of HPLC System

Sample Preparation

The proper solution concentrations (mg/mL) of the compound dissolved in the mobile phase: 100% acetonitrile or a mixture of acetronitrile/methanol in the ratio of 98/2%, and a mixture of acetronitrile/methanol in the ratio of 95/5%, were prepared. Samples were taken from those solutions and used for injections by an autosampler.

Results

Computer processing of the obtained chromatograms allowed us to determine separation parameters for compounds **1**–**4**. The results are shown in Tables 1–3.

Table 1. The obtained values of t_r, w, k, n for the system of 100% (v/v) acetonitirile

Concentration 10^{-1} (mg/mL)	t_{Rmean} (min)	w_{mean} (s)	k_{mean}	n_{mean}
com. **4**	4,156	67,00	1,352 e + 00	3585
com. **3**	3,029	17,00	7,144 e-01	5436
com. **1**	2,106	14,00	1,920 e-01	3115
com. **2**	2,027	139,00	1,477 e-01	3692

Table 2. The obtained values of t_r, w, k, n for the system of acetonitirile/methanol 98/2% (v/v)

Concentration 10^{-1} (mg/mL)	t_{Rmean} (min)	w_{mean} (s)	k_{mean}	n_{mean}
com. **2**	1,946	47,00	1,566 e−01	1854
com. **4**	4,017	66,00	1,387 e + 00	3665

Validation

Specificity

Sample Preparation

A solution of 10^{-1} mg/mL of compound **1** and **3**, dissolved in 100% acetonitrile, and a solution of 10^{-1} mg/mL of compound **2** and **4**, dissolved in the mixture of acetonitrile/methanol, in the ratio of 95/5% were prepared. A mobile phase used for the analysis of compound **1** and **3** contained 100% acetonitrile, and the mixture of acetonitrile/methanol in the ratio of 95/5%, for compound **2** and **4**, respectively. A control chromatogram without any compound studied was obtained as well.

Results

An analytical interpretation of chromatographic data was performed determining time retention (t_R), peak width (w), retention coefficient (k), number of theoretical plates (n) (Figures 2–6).

Precision

Sample Preparation

Solutions were prepared containing 25% to 100% (v/v) of the studied compound content.

Table 3. The obtained values of t_r, w, k, n for the system of acetonitirile/methanol 95/5% (v/v)

Concentration 10^{-1} (mg/mL)	t_{Rmean} (min)	w_{mean} (s)	k_{mean}	n_{mean}
com. **2**	1,879	12,00	6,002 e − 01	3404
com. **4**	4,130	25,00	2,519 e + 00	5485

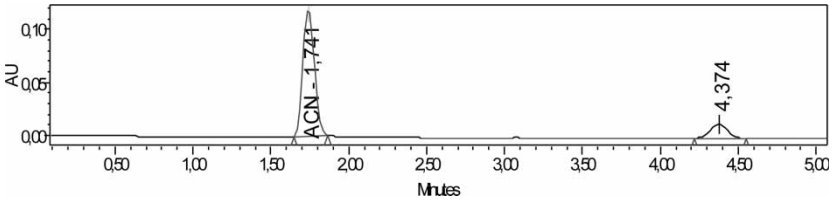


Figure 2. A control sample.

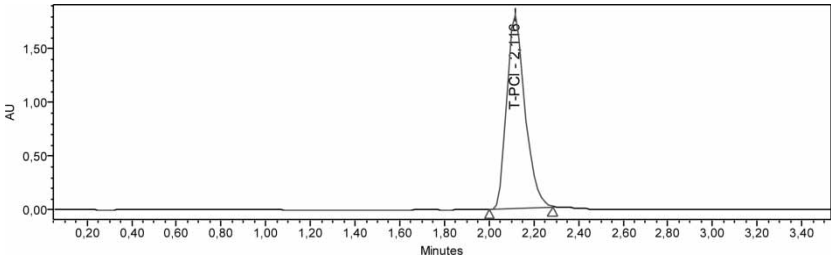


Figure 3. Compound **1**, eluent—acetonitrile 100%.

Results

To determine the compound content in the sample, a calibration curve was generated for a given substance within the concentration varied from 0.1 mg/mL to 0.1 μg/mL. The following parameters: $\bar{x}$, s, μ, RSD, were used to define accuracy of the method for a particular compound. The results are presented in Table 4.

Accuracy

Sample Preparation

Solutions were prepared containing 25% to 100% (v/v) of the studied compound content.

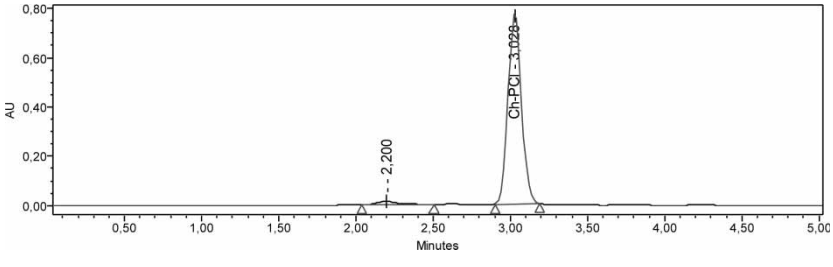


Figure 4. Compound **3**, eluent—acetonitrile 100%.

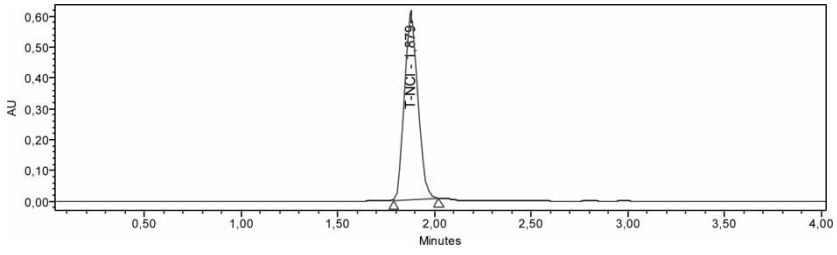


Figure 5. Compound **2**, eluent—acetonitrile/methanol 95/5%.

Results

A calibration curve was generated, for a given substance, within the concentration from 0.1 mg/mL to 0.1 μg/mL, in order to determine the compound content in the sample.

A recovery degree defined for a given compound content was expressed in mg/mL for a single compound. The parameters: $\bar{x}$, s, μ, RSD, were used to determine the accuracy of the method for a particular compound. The results are presented in Table 5.

Linearity

Sample Preparation

Solutions of a particular compound (**1**–**4**) in concentrations of: 0.1 mg/mL, 0.05 mg/mL, 0.01 mg/mL, 0.005 mg/mL, 0.001 mg/mL, 0.5 μg/mL, 0.1 μg/mL were prepared.

Results

A linear relationship between the particular sample concentration and a peak area was plotted. A coefficient of a straight line displacement value (a) and an inclination parameter value (b), expressed by the equation of $y = bx + a$, were

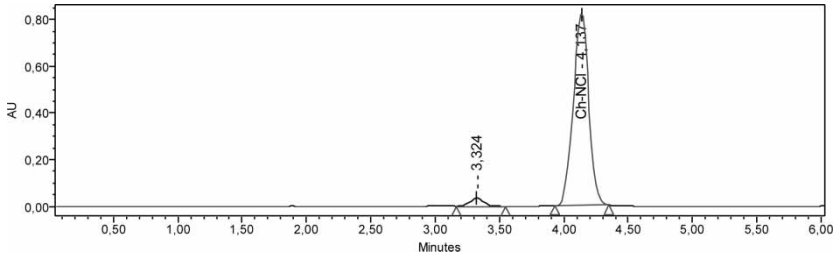


Figure 6. Compound **4**, eluent—acetonitrile/methanol 95/5%.

Table 4. Precision of the analytical method

Sample	25,00	37,50	50,00	60,00	75,00	100,00
Concentration % (v/v) of com. **2**						
1	0,043	0,066	0,089	0,111	0,145	0,186
2	0,043	0,066	0,089	0,112	0,145	0,186
3	0,043	0,067	0,089	0,112	0,145	0,187
$\bar{x}$	0,043	0,066	0,089	0,112	0,145	0,186
S	0,0007	0,0007	0,00	0,0007	0,0007	0,0007
μ	0,043 $\pm$ 0,0014	0,066 $\pm$ 0,0014	0,089 $\pm$ 0,00	0,112 $\pm$ 0,0014	0,145 $\pm$ 0,0014	0,186 $\pm$ 0,0014
RSD(%)	1,63	1,07	0	0,625	0,483	0,376
	25,00	37,75	50,00	60,20	75,00	100,00
Concentration % (v/v) of com. **4**						
1	0,048	0,073	0,096	0,118	0,147	0,195
2	0,048	0,073	0,097	0,117	0,146	0,197
3	0,048	0,073	0,097	0,118	0,145	0,195
$\bar{x}$	0,048	0,073	0,097	0,118	0,146	0,196
S	0,00	0,00	0,0007	0,0007	0,001	0,001
μ	0,048 $\pm$ 0,00	0,073 $\pm$ 0,00	0,097 $\pm$ 0,0014	0,118 $\pm$ 0,0014	0,146 $\pm$ 0,002	0,196 $\pm$ 0,002
RSD (%)	0	0	0,72	0,59	0,68	0,51

(*continued*)

Table 4. Continued

Sample	25,27	37,63	50,00	60,21	75,27	100,00
Concentration % (v/v) of com. **3**						
1	0,048	0,071	0,096	0,115	0,144	0,185
2	0,048	0,072	0,096	0,115	0,144	0,187
3	0,048	0,071	0,095	0,115	0,143	0,187
$\bar{x}$	0,048	0,071	0,096	0,0115	0,144	0,186
S	0,00	0,0007	0,0007	0,00	0,0007	0,001
μ	0,048 $\pm$ 0,00	0,071 $\pm$ 0,0014	0,096 $\pm$ 0,0014	0,115 $\pm$ 0,00	0,144 $\pm$ 0,0014	0,186 $\pm$ 0,002
RSD (%)	0	0,99	0,73	0	0,49	0,54
	25,00	37,25	49,50	59,80	74,51	100,00
Concentration % (v/v) of com. **1**						
1	0,050	0,077	0,104	0,129	0,156	0,203
2	0,050	0,078	0,104	0,128	0,157	0,205
3	0,050	0,077	0,105	0,129	0,157	0,203
$\bar{x}$	0,050	0,077	0,104	0,129	0,157	0,204
S	0,00	0,0007	0,0007	0,0007	0,0007	0,001
μ	0,050 $\pm$ 0,00	0,077 $\pm$ 0,0014	0,104 $\pm$ 0,0014	0,129 $\pm$ 0,0014	0,157 $\pm$ 0,0014	0,204 $\pm$ 0,002
RSD (%)	0	0,91	0,67	0,5	0,44	0,49

Table 5. Accuracy of the analytical method

	Real value	Value determined	Real value	Value determined	Real value	Value determined	Real value	Value determined	Real value	Value determined	Real value	Value determined
Sample	25,00%		37,50 %		50,00 %		60,00 %		75,00 %		100,00 %	
Contents of the determined substance 2 in relation to the declared (mg/mL)												
1	0,052	0,052	0,078	0,079	0,104	0,105	0,125	0,129	0,157	0,157	0,209	0,209
2	0,060	0,060	0,091	0,091	0,121	0,121	0,145	0,145	0,181	0,183	0,242	0,242
3	0,043	0,045	0,065	0,065	0,086	0,087	0,104	0,104	0,130	0,130	0,173	0,173
	Mean recover (%) = 100,62, s = 0,50%, μ = 100,62% $\pm$ 1,00%, RSD = 0,50%											
	25,00 %		37,75 %		50,00 %		60,20 %		75,00 %		100,00 %	
Contents of the determined substance 4 in relation to the declared (mg/mL)												
1	0,048	0,048	0,073	0,073	0,098	0,097	0,118	0,118	0,147	0,146	0,196	0,196
2	0,049	0,049	0,074	0,074	0,099	0,098	0,118	0,118	0,149	0,155	0,198	0,198
3	0,043	0,043	0,064	0,064	0,086	0,087	0,103	0,103	0,129	0,130	0,172	0,172
	Mean recover (%) = 100,26, s = 0,50%, μ = 100,26% $\pm$ 1,00%, RSD = 0,50%											
	25,00%		37,25 %		49,50 %		59,80 %		74,51 %		100,00 %	
Contents of the determined substance 1 in relation to the declared (mg/mL)												
1	0,053	0,053	0,082	0,081	0,110	0,109	0,129	0,129	0,164	0,129	0,214	0,214
2	0,022	0,022	0,030	0,030	0,010	0,041	0,052	0,052	0,068	0,052	0,086	0,086
3	0,082	0,018	0,028	0,029	0,037	0,037	0,044	0,045	0,059	0,045	0,072	0,072

(*continued*)

Table 5. Continued

	Real value	Value determined	Real value	Value determined	Real value	Value determined	Real value	Value determined	Real value	Value determined	Real value	Value determined
Sample	25,00%		37,50 %		50,00 %		60,00 %		75,00 %		100,00 %	
	Mean recover (%) = 100,34, s = 0,35%, μ = 100,34% $\pm$ 0,70%, RSD = 0,35%											
	25,27 %		37,63 %		50,00 %		60,21 %		75,27 %		100,00 %	
Contents of the determined substance 3 in relation to the declared (mg/mL)												
1	0,047	0,047	0,070	0,070	0,093	0,094	0,112	0,112	0,140	0,140	0,187	0,187
2	0,050	0,050	0,075	0,075	0,101	0,101	0,122	0,121	0,152	0,151	0,200	0,200
3	0,047	0,047	0,070	0,070	0,093	0,094	0,112	0,112	0,140	0,141	0,187	0,187
	Mean recover (%) = 100,08, s = 0,30%, μ = 100,089% $\pm$ 0,60%, RSD = 0,30%											

calculated for the analysed substance. Correlation coefficients were also determined.

Limit of Detection (LOD)

Sample Preparation

A concentration of 0.1 μg/mL of a particular compound in a proper solvent was prepared.

Results

The LOD parameter was determined as an arithmetical mean from the three individual measurements, each one having been an arithmetical mean from the three subsequent injections. The limits of detection, at a signal-to-noise ratio of 3, were: 0.04 μg/mL, 0.10 μg/mL, 0.05 μg/mL, and 0.08 μg/mL, for compounds **1**, **2, 3**, and **4**, respectively.

Limit of Quantification (LOQ)

Sample Preparation

0.1 μg/mL concentration of a given compound in a proper solvent was prepared.

Results

The LOQ parameter was determined as an arithmetical mean from the three individual measurements, each one having been an arithmetical mean from the three subsequent injections. The limits of quantification at a signal-to-noise ratio of 10, were: −0.10 μg/mL, −0.50 μg/mL, −0.18 μg/mL, −0.30 μg/for compound **1**, **2**, **3**, **4**, respectively.

Investigation of the Stability of the Quinazoline Derivative in 0.2% DMSO

Sample Preparation

A sample of the analysed compound was weighed on the analytical scale with an accuracy of 0.01 mg. To a 10 mL volumetric flask with a weighted substance 1 mL of prepared 0.2% DMSO was added. After 96 h the flask was filled up to 10 mL with a solution of the proper solvent or a mixture of solvents.

Results

Homogeneity of compounds **1**–**4** was confirmed by the analytical interpretation of the obtained chromatograms. Retention times and UV spectra for additional peaks appearing on the chromatograms were compared to retention times and UV spectra characteristic for the analysed derivatives. A recovery degree of the analysed compound was calculated (Table 6, Figures 11–14).

RESULTS AND DISCUSSION

Optimisation of the Chromatographic System for the Analysed Quinazoline Derivatives

Compounds assigned for the chromatographic analysis are derivatives of quinazoline (**1**–**2**) and N^3-quinazoline oxide (**3**–**4**). The study was performed on

Table 6. Sample recovery after 96 h of staying in the environment of 0.2% DMSO

Compound	Content (mg/mL)	Value determined (mg/mL)	$\bar{x}$, Recovery
com. **1**	0,145	0,145	$\bar{x} = 0{,}145$
		0,144	100%
		0,146	
	0,133	0,133	$\bar{x} = 0{,}133$
		0,133	100%
		0,133	
com. **3**	0,113	0,112	$\bar{x} = 0{,}113$
		0,113	100%
		0,113	
	0,106	0,106	$\bar{x} = 0{,}106$
		0,106	100%
		0,107	
com. **2**	0,119	0,119	$\bar{x} = 0{,}119$
		0,119	100%
		0,119	
	0,139	0,137	$\bar{x} = 0{,}139$
		0,140	100%
		0,139	
com. **4**	0,107	0,107	$\bar{x} = 0{,}107$
		0,107	100%
		0,107	
	0,127	0,126	$\bar{x} = 0{,}127$
		0,127	100%
		0,127	

columns with a reversed phase (RP-18) due to the necessity of analysing two compound groups with the different polar properties in the same chromatographic system, in which decomposition of N^3-quinazoline oxide derivatives into their analogues of the quinazoline structure could be confirmed or excluded.

Chromatograms in pure acetonitrile initiated optimisation of the system for chromatographic separation of the described quinazoline derivatives (Table 1).

Results of the Analysis in Pure Acetonitrile

The eluent used in the experiments obtained satisfactory separation parameters for 6-chloro-4-phenyl-2-piperonyloquinazoline **(3)** and N^3-oxide 6-chloro-4-phenyl-2-piperonyl-l,2-dihydroquinazoline **(1)** for all prepared concentrations (Table 1). Signal characteristics for the analysed samples are sharp. The number of theoretical plates for compound **3** equals $n = 5000$, and for compound **1** $n = 3000$. Retention time is specific for a given compound. The system can be utilised in further analytical experiments with these substances. Separation of the two remaining compounds, i.e., 6-chloro-4-phenyl-2-(2-naphthyl)quinazoline **(4)** and N^3-oxide 6-chloro-4-phenyl-2-(2-naphthyl)-l,2-dihydro-quinazoline **(2)** was unsuccessful. Low values of the retention coefficient k and the very low values of the number of theoretical plates—n, as well as the broadened peaks appearing on the chromatograms, rendered this system useless for optimisation of the studied compound of N^3-oxide 6-chloro-4-phenyl-2-(2-naphthyl)-l, 2-dihydroquinazoline **(4)** (Table 1).

Results of the Analysis in the System Acetonitrile/ Methanol −98/2% (v/v) and Acetonitrile/ Methanol −95/5% (v/v) for Compounds 2 and 4

The obtained chromatograms stated that, due to the unsatisfactory results of developing N^3-oxide 6-chloro-4-phenyl-2-(2-naphthyl)-1-dihydroquinazoline (**2**) with acetonitrile, the composition of the mobile phase should be modified by increasing the elution strength. Because of the stationary non-polar phase and the fact that aqueous environment does not suit the studied compounds, particularly derivatives of N^3-oxide 1,2-dihydroquinazoline, methanol has become a modifier of choice. Further analyses with the use of the modifier included, also, an analogue of the compound **2**, namely 6-chloro-4-phenyl-2-(2-naphthyl)quinazoline (**4**) to determine retention times of both analysed compounds in the same separation conditions.

The system acetonitrile/methanol in the ratio of 98/2% (v/v) decisively effected improvement of the parameters essential for the optimal chromatographic system of the compound **2** and **4**, i.e., the number of theoretical plates, n, but the addition of the modifier shortened the retention time, that

resulted in a reduction of the value of the retention coefficient k (Table 2); furthermore, peaks appearing on chromatograms were still broadened. Therefore, in order to further modify the composition of a mobile phase, the analysis of these compounds with the use of a mixture of acetonitrile/methanol 95/5% (v/v) was performed. An increase in percentage participation of the modifier in the eluent shortened retention time to a still greater degree, and decreased the value of the retention coefficient k, together with a distinct improvement of the theoretical plates n in the analysis of compound **2**. However, in the case of compound **4**, addition of the modifier to the eluent caused a decrease in elution process, prolongation of retention time (t_R), and a rise in the value of retention coefficient k, with a simultaneous decrease in the number of theoretical plates n. The achieved separation parameters of both compounds (**2** and **4**), using a mixture of acetonitrile/methanol 95/5% (v/v) as a mobile phase, were regarded as satisfactory and provided the basis for their identification in a mixture (Table 3).

The HPLC analysis of the selected compounds in various systems, with a mobile phase composition as a variable, led to the following conclusions:

- compounds **1** and **3** should be analysed in a reversed phase system with 100% (v/v) acetonitrile as an eluent;
- compounds **2** and **4** should be analysed in a reversed phase system with participation of mobile phase in the form of a mixture of acetonitrile/methanol in the ratio of 95/5% (v/v),
- in the selected systems, retention times are specific for the analysed substances, and low values of the retention coefficient k′ for compounds **1** and **2** result from their polar characteristics.

Validation of the Established HPLC Method for the Purpose of the Analysis of the Compound Stability in the DMSO Medium

Establishment of the exact analytical method and its optimisation with regard to satisfactory parameter values, essential for the undertaken HPLC method, has provided the basis for performing the characteristics of this method in order to demonstrate its scientific suitability in the investigations of the stability of the obtained quinazoline derivatives with the anticancer activity.

Validation of the established HPLC method was conducted through determining individual process parameters: specificity, precision, linearity, accuracy, detectability, and determinability.

The conducted characteristic of the method has proven that it is:

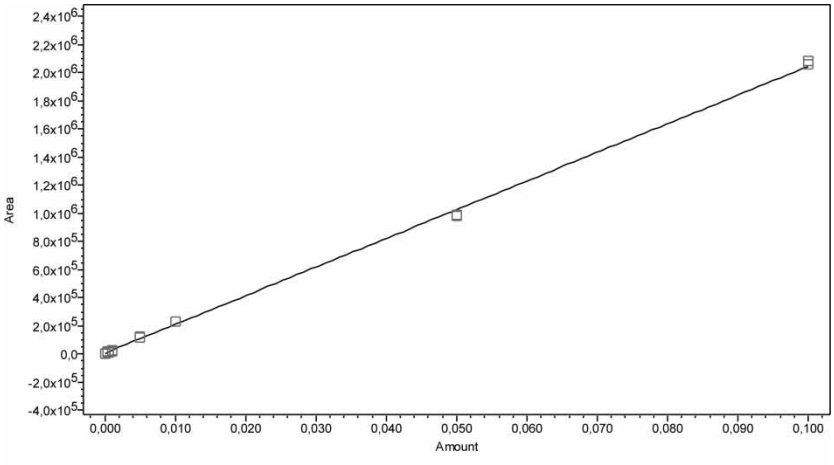
- specific, as additional peaks in the sample chromatogram are well separated from the main peak and from one another; also, a control sample chromatogram does not depict peaks within the studied ranges (Figures 2–5),

- precise – s $\leq$0.001 and RSD $\leq$2% (Table 4);
- accurate, as mean percentage recovery is close to 100%, and the results are within 95 – 105% (Table 5);
- linear, due to the fact that the measured values lie on the regression curve, $y = a + bx$, and the correlation coefficient, r, approaches a value of 1 (Figures 6 – 10).

A level of (LOD) detectability and (LOQ) determinability for the particular compound defined with the worked out analytical method has also been determined.

Stability of Quinazoline Derivatives in the 0.2% DMSO Medium

The established analytical method allows performing the study on stability of quinazoline derivatives in the environment of 0.2% DMSO with satisfactory precision, accuracy, and specificity. Due to the time in which the biochemical investigation of the compounds would to be conducted, the analysis of their stabilities in a given environment had been performed



$a = 2{,}04\ e{+}007$

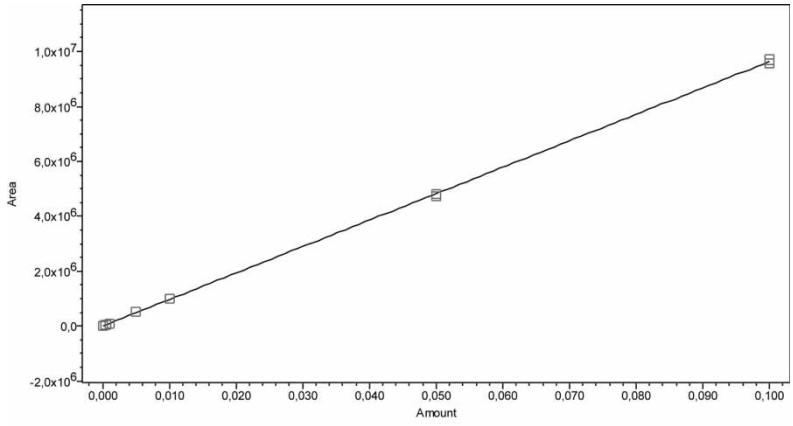
$b = 6{,}56\ e{+}003$

$a \pm t_{\alpha f} \bullet s_a \qquad a = 2{,}04\ e{+}007 \pm 0$

$b \pm t_{\alpha f} \bullet s_b \qquad b = 6{,}56\ e{+}003 \pm 0$

$r = 0{,}9997$

Figure 7. Regression curve $y = a + bx$ for compound **2**.



$a = 9{,}63\ e{+}007$

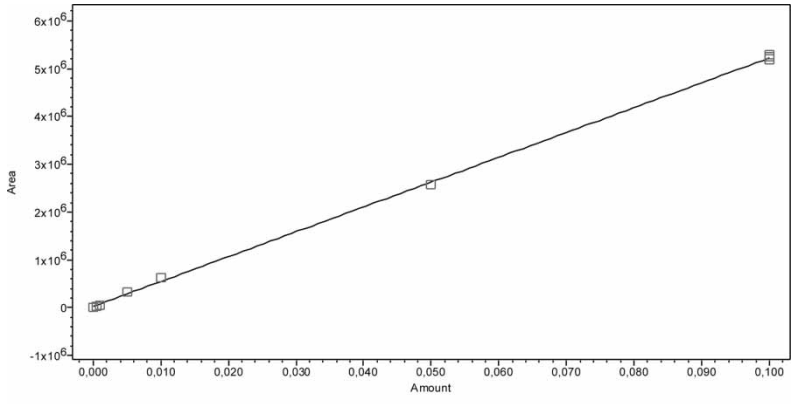
$b = 8{,}59\ e\ {+}003$

$a \pm t_{\alpha f} \bullet s_a \qquad a = 9{,}63\ e{+}007 \pm 0$

$b \pm t_{\alpha f} \bullet s_b \qquad b = 8{,}59\ e\ {+}003 \pm 0$

$r = 0{,}9999$

Figure 8. Regression curve $y = a + bx$ for compound **1**.



$a = 6{,}80\ e{+}007$

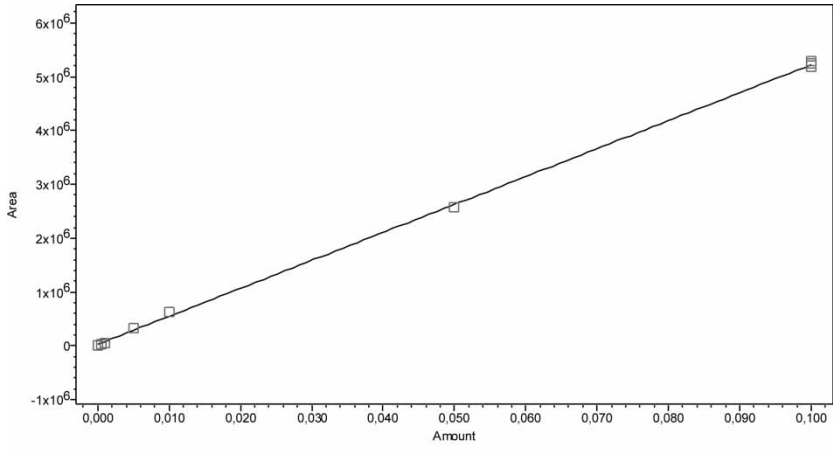
$b = 3{,}37\ e\ {+}004$

$a \pm t_{\alpha f} \bullet s_a \qquad a = 6{,}80\ e{+}007 \pm 0$

$b \pm t_{\alpha f} \bullet s_b \qquad b = 3{,}37\ e\ {+}004 \pm 0$

$r = 0{,}9995$

Figure 9. Regression curve $y = a + bx$ for compound **4**.



$a = 5{,}15\ e{+}007$

$b = 3{,}09\ e{+}004$

$a \pm t_{\alpha f} \bullet s_a \qquad a = 5{,}15\ e{+}007 \pm 0$

$b \pm t_{\alpha f} \bullet s_b \qquad b = 33{,}09\ e{+}004 \pm 0$

$r = 0{,}9997$

Figure 10. Regression curve $y = a + bx$ for compound **3**.

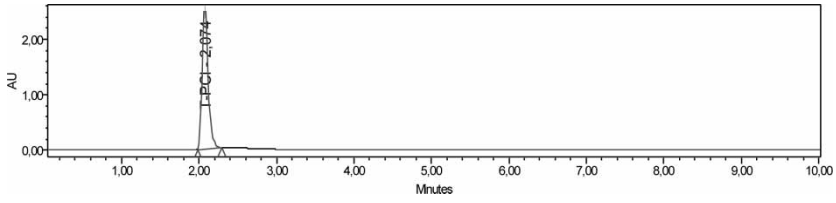


Figure 11. Stability of compound **1** in 0.2% DMSO.

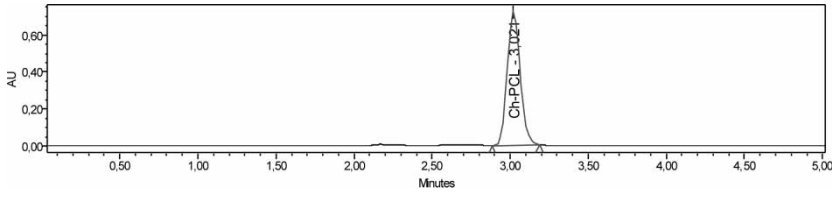


Figure 12. Stability of compound **3** in 0.2% DMSO.

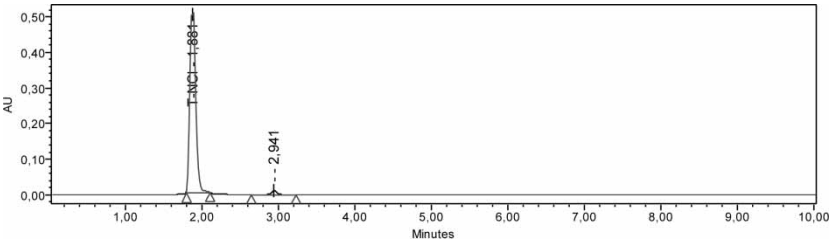


Figure 13. Stability of compound **2** in 0.2% DMSO.

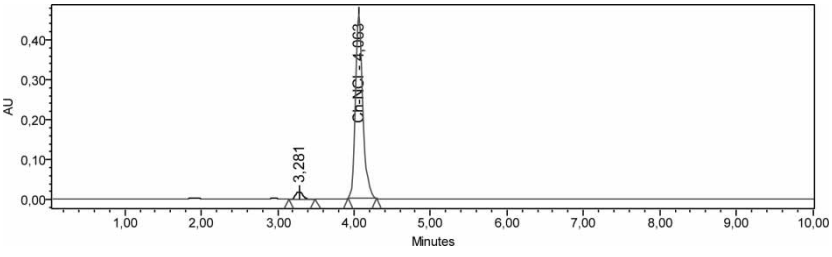


Figure 14. Stability of compound **4** in 0.2% DMSO.

after 96 h, since the moment when the sample was dissolved in 0.2% DMSO.

We expected that the chromatographic analysis would reveal sample recovery in relation to the baseline content and possible decomposition of the compound through the appearance of additional significant peaks on the chromatogram. We were particularly interested in chromatograms of N^3-oxide quinazoline derivatives (compounds **3**–**4**) because of the possibility of their decomposition into their analogues of the quinazoline structures in this environment.

The chromatographic analysis with the use of the validated HPLC method of N^3-oxide quinazoline derivatives (compounds **3**–**4**) and their structural analogues – quinazoline derivatives (compounds **1**–**2**), has proven that these compounds in 0.2% DMSO and in the time up to 96 h do not undergo decomposition. The sample recovery was 100% (v/v) for each compound. Comparison of the derivative UV spectra, the retention time characteristic for the compounds analysed with UV spectra, and the retention time of additional peaks appearing on chromatograms, led to the conclusion that additional peaks present on chromatograms do not originate from the compound analysed and are analytically unimportant. The most evident fact is that chromatograms of N^3-oxide quinazoline derivatives have not revealed the presence of an appropriate quinazoline derivative in the environment studied (Table 6, Figures 11–14).

ACKNOWLEDGMENT

The HPLC analytical research was supported by the Medical University of Łódź, Poland (Grant No. 507 13 028).

REFERENCES

1. Braun, J.M. SR 4233 (Tirapazamina): a new anticancer drug exploiting hypoxia solid tumors. Br. J. Cancer **1993**, *67*, 1163–1170.
2. Monge, A.; Palop, J.A.; Lopez De Garin, X.; Senador, V.; Sainz, J.; Narro, S.; Hamilton, E.; Clarke, E.D.; Barker, A.J.; Greenhaw, D.T. Hipxia-selective agents derived from quinoxaline 1,4-Di-N-oxides. J. Med. Chem. **1995**, *38*, 1786–1792.
3. Nunn, A.; Linder, K.; Strauss, H.W. Nitroimidazoles and imaging hypoxia. Eur. J. Nuci. Med. **1995**, *22*, 265.
4. Cerecotto, H.; Gonzales, M. N-oxides as hypoxia selective cytotoxin. Mini Rev. Med. Chem. **2001**, *1* (3), 219–231.
5. Papadopoulou, M.V.; Ji, M.; Rao, M.K.; Bloomer, W.D. Reductive metabolism of the nitroimidazole-based hypoxia-selective cytotoxin NLCQ-1 (NSC 709257). Oncol. Res. **2003**, *14* (1), 21–29.
6. Mikiciuk-Olasik, E.; Błaszczak-Świątkiewicz, K.; Żurek, E.; Krajewska, U.; Różalski, M.; Kruszyński, R.; Bartczak, T.J. New derivatives of quinazoline and 1,2-dihydroquinazoline N^3–oxide with expected antitumour activity. Arch. Pharm. Pharm. Med. Chem. **2004**, *337*, 239.
7. Mikiciuk-Olasik, E.; Żurek, E.; Mikołajczak, R.; Zakrzewska, E.; Błaszczak-Świątkiewicz, K. New derivatives of 2-(N-arylmethylideneamine)benzophenone oxime as potential radiopharmaceuticals for brain imaging. Nuclear Med. Rev. **2000**, *3* (2), 149.
8. Błaszczak-Świątkiewicz, K.; Mikiciuk-Olasik, E.; Chomka, A. Planar chromatography for new quinazoline derivatives. J. Liq. Chromatogr. & Rel. Technol. **2004**, *27* (19), 3121.

Received December 6, 2005
Accepted April 24, 2006
Manuscript 6775