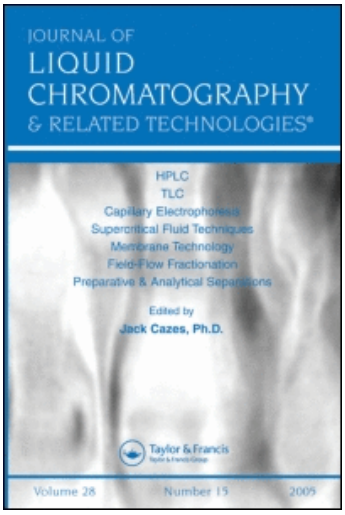

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Planar Chromatography for New Quinazoline Derivatives

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ABSTRACT

The quinazoline derivatives (**1**–**6**) are potential antitumour preparations. The selectivity of the TLC parameters of six new quinazoline derivatives (**1**–**6**) has been investigated on silica gel 60 F 254. Optimisation of the retention and efficiency of these compounds was performed by changing the mixture of eluents and the concentration of modifier in organic diluent (v/v). Chromatograms were developed in the DS chamber and the substances were detected using UV light (λ 254 nm). The best separation parameters for compounds **1**–**3** were obtained with chloroform : ethyl

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acetate [5% (v/v)] as a mobile phase and for compounds **4**–**6** with ethyl acetate : acetonitrile [65% (v/v)] as a mobile phase.

Key Words: TLC; Quinazoline; 1,2-Dihydroquinazoline N^3-oxides; Silica.

INTRODUCTION

Neoplastic diseases are among the most puzzling problems, which 21-century medicine must cope with. Malignant neoplasms, in spite of a very dynamic development of science and better knowledge of oncogenesis mechanisms, are still the second most frequent cause of death after cardiovascular diseases.[1]

A frequent factor, which limits the possibility of obtaining beneficial results of chemotherapy, is the presence of cells—within a neoplasm—in a state of hypoxia, which are less sensitive to conventional cytotoxic drugs and radiotherapy.[2]

Nowadays, a belief dominates that from a medical viewpoint hypoxia can be advantageous because it permits application of new diagnostic methods,[3] and also allows looking for substances of more selective activity. A difference in the cell oxidation level is a target in treatment. The huge achievement of medicine in this field is tirapazamine (TPZ)-a leading compound from the group of bioreductive activation.[4]

Taking this information into account and knowing that quinazoline derivatives exhibit antineoplastic activity[5] we planned a synthesis of new quinazoline derivatives of potential antineoplastic properties and their analogues, quinazoline N^3-oxides, potential therapeutics of bioreductive effects. Biochemical experiments that we performed allowed us to confirm the expected activity under normoxia and hypoxia conditions of obtained quinazoline derivatives. We demonstrated that quinazoline N^3-oxide derivatives exerted the higher cytotoxic action under hypoxia,[6] but further studies are needed to evaluate the mechanism of their activity.

Syntheses of compounds having biological effects, are tightly bound up with analytical methods, which serve for identification, isolation, and quantitative interpretation of performed reaction efficiency.

The aim of this analytical study was to optimise the chromatographic system (TLC) of obtained quinazoline derivatives, and to process the chromatographic separation method for two groups of chemical compounds. Results we achieved were used for the examination of the synthesis process of quinazoline N^3-oxide derivatives, in order to define the exact moment of their isolation from the reaction medium.[6]

EXPERIMENTAL

Equipment and Reagents

Measurements were carried out on ready-made TLC plates (silica gel 60 F 254) produced by Merck, 10 cm long and 0.2 mm thick, in the TLC chamber. Substances dissolved in an adequate mobile phase [5–70% (v/v)] (10 μL) were transferred to the plate in the form of drops by means of a Hamilton microsyringe (20 μL). The plate was developed to a distance of 10 cm in the DS chamber (Chromodes Lublin, Poland), previously saturated with vapourated mobile phase. Chloroform, acetonitrile, ethyl acetate, acetone, toluene, methanol, and analytical grade were purchased from P.O.Ch. Gliwice, Poland. The mixtures of: chloroform : acetonitrile, chloroform : ethyl acetate, chloroform : acetone, acetonitrile toluene : acetone, ethyl acetate : methanol, ethyl acetate : acetonitrile, acetone : ethyl acetate were applied as mobile phases. They were prepared separately for each development. After development plates were dried in air, spots were localised using UV light ($\lambda = 254$ nm). Migration distances were estimated with an accuracy of 1 mm for three independent chromatograms. R_f values of examined compounds were calculated for different mobile phases are shown graphically in diagrams.

RESULTS AND DISCUSSION

A basic aspect of optimisation in thin-layer chromatography is the regulation of retention (R_f) by the selection of elution force of the mixture of solvents, as described by the following formula:

$$\varepsilon^{o}_{AB} = \varepsilon^{o}_{A} + \log N_B 10^{a/n_b(\varepsilon A - \varepsilon B)} + 1 - N_B) a n_b$$

where N_B—mole fraction of B component in the mixture; a—adsorbent activity parameter; n_b—area of adsorbent taken by B molecule; A—weak pure solvent; B—strong pure solvent.

After obtaining R_f values for quinazoline derivatives (compounds **1**–**3**, Fig. 1) and quinazoline N^3-oxide derivatives (compounds **4**–**6**, Fig. 1) in pure solvents, several chromatograms were performed with an increasing modifier amount in the mixture for phases.

Chloroform : Acetonitrile (Fig. 2)

This phase is characterised by a significant selectivity in the range of 5–15% (v/v) of acetonitrile content. In the majority, the curves run parallel to

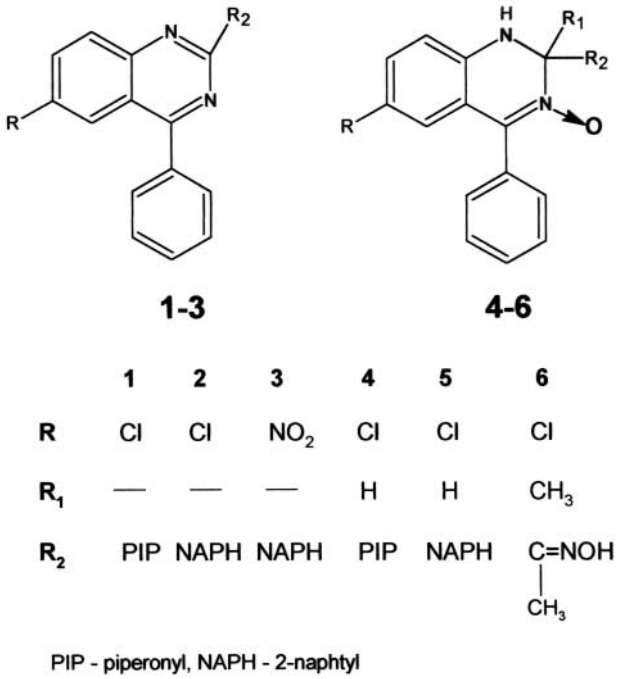

Figure 1. Chemical structures.

each other, and at a concentration of 70% (v/v) they cross, which indicates that with increasing of modifier content the system selectivity decreases. The data suggest that compound **1** is more polar than two other compounds and that acetonitrile is too polar as a modifier for examined quinazoline derivatives because with the increase of acetonitrile percentage content, R_m values decrease.

Chloroform : Ethyl Acetate (Fig. 3)

In this system, there is a very good possibility of separation of compound number **2** from compounds **1** and **3** in the whole range of concentrations, whereas all three derivatives can be totally separated only using ethyl acetate at a concentration of 5% (v/v). The curves in the whole range of concentrations run parallel, thus, with increase of modifier concentration no significant changes in the system selectivity can be observed. This is similar

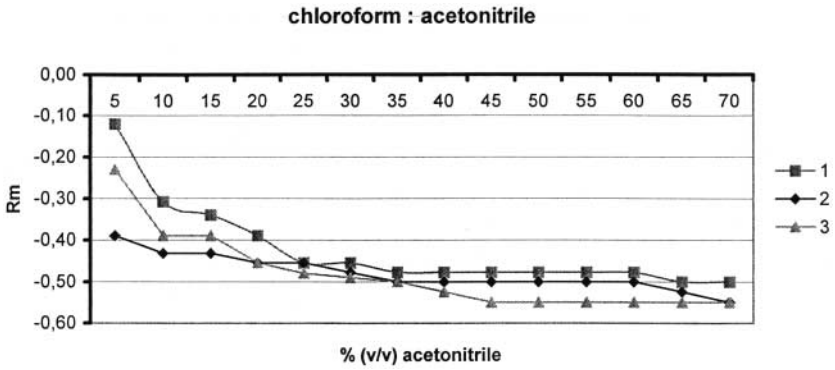

Figure 2. Dependence of R_m vs. % (v/v) acetonitrile in chloroform.

to the case of chloroform : acetonitrile, in which we demonstrated that the increase of ethyl acetate percentage content caused elution of the compounds from the adsorbent surface (R_m values decrease).

Chloroform : Acetone (Fig. 4)

This phase, in comparison with mixture of chloroform : ethyl acetate and chloroform : acetonitrile, is characterised by a significantly reduced selectivity. Only at acetone concentration of 10% (v/v), a possibility of separation of all three compounds exists. Besides, in the range of 5–10% (v/v)

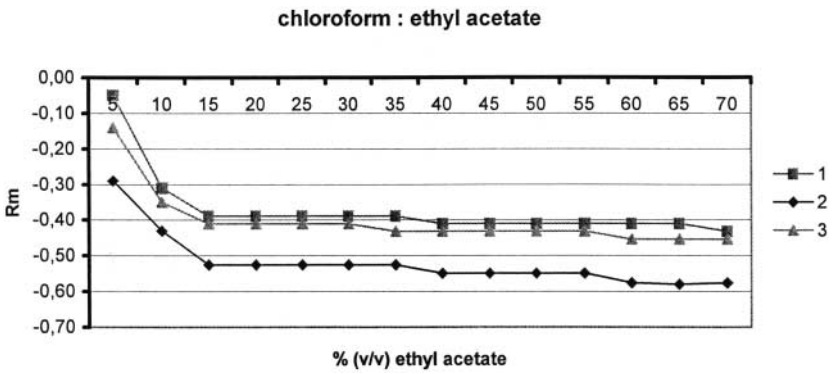

Figure 3. Dependence of R_m vs. % (v/v) ethyl acetate in chloroform.

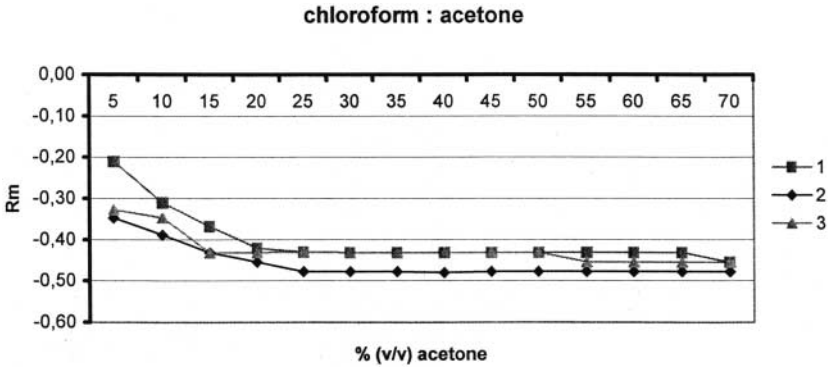

Figure 4. Dependence of R_m vs. % (v/v) acetone in chloroform.

compound **1** and in the range of 25–50% (v/v) compound **2** can be separated from two other compounds. The curves in almost the whole range of concentrations coincide or cross, which signifies that with the increase of modifier percentage content (acetone), the system selectivity decreases significantly. As in the above-mentioned systems the modifier was too polar.

Toluene : Acetone (Fig. 5)

This phase is characterised by the lowest selectivity compared with all systems we presented. It is not feasible to calculate the concentration at

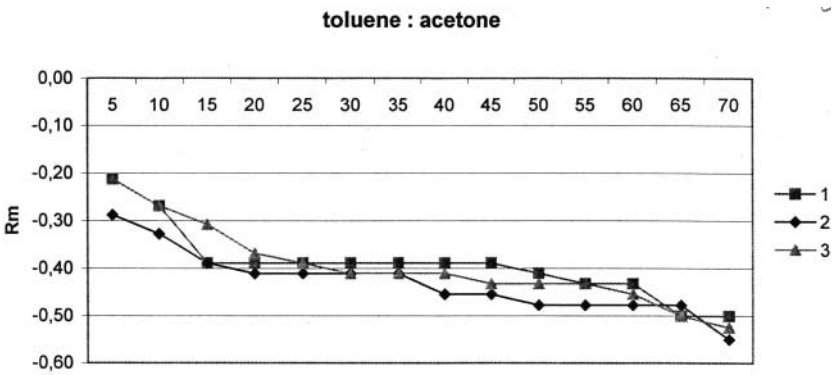

Figure 5. Dependence of R_m vs. % (v/v) acetone in toluene.

which all compounds are separated. As in other phases, the modifier was more polar than quinazoline derivatives. In order to select the optimal phase, correlation diagrams were made (Figs. 6 and 7), which presented clearly in which systems each quinazoline derivatives were better separated (compounds **1**–**3**). To prepare correlations, phases were selected in which we used chloroform as a solvent. To select concentrations, R_m values were taken into consideration, for which the best separation of all compounds was achieved.

Correlation of R_m Values for Acetonitrile and Ethyl Acetate Concentration of 5% (v/v) in Chloroform (Fig. 6)

Figure 6 demonstrates that generally better parameters of separation (R_m values close to zero) of all compounds are obtained, using ethyl acetate as a modifier [at a concentration of 5% (v/v)]. Additionally, ethyl acetate in comparison with acetonitrile exerted higher elution force and, thus, lower R_m values and higher efficiency, measured by means of the number of theoretical plates N (Table 1). It can be assumed that chloroform : ethyl acetate at a modifier concentration of 5% was a better system when compared with chloroform : acetonitrile.

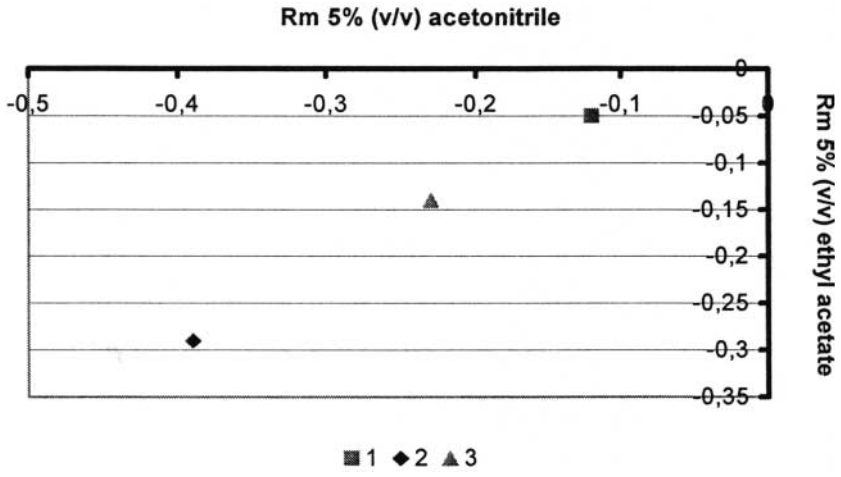

Figure 6. Correlation of R_m values for ethyl acetate and acetonitrile of concentration 5% (v/v) in chloroform.

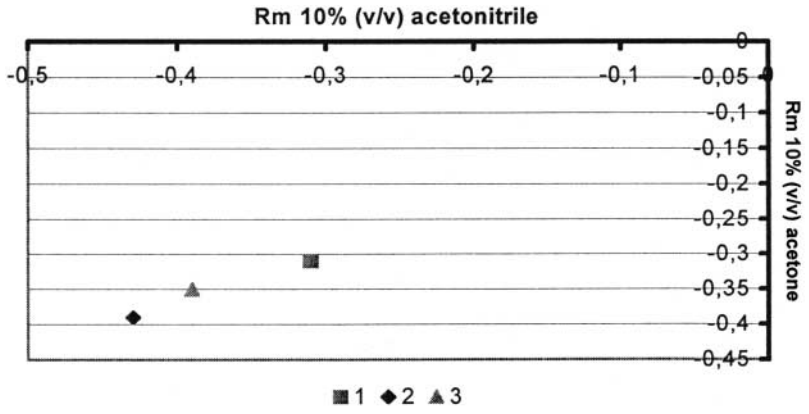

Figure 7. Correlation of R_m values at acetone and acetonitrile concentration of 10% (v/v) in chloroform.

Correlation of R_m Values at Acetonitrile and Acetone Concentration of 10% (v/v) in Chloroform (Fig. 7)

According to data presented in Fig. 7, better conditions of separation of compounds **2** and **3** are achieved using acetone as a modifier [at a concentration of 10% (v/v)]. However, in the case of compound **1**, the conditions of separation using acetone or acetonitrile are identical. Comparing R_m values we demonstrated that acetone had a higher elution force. It can be assumed that chloroform : acetone at modifier content of 10% (v/v) is an optimal phase for separation of obtained quinazoline derivatives. This system is characterised by both high selectivity and efficiency.

Table 1. Obtained *N* values for systems.

Compounds	Chloroform : 5% (v/v) acetonitrile, *N*	Chloroform : 5% (v/v) ethyl acetate, *N*
1	2,472	4,516
2	2,916	3,532
3	2,885	3,927

In a similar way, the optimisation of separation of quinazoline N^3-oxides were carried out (Fig. 1, compounds, **4**–**6**), preparing chromatograms in the following mixtures.

Ethyl Acetate : Methanol (Fig. 8)

This phase is characterised by very good selectivity, because the curves run parallel to each other with a large distance between them in the whole range of concentrations. R_m coefficient takes on a value closest to zero in the range of 5–10% (v/v) for compounds **4** and **5** and in the range of 15–20% (v/v) for compound **6**. The diagram presents the most polar compound among N^3-oxides is compound **6**, and the least—compound **4**.

Ethyl Acetate : Acetonitrile (Fig. 9)

This phase is characterised by a very good separation between compound **6** and other quinazoline N^3-oxides in the whole range of concentrations. On the other hand, separation between compound **4** and **5** takes place in the mixture of solvents in the range of 40–70% (v/v) of acetonitrile concentration. Similarly, as in the previous system with the increase of modifier content (acetonitrile), R_m values decrease. The optimal range of modifier concentration at which separation between all three compounds takes place is within the range of 65–70% (v/v) of acetonitrile.

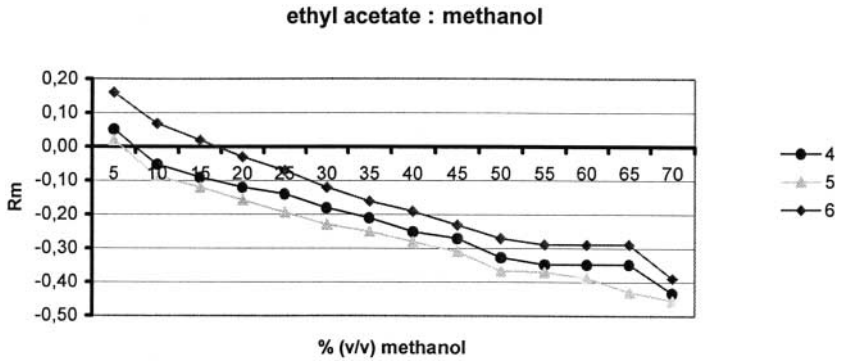

Figure 8. Dependence of R_m vs. % (v/v) methanol in ethyl acetate.

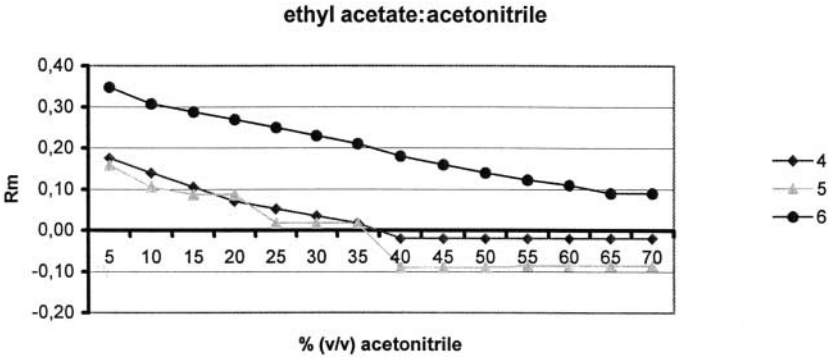

Figure 9. Dependence of R_m vs. % (v/v) acetonitrile in ethyl acetate.

Chloroform : Acetonitrile (Fig. 10)

This phase, similar to ethyl acetate : methanol, is characterised by very good selectivity in the whole range of concentrations. Comparison between R_m coefficients for these two phases (chloroform : acetonitrile, ethyl acetate : methanol) reveals that the above-mentioned mixture (chloroform :acetonitrile in the range of 5–35% (v/v) for compound **6** and in the range of 5–10% (v/v) for compounds **4** and **5**, does not comply with basic requirements of optimisation of conditions and exceeds the range of R_m values. The best parameters of separation of mobile compounds are achieved at acetonitrile concentration of 70% (v/v).

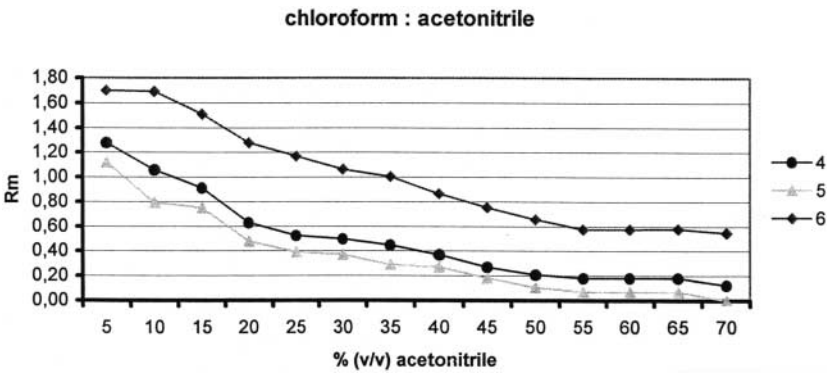

Figure 10. Dependence of R_m vs. % (v/v) acetonitrile in chloroform.

Acetone : Ethyl Acetate (Fig. 11)

This phase is characterised by the lowest selectivity when compared with other phases presented. Curves cross many times or coincide. The separation of all three substances can be achieved only at modifier (ethyl acetate) concentrations of 5%, 25%, and 65% (v/v), respectively. As opposed to other phases with the increase of percentage content of ethyl acetate, R_m values are also augmented, not exceeding the admissible range from +1 to −1, and taking on values close to zero at a concentration of ethyl acetate of above 65%. Subsequently, to select the optimal mixture of eluents for quinazoline N^3-oxide derivatives, the correlation diagrams for the best phases were made (Figs. 12 and 13).

Correlation of R_m Values at Methanol and Acetonitrile Concentrations of 25% in Ethyl Acetate

According to data presented in Fig. 12, better conditions of separation of compounds **4** and **5** are achieved using acetonitrile as a modifier (at a concentration of 25% v/v). However, in the case of compound **6** much better conditions of separation are obtained using methanol as a modifier at the same concentration. It can be assumed that ethyl acetate : acetonitrile at the modifier content of 25% is the optimal system to separate compounds **4** and **5**, and for compound **6** ethyl acetate : methanol (25% v/v) should be applied. Similar conclusions can be drawn when we analyse Table 2. They demonstrate that the efficiency measured by the number of theoretical plates reaches the optimal range (from 2500 to 5000) in the case of compounds **4** and **5** separation in the phase: ethyl acetate : acetonitrile (25% v/v), and compound **6** in the phase: ethyl acetate : methanol (25% v/v).

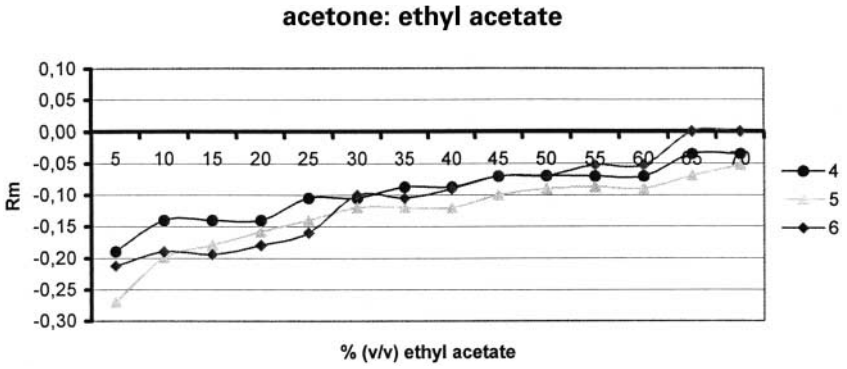

Figure 11. Dependence of R_m vs. % (v/v) ethyl acetate in acetone.

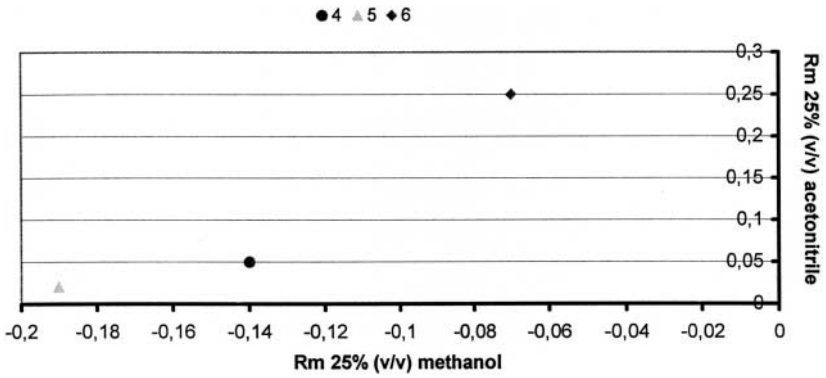

Figure 12. Correlation of R_m values at methanol and acetonitrile concentrations of 25% in ethyl acetate.

Correlation of R_m Values at Methanol and Acetonitrile Concentration of 65% in Ethyl Acetate (Fig. 13)

In Fig. 13, better parameters of all compounds separation (**4**–**6**) in the mixture of acetonitrile in ethyl acetate at a concentration of 65% (v/v) can be noticed. It can be assumed that ethyl acetate : acetonitrile at a concentration of 65% (v/v) of modifier content is a better phase than ethyl acetate : methanol. However, methanol when compared with acetonitrile exerted higher elution force and, thus,

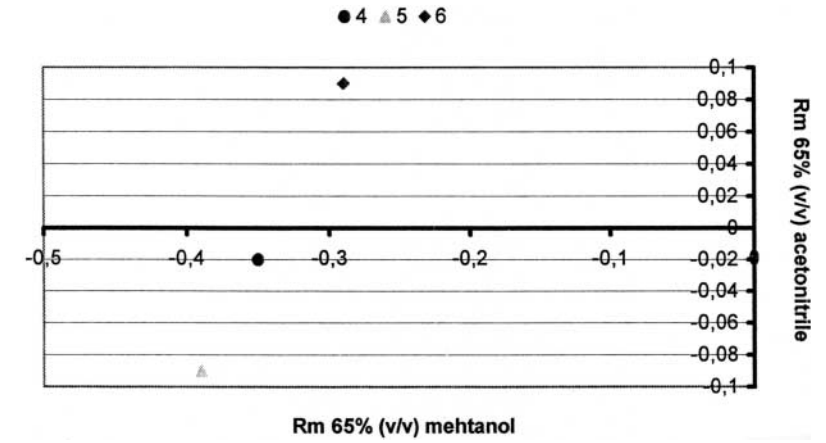

Figure 13. Correlation of R_m values at methanol and acetonitrile concentration of 65% in ethyl acetate.

Table 2. Received *N* values for systems.

Compounds	Ethyl acetate : 25% (v/v) methanol, *N*	Ethyl acetate : 25% (v/v) acetonitrile, *N*
4	4,444	2,621
5	2,500	3,318
6	4,096	2,178

lower R_m values. Ethyl acetate : acetonitrile [at a concentration of 65% (v/v)] is characterised, apart from good selectivity, also by high efficiency (Table 3).

CONCLUSION

The analytical experiments we carried out permitted for optimisation of conditions of chromatographic separation of six quinazoline derivatives (compounds **1**–**6**). Selection of optimal phase was commenced with chromatogram preparation in pure solvents in order to establish approximate compound polarity and R_f value. Subsequently, mixtures of solvent in accordance with increasing modifier content (v/v) (solvent of higher polarity) were prepared. In the next stage, correlation of phases containing the same diluent at the adequate concentration were assessed (diluent at lower polarity when compared with a modifier). To select the correlation concentration such values were taken into account, at which selectivity of both phases were relatively the best. Obtained correlations allowed us to establish which system at the same modifier content (%, v/v) is characterised by the best parameters of separation. The results of chromatographic separation reveal that:

The best phases for the separation of compounds **1**, **2**, **3** are: chloroform : ethyl acetate at the modifier content of 5% (v/v) and chloroform : acetone at the modifier content of 10% (v/v), while the former is optimal (better parameters of R_m coefficient).

Table 3. Obtained *N* values for systems.

Compounds	Ethyl acetate : 65% (v/v) methanol, *N*	Ethyl acetate : 65% (v/v) acetonitrile, *N*
4	7,465	2,988
5	8,028	3,600
6	3,464	3,464

Ethyl acetate : acetonitrile at the modifier content of 25% (v/v) is an optimal phase for the separation of compounds **4** and **5**, and for compound **6** ethyl acetate : methanol [at a concentration of 25% (v/v)] should be applied.

Ethyl acetate : acetonitrile at the modifier concentration of 65% is an optimal phase for separation of all three quinazoline N^3-oxides (compounds **4**–**6**).

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