ELSEVIER

Journal of Chromatography B, 770 (2002) 183–189

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Quantification and purity determination of newly synthesized thioacridines by capillary liquid chromatography

Pavel Coufal[a,*], Zuzana Bosáková[a], Eva Tesařová[b], Božena Kafková[a], Jana Suchánková[a], Jacques Barbe[c]

[a]*Department of Analytical Chemistry, Faculty of Science, Charles University, Albertov 2030, 128 43 Prague 2, Czech Republic*
[b]*Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Albertov 2030, 128 43 Prague 2, Czech Republic*
[c]*Gerctop Upres A CNRS 6009, Faculty of Pharmacy, 27 Bd Jean-Moulin, 133 85 Marseilles, France*

Abstract

Capillary liquid chromatography (CLC) was applied for quantification and impurity profile determination of ten newly synthesized acridine thioderivatives. A reversed-phase CLC system employing two different stationary phases, Nucleosil C_{18} and LiChrosorb RP-select B, was used. The mobile phase composition was optimized to get a satisfactory separation of impurities from the main acridine component in a reasonable analysis time. Significant differences in the chromatographic behavior between acridine derivatives containing and lacking amino groups were observed. Optimized separation conditions were used in CLC to measure the calibration curves of the acridine derivatives in a concentration range from $1.0 \cdot 10^{-6}$ to $1.0 \cdot 10^{-3}$ *M* at two different detector wavelengths (214 and 230 nm). Limits of detection and quantification of all the substances were determined. The detection limits went down to units of μM for most of the derivatives. CLC was also demonstrated to be a suitable method for the purity determination of test batches of the acridine thioderivatives.

Keywords: Quantification; Thioacridines

1. Introduction

Some acridine derivatives are considered to be important chemotherapeutics because they show bactericidal, fungicidal, and especially, anti-malarial effects [1]. Derivatives of acridine have been applied for the treatment of protozoal infections caused by Plasmodium microorganisms for many years [2]. Additionally, there is the growing importance of acridine derivatives with antineoplasmatic properties, which have been identified recently [3]. An increasing number of unhealthy factors in our environment, a decreasing immunity of all human beings to diseases and an increasing resistance of pathogens to chemotherapeutics used at present are the reasons why the pharmaceutical companies continue to develop and produce new derivatives with more efficient, effective and specific actions against pathogens. At the same time, analytical methods exhibiting higher selectivity and sensitivity are needed for purity determination of chemotherapeutics and their detection and quantification in biological materials.

Acridines are chemically derived from dibenzopyridine and contain at least one nitrogen atom in the aromatic cycle. Aqueous solutions of acridine show

*Corresponding author.
E-mail address: pcoufal@natur.cuni.cz (P. Coufal).

1570-0232/02/$ – see front matter
PII: S0378-4347(01)00523-0

weak basic properties ($pK_b = 8.4$). Derivatives and degradation products of acridine also behave as weak bases having altered dissociation constants determined through the character of substituents and their location in the acridine molecule [4].

High-performance liquid chromatography (HPLC) has most often been utilized to analyze acridine derivatives with respect to their identity, purity [5] and pharmacological and pharmacokinetic properties [6]. Capillary zone electrophoresis has been applied for the successful separation of acridines [7] as they can exhibit a positive charge in acidic solutions owing to their basic properties. Electrochemical oxidation of acridine without any substituents has also been studied [8]. However, capillary liquid chromatography (CLC) has not been employed for analysis of acridine and its derivatives to date.

CLC has some advantages, such as a low consumption of organic modifiers and a reduced sample amount (tens of nanoliters) compared to conventional HPLC [9,10]. These benefits of CLC can be especially advantageous for monitoring metabolites in body fluids and for determination of the main component and the impurities profile, since each analysis must be repeated several times to ensure reliability of the results in the frame work of good laboratory practice. Moreover, CLC seems to be more compatible with mass spectrometric detection than conventional HPLC.

Based on these facts, CLC was investigated in this work as a possible analytical technique for the quantification and the purity determination of ten newly synthesized acridine derivatives.

2. Experimental

2.1. Chemicals

Acetonitrile (gradient grade purity) for preparation of eluents was purchased from Merck (Darmstadt, Germany). Acetic acid (p.a.) and sodium hydroxide (p.a.) were supplied by Lachema (Brno, Czech Republic). Uracil (99%) for determination of the dead retention time was provided by Sigma (St. Louis, MO, USA). The water used for preparation of all mobile phases and solutions was purified with a Milli-Q water purification system (Millipore, USA).

Acetonitrile–water and acetonitrile–0.2 *M* acetate buffer (pH 3.5) eluents containing various percentages of acetonitrile were used as mobile phases. The eluents were sonicated just before use for at least 10 min.

All the studied thioacridine derivatives, the structures and labels of which are given in Fig. 1, were synthesized at the Faculty of Pharmacy in Marseilles using the synthetic procedure depicted in Fig. 2. A substituted diphenylaminecarboxylic acid is formed by the reaction of a substituted *o*-chlorobenzoic acid and an aniline derivative in accordance to the Ullmann reaction [11]. The diphenylaminecarboxylic acid is cyclized to acridone using phosphoryl chloride and a substituted thioacridone is obtained by treating the acridone with phosphorus sulfide in

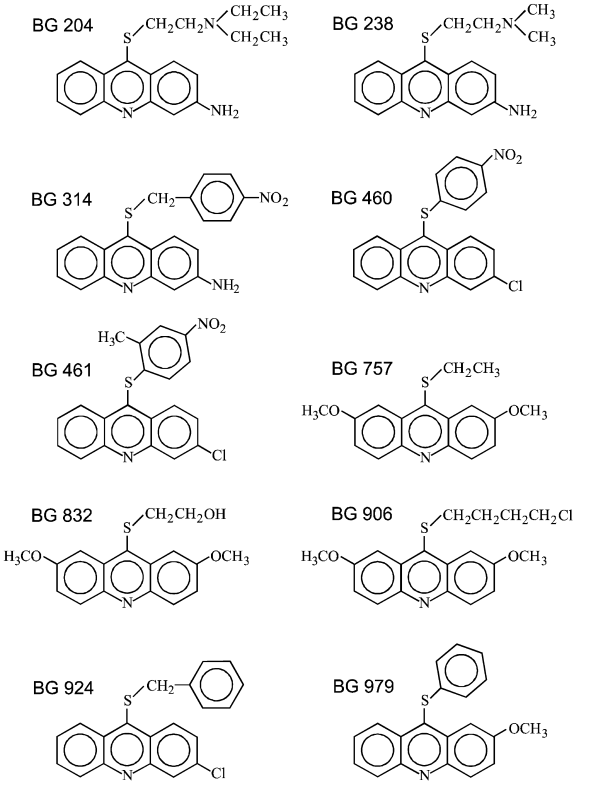

Fig. 1. Structures and labels of the acridine derivatives.

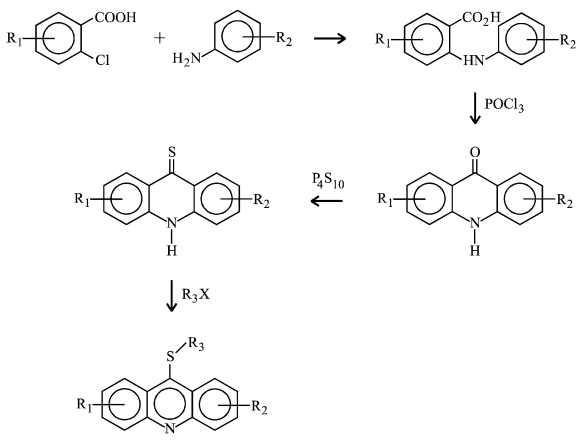

Fig. 2. Reaction scheme of the synthesis of acridine derivatives.

pyridine [12]. An alkylation or arylation of the thioacridone leads to a thioacridine derivative [13].

2.2. Equipment

An ISCO syringe pump model 100 DM (Lincoln, NE, USA), a Valco injection valve with a 60-nl internal loop (Schenkon, Switzerland) and a Linear UV–VIS 205 dual absorbance detector equipped with a CE on-column flow cell (San Jose, CA, USA) were applied for the CLC experiments. A fused-silica capillary column of 36 cm×320 μm I.D. packed with 5 μm Nucleosil 100-5 C_{18} was prepared at the Laboratory of Instrumental Analysis in Eindhoven Technical University, The Netherlands [14]. A stainless steel capillary column of 25 cm×300 μm I.D. packed with 5 μm LiChrosorb RP-select B was purchased from Grom (Herrenberg, Germany). The column inlet was installed in the injection valve using a 5-cm polyether ether ketone (PEEK) sleeve (500 μm I.D.) and a PEEK finger-tight fitting and the column outlet was connected by PTFE tubing to a 220 μm I.D. fused-silica capillary with detection window located 7 cm from the column outlet. This capillary was placed into the absorbance detector operated at wavelengths of 214 and 230 nm. Chromatograms were recorded and evaluated employing CSW 1.7 computer software provided by DataApex (Prague, Czech Republic).

3. Results and discussion

3.1. Investigation of the separation conditions

The influence of the acetonitrile (ACN) content in the ACN–water eluent on retention of the studied acridine derivatives was investigated within the range 60–100% of ACN on the Nucleosil C_{18} column. Thioacridines without any amino group (i.e. BG 460, BG 461, BG 757, BG 832, BG 906, BG 924 and BG 979, see Fig. 1) were eluted using all the mobile phases, however, long analysis times (over 50 min) were observed if eluents with a low ACN content were applied. When the ACN–water mobile phases were replaced with ACN–0.2 *M* acetate buffer (pH 3.5) eluents, only a negligible change in retention behavior and a small improvement considering the separation efficiency and peak symmetry were observed for acridine derivatives without amino groups [15,16].

On the other hand, thioacridines containing amino groups (i.e. BG 204, BG 238 and BG 314, see Fig. 1) were not eluted in a reasonable analysis time of 60 min on the Nucleosil C_{18} column, not even with the ACN–0.2 *M* acetate buffer eluents. The high silanol activity of this stationary phase induces its affinity to basic compounds which can be retained strongly or even irreversibly in the Nucleosil C_{18} column. Based on these results and experience, the LiChrosorb RP-select B column was chosen for the analysis of the acridine derivatives with amino groups. The latter type of stationary phase is generally recommended for separation of basic compounds since the silanol activity of the silica gel support is considerably diminished. Indeed, the basic thioacridines eluted from the LiChrosorb RP-select B column in acceptable analysis times using the ACN–0.2 *M* acetate buffer mobile phases.

To find separation conditions under which impurities from synthesis can effectively be separated from the main acridine derivative, the ACN content in the mobile phases was tuned within the range 60–100% (v/v). The ACN–0.2 *M* acetate buffer, pH 3.5 (90:10, v/v) eluent was able to resolve, in a reasonable time, the impurities from the main component on the Nucleosil C_{18} column and on the LiChrosorb RP-select B column in the case of

Table 1
Parameters of the calibration curves (including standard deviations in parentheses) and linear dynamic ranges of acridines containing amino groups. Stationary phase, LiChrosorb RP-select B; mobile phase, ACN–0.2 *M* acetate buffer, pH 3.5 (90:10, v/v); flow-rate, 3 μl/min; detection wavelength, 214 nm

Derivative	Intercept (SD) (mV s)	Slope (SD) (mV s l/mol)	*r*	Linear dynamic range (*M*)
BG 204	6.973 (3.567)	152 000 (4400)	0.9995	$4.0\cdot10^{-5}$–$1.0\cdot10^{-3}$
BG 238	−5.114 (4.737)	120 000 (13 500)	0.9877	$8.0\cdot10^{-5}$–$1.0\cdot10^{-3}$
BG 314	−4.047 (4.403)	253 000 (14 300)	0.9952	$2.0\cdot10^{-5}$–$1.0\cdot10^{-3}$

thioacridines without and with amino groups, respectively. The decrease of ACN content in the mobile phases did not lead to a separation of further impurities from the main component, but a longer analysis time and a greater peak asymmetry of the acridine derivatives were observed under these conditions. Van Deemter curves for some of the acridine derivatives were measured in an interval from 2 to 5 μl/min for optimization of the eluent flow-rate [16]; e.g. with the derivative BG 461, HETP values of 26, 31, 35 and 41 μm were reached for the flow-rates of 2, 3, 4 and 5 μl/min, respectively. The linear dependences of the height equivalent to a theoretical plate on the flow-rate obtained within this interval indicated that the resistance to mass transfer was the major mechanism of band broadening of the analyte zones. A mobile phase flow-rate of 3 μl/min was selected as a compromise between the separation efficiency and the analysis time.

The results showed that the ACN–0.2 *M* acetate buffer, pH 3.5 (90:10, v/v) eluent at a flow-rate of 3 μl/min can be applied for separation and quantification of impurities from the main acridine component in an analysis time not exceeding 30 min.

3.2. Quantification of the thioacridine derivatives

Calibration curves for all the studied thioacridine derivatives were measured in the concentration interval from $1.0\cdot10^{-6}$ to $1.0\cdot10^{-3}$ *M* at two different wavelengths, 214 and 230 nm. The optimized separation systems composed of the Nucleosil C_{18} column (for the seven derivatives lacking amino groups) and on the LiChrosorb RP-select B column (for the three thioacridines containing amino groups) and the ACN–0.2 *M* acetate buffer, pH 3.5 (90:10, v/v) mobile phase at the flow-rate of 3 μl/min were applied. Measurements at each concentration level were repeated four times and average values of the peak area were subjected to linear regressions. Intercepts, slopes and correlation coefficients of the calibration curves (peak area versus molar concentration) and linear dynamic ranges for acridines containing amino groups determined at the wavelengths of 214 and 230 nm are summarized in Tables 1 and 2, respectively. Parameters of the calibration curves and linear dynamic ranges for acridines lacking amino groups obtained at the same two wavelengths are listed in Tables 3 and 4.

As an example, calibration curves in logarithmic coordinates for the acridine derivative BG 832 within the linear concentration range $6.0\cdot10^{-6}$–$8.0\cdot10^{-4}$ *M* are depicted in Fig. 3. An excellent linearity was observed for the calibration curves of thioacridine BG 832 at both wavelengths over the three concentration orders. A very good agreement between the experimental points and the linear calibration curves was indicated through the correlation co-

Table 2
Parameters of the calibration curves (including standard deviations in parentheses) and linear dynamic ranges of acridines with amino groups. Detection wavelength, 230 nm; for other conditions see Table 1

Derivative	Intercept (SD) (mV s)	Slope (SD) (mV s l/mol)	*r*	Linear dynamic range (*M*)
BG 204	−4.043 (7.915)	275 000 (9700)	0.9993	$4.0\cdot10^{-5}$–$1.0\cdot10^{-3}$
BG 238	−4.898 (9.065)	170 000 (13 400)	0.9938	$8.0\cdot10^{-5}$–$1.0\cdot10^{-3}$
BG 314	−4.428 (5.241)	309 000 (17 000)	0.9954	$2.0\cdot10^{-5}$–$1.0\cdot10^{-3}$

Table 3
Parameters of the calibration curves (including standard deviations in parentheses) and linear dynamic ranges of acridines without amino groups. Stationary phase, Nucleosil C_{18}; mobile phase, ACN–0.2 *M* acetate buffer, pH 3.5 (90:10, v/v); flow-rate, 3 μl/min; detection wavelength, 214 nm

Derivative	Intercept (SD) (mV s)	Slope (SD) (mV s l/mol)	*r*	Linear dynamic range (*M*)
BG 460	0.003 (0.689)	353 000 (13 100)	0.9959	$6.0\cdot10^{-6}$–$1.0\cdot10^{-4}$
BG 461	−0.551 (0.232)	633 000 (4700)	0.9998	$4.0\cdot10^{-6}$–$1.0\cdot10^{-4}$
BG 757	0.034 (0.512)	280 000 (1500)	0.9998	$1.0\cdot10^{-5}$–$8.0\cdot10^{-4}$
BG 832	−0.001 (0.661)	183 000 (2100)	0.9993	$6.0\cdot10^{-6}$–$8.0\cdot10^{-4}$
BG 906	0.466 (1.738)	232 000 (5000)	0.9981	$1.0\cdot10^{-5}$–$8.0\cdot10^{-4}$
BG 924	1.306 (1.515)	448 000 (3500)	0.9996	$8.0\cdot10^{-6}$–$1.0\cdot10^{-3}$
BG 979	−1.351 (2.141)	601 000 (5400)	0.9995	$4.0\cdot10^{-6}$–$1.0\cdot10^{-3}$

efficients for all the calibrations. The worst value of the correlation coefficient (i.e. $r=0.9877$) was obtained for acridine BG 238 at 214 nm, the variation of experimental points was explained from 97.6% (i.e. $100r^2$) by the calibration curve variation.

Based on the results summarized in Tables 1–4, a more sensitive detection for all the acridine derivatives with amino groups was obtained at 230 nm compared to 214 nm. The most sensitive detection was observed for the thioacridines BG 461 and BG 979 at 214 nm. The lowest slope values were obtained for the calibration curves measured with derivatives BG 204 and BG 238 at the same detection wavelength. None of the intercepts of the calibration curves in Tables 1–4 were found to be significantly different from zero at a significance level of $\alpha=0.01$ applying the *t*-test for intercepts, $t=\alpha/\mathrm{SD}$.

The dependences of the peak height on the molar concentration of the studied acridines were plotted for determination of the limits of detection (LOD) and the limits of quantification (LOQ) under the experimental conditions described previously. The LOD and LOQ values were obtained from these dependences as concentrations corresponding to the three-multiple and ten-multiple of root mean squared baseline noise, respectively [17]. The obtained values are summarized in Table 5. The detection limits for most of the acridine derivatives were as low as units of μ*M*.

3.3. Impurity profile of test batches of the acridine derivatives

The optimized separation conditions were used to analyze the impurity profiles of test batches of the thioacridine derivatives containing various impurities from the synthetic procedure. The profiles of test batches of derivatives BG 314 and BG 832 are presented in Fig. 4. Since the chemical structures of the impurities were not known and therefore any standards for the impurities were not available, an

Table 4
Parameters of the calibration curves (including standard deviations in parentheses) and linear dynamic ranges of acridines lacking amino groups. Detection wavelength, 230 nm; for other conditions see the caption to Table 3

Derivative	Intercept (SD) (mV s)	Slope (SD) (mV s l/mol)	*r*	Linear dynamic range (*M*)
BG 460	0.065 (0.578)	321 000 (11 000)	0.9965	$6.0\cdot10^{-6}$–$1.0\cdot10^{-4}$
BG 461	−0.098 (0.206)	510 000 (4100)	0.9997	$4.0\cdot10^{-6}$–$1.0\cdot10^{-4}$
BG 757	0.096 (0.725)	491 000 (2100)	0.9999	$1.0\cdot10^{-5}$–$8.0\cdot10^{-4}$
BG 832	−0.044 (0.838)	322 000 (2600)	0.9996	$6.0\cdot10^{-6}$–$8.0\cdot10^{-4}$
BG 906	0.658 (1.375)	405 000 (4100)	0.9995	$8.0\cdot10^{-6}$–$8.0\cdot10^{-4}$
BG 924	0.683 (1.198)	323 000 (2800)	0.9996	$8.0\cdot10^{-6}$–$1.0\cdot10^{-3}$
BG 979	−0.920 (1.629)	540 000 (4100)	0.9996	$4.0\cdot10^{-6}$–$1.0\cdot10^{-3}$

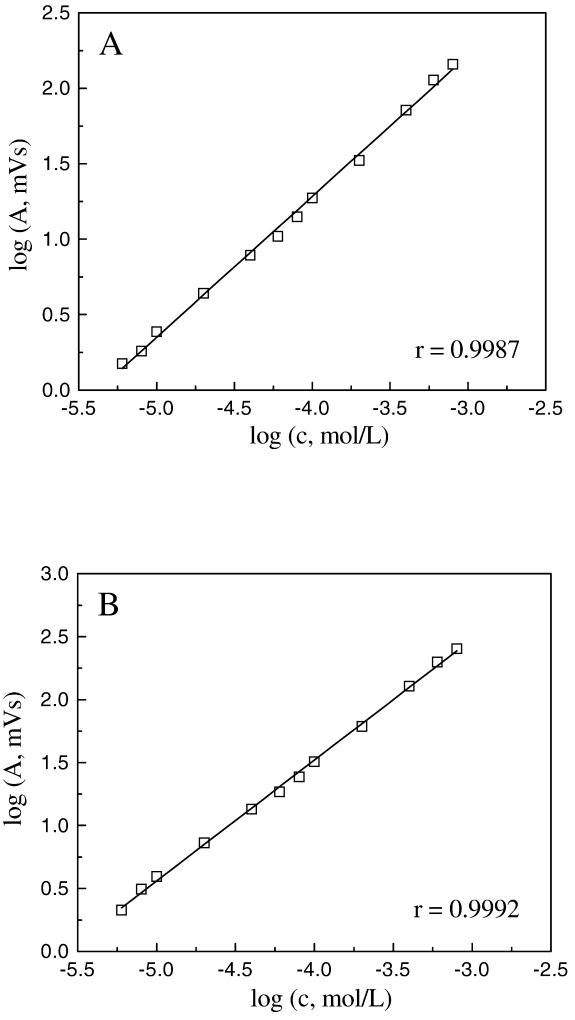

Fig. 3. Dependencies of the decadic logarithm of the peak area on the decadic logarithm of the molar concentration of the acridine derivative BG 832 in the concentration range $6.0 \cdot 10^{-6}$–$8.0 \cdot 10^{-4}$ *M* measured at detection wavelengths of 214 (A) and 230 nm (B).

internal normalization evaluation method was applied to quantify the percentage of impurities in the test batches. Based on this evaluation method the following contents of the main components were determined in the corresponding test batches of thioacridines: 95.9% for BG 204, 96.7% for BG 238, 96.5% for BG 314, 100.0% for BG 460, 100.0% for BG 461, 100.0% for BG 757, 90.2% for BG 832, 92.9% for BG 906, 92.3% for BG 924 and 100.0% for BG 979 of the main component was determined in the corresponding test batch of thioacridine.

Table 5
Limits of detection (LOD) and limits of quantification (LOQ) of the thioacridines expressed in *M*. Stationary phase, Nucleosil C_{18} or LiChrosorb RP-select B; mobile phase, ACN–0.2 *M* acetate buffer, pH 3.5 (90:10, v/v); flow-rate, 3 μl/min; detection wavelengths, 214 and 230 nm

Derivative	214 nm		230 nm	
	LOD	LOQ	LOD	LOQ
BG 204[a]	$4.9 \cdot 10^{-5}$	$1.6 \cdot 10^{-4}$	$2.8 \cdot 10^{-5}$	$9.4 \cdot 10^{-5}$
BG 238[a]	$3.6 \cdot 10^{-5}$	$2.1 \cdot 10^{-4}$	$2.7 \cdot 10^{-6}$	$1.1 \cdot 10^{-4}$
BG 314[a]	$8.0 \cdot 10^{-6}$	$2.7 \cdot 10^{-5}$	$6.5 \cdot 10^{-6}$	$2.2 \cdot 10^{-5}$
BG 460	$9.1 \cdot 10^{-6}$	$3.1 \cdot 10^{-5}$	$1.0 \cdot 10^{-5}$	$3.5 \cdot 10^{-5}$
BG 461	$6.2 \cdot 10^{-6}$	$2.1 \cdot 10^{-5}$	$7.3 \cdot 10^{-6}$	$2.5 \cdot 10^{-5}$
BG 757	$7.8 \cdot 10^{-6}$	$2.6 \cdot 10^{-5}$	$5.9 \cdot 10^{-6}$	$2.0 \cdot 10^{-5}$
BG 832	$9.5 \cdot 10^{-6}$	$3.2 \cdot 10^{-5}$	$7.1 \cdot 10^{-6}$	$2.4 \cdot 10^{-5}$
BG 906	$7.6 \cdot 10^{-6}$	$2.5 \cdot 10^{-5}$	$5.4 \cdot 10^{-6}$	$1.8 \cdot 10^{-5}$
BG 924	$6.3 \cdot 10^{-6}$	$2.1 \cdot 10^{-5}$	$8.6 \cdot 10^{-6}$	$2.9 \cdot 10^{-5}$
BG 979	$5.3 \cdot 10^{-6}$	$1.8 \cdot 10^{-5}$	$6.0 \cdot 10^{-6}$	$2.0 \cdot 10^{-5}$

[a] Determined on LiChrosorb RP-select B.

4. Conclusion

An analytical method for quantification and impurity profile determination of ten acridine thioderivatives by CLC has been developed. An ACN–0.2 *M* acetate buffer, pH 3.5 (90:10, v/v) eluent was found to sufficiently separate acridine impurities from the main component on the Nucleosil C_{18} column in the case of thioacridines without amino groups or on the LiChrosorb RP-select B column in the case of all ten acridine derivatives. These separation conditions were applied to measure the calibration curves for the thioacridines at two different wavelengths (214 and 230 nm) with linear dynamic ranges from $1.0 \cdot 10^{-6}$ to $1.0 \cdot 10^{-3}$ *M* and with detection and quantification limits reaching units and tens of μ*M*, respectively. Additionally, the content of impurities in the test batches of the thioacridine derivatives was determined by CLC under the same separation conditions. CLC was demonstrated to be a powerful separation technique for pharmaceutical applications, moreover, it guarantees a considerable saving in analysis time, organic modifiers and the use of mobile phases.

5. Nomenclature

A absorbance

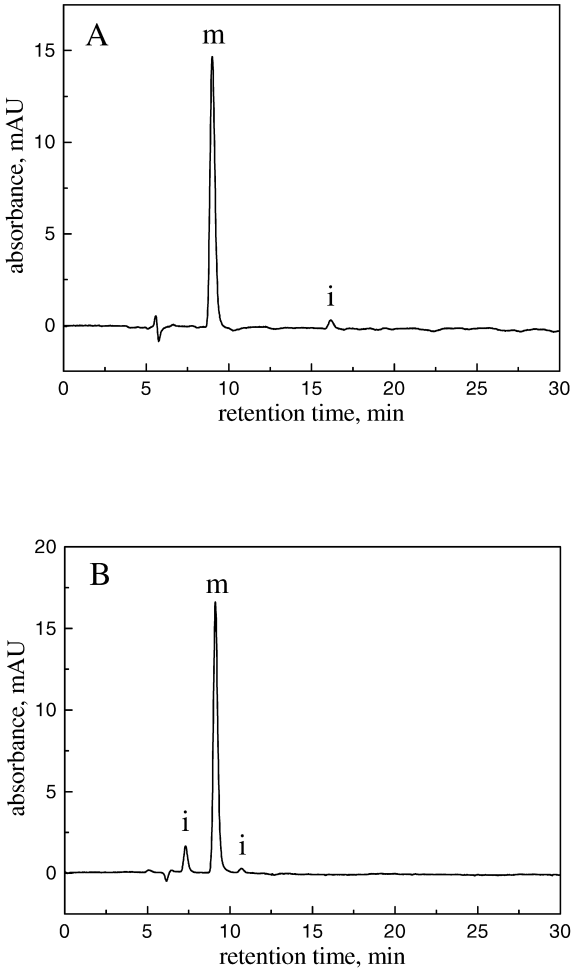

Fig. 4. Chromatograms of the impurity profile of the test batches of the derivatives BG 314 (A) and BG 832 (B). Stationary phases, LiChrosorb RP-select B (A) or Nucleosil C_{18} (B); mobile phase, acetonitrile–0.2 *M* acetate buffer, pH 3.5 (90:10, v/v); flow-rate, 3 μl/min; concentration, $1.0 \cdot 10^{-3}$ *M*; injection volume, 60 nl; detection wavelength, 230 nm; m, main component; i, impurity.

α	intercept of calibration curve
ACN	acetonitrile
CLC	capillary liquid chromatography
HETP	height equivalent to a theoretical plate
HPLC	high-performance liquid chromatography
i	impurity
I.D.	inner diameter
LOD	limit of detection
LOQ	limit of quantification
m	main component
PEEK	polyether ether ketone
RP	reversed-phase
r	correlation coefficient
SD	standard deviation
t	Student distribution

Acknowledgements

This work was financially supported by grant number 203/00/1569 of the Grant Agency of the Czech Republic. The study is a part of the international project COST Action B 16. The authors would like to thank Mrs. Marcela Aussenbergová for her technical assistance.

References

[1] Z. Moder, Remedia 2 (1994) 821.
[2] J. Barbe, in: Bulletin 1st Acrival Meeting on Chemotherapy of Trypanosomatidae, Marseilles, France, 1992, p. 63.
[3] P.C. Schofield, I.G.C. Robertson, J.W. Paxton, Cancer Chemother. Pharmacol. 44 (1999) 51.
[4] J. Kovác, Š. Kovác, Org. Chem., SNTL, Prague, 1977.
[5] J.L. Jurlina, J.W. Paxton, J. Chromatogr. 342 (1985) 431.
[6] A.L. Jayewardene, J.E. Santoro, J.G. Gambertoglio, J. Chromatogr. B 702 (1997) 203.
[7] I. Koštálová, I. Jelínek, N. Zimová, J. Barbe, Chem. Listy 91 (1997) 369.
[8] K. Yakosouchi, I. Taniguchi, H. Yamaguchi, K. Aeakawa, J. Electroanal. Chem. 121 (1981) 230.
[9] J.P.C. Vissers, H.A. Claessens, C.A. Cramers, J. Chromatogr. A 779 (1997) 1.
[10] J.P.C. Vissers, J. Chromatogr. A 856 (1999) 117.
[11] F. Ullmann, Liebigs Ann. Chem. 355 (1907) 327.
[12] R.R. Smolders, J. Hanuise, R. Coonians, V. Proietto, N. Voglet, A. Waefelaer, Synthesis (1982) 493.
[13] N. Bisiri, C. Johnsos, M. Kayiréné, Ann. Pharm. Fr. 54 (1996) 27.
[14] J.P.C. Vissers, H.A. Claessens, J. Laven, C.A. Cramers, Anal. Chem. 67 (1995) 2103.
[15] P. Coufal, E. Tesařová, Z. Bosáková, B. Kafková, J. Barbe, in: Proceedings of the ITP 2000 Symposium, Bratislava, September, 2000, p. B26.
[16] Z. Bosáková, E. Tesařová, P. Coufal, B. Kafková, J. Barbe, Chem. Listy 95 (2001) 569.
[17] R.C. Graham, in: Data Analysis for the Chemical Sciences — A Guide To Statistical Techniques, VCH, Weinheim, 1993, p. 161, 269.