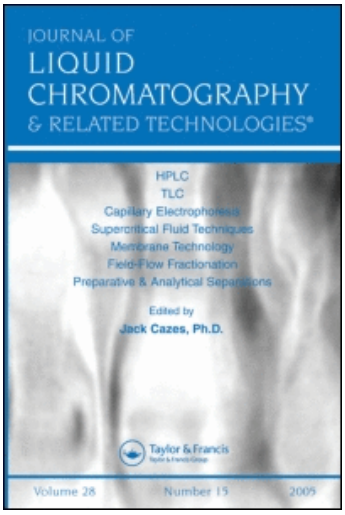

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Isotachophoresis of Chosen Biologically Active (E)-Azastilbenes

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Abstract: Biological activity of (E)-azastilbenes and their numerous derivatives appears in antimicrobial and anticancer effects. They also show the behavior of liquid crystals and the ability of formation of complex compounds. In this work are presented the conditions of optimum separation and determination of chosen isomers of (E)-azastilbenes by the isotachophoretic method. In the process of optimization, the length of steps of analysis, intensity (it was justified by the fact of dependence of ion mobility on electric field intensity), and pH of solutions of electrolytes and samples were changed. A new terminating electrolyte was used for the determinations. By a process of trial and error it was proven that optimum pH for analyzed isomers are 3.8. The shortest time of analysis was obtained for individual isomers. However, for mixtures of isomers the optimum time of analysis was twice as long.

Keywords: (E)-Azastilbenes, Isomers, Isotachophoresis, Optimization, Terminating

INTRODUCTION

A need of the synthesis for more and more new medicines counteracting diseases typical for contemporary civilization (often incurable) lead to a

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new development. This development includes synthesis of new compounds, testing of their biological activity, as well as their determination in different matrices by different techniques.

One of an important group of compounds are azastilbenes. They are a research subject of scientists world wide scientists. This is shown in numerous papers published in chemical, electronic, and other periodicals.[1–8]

The biological activity of (E)-azastilbenes, first of all antimicrobial and anticancer effects, their behavior as liquid crystals, and their ability of complex compounds formation are the reasons of especial interest in this group of compounds.

Until now, all synthesized (E)-azastilbenes showed biological activity. For example trans-styrylpyridines are effective inhibitors of acetyltransferase of choline.[1–3] It was shown that N-substituted derivatives of (E)-4′ (3′,2′) hydroxystibazoles-4 have antibacterial and fungistatic activity. The tests were carried out on the following strains: Gram-positive cocci (*Staphylococus aureus* 209P FDA, *Streptococus faecalis* ATCC 8040), aerobis bacilli (*Bacillus subtilis* ATCC 1633), Gram-negative rods (*Escherichia coli* PZH 026B6, *Klebsiella pneumoniae* 231, *Pseudomonas aeruginosa* SR_1), yeasts (*Candida albicans* PCM 1409 PZH), dermatophytes (*Microsporum gypseum* K_1), and moulds (*Aspergillus fumigatus* C_1).[2,3]

(E)-azastilbenes unsubstituted in position N-have a free pair of electrons on the nitrogen atom and belong to bases according to Lewis theory. Free pairs of electrons of the cause of these compounds being good ligands. For example, complexes of trans-styrylpyridine with some transition metals (like silver, ruthenium, osmium, zinc, wolfram, molybdenum) are well known.[9–12] Other examples of compounds with (E)-azastilbenes as ligands are metalloporphyrins. Porphyrin axially substituted by phenyl groups and complexed with zinc can form two kinds of complexes with two different (E)-azastilbenes.[11] Other metalloporhyrins that form complexes with (E)-azastilbenes are porphyrins complexed with ruthenium (II) and osmium (II).[11]

Some complex compounds of (E)-azastilbenes show properties of liquid crystals. This interesting behavior exhibit complexes with silver, molybdenum, and wolfram. During transformation from the solid state of aggregation into a liquid state, they form a liquid crystalline phase. Every of complex of silver and wolfram described in the literature show that at adequate temperature there is transformation from crystalline phase, by smectic phase C, smectic phase A, nematic phase, to isotropic phase. It enables further investigations in the use of these compounds in the developing electronic industry that takes advantage of liquid crystals.[9,10,12]

Quoted above, the information concerning (E)-azastilbenes, however only fragmentary, confirm the importance of this group of compounds

and of the necessity of further research in this area. Described in our work, the optimization of the process of separation and determination of (E)-azastilbenes by various techniques certainly will be a contribution to development of the chemistry of these compounds. Until now, no attempt of determination of these compounds by isotachophoresis has been undertaken; no papers concerning this problem have been published. Therefore, our main aim of the investigation was to elaborate optimum conditions of separation and determination of chosen N-substituted derivatives of (E)-azastilbenes by the isotachophoretic method (ITP). In the investigation a new terminating electrolyte was used. In the process of optimization two isomers were considered: chloride of (E)-N-(m-chlorobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(m-chlorobenzyl)-2′-hydroxystilbazole-4.

EXPERIMENTAL

Electrophoretic Analysis of (E)-azastilbenes

Samples of compounds: chloride of (E)-N-(m-chlorobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(m-chlorobenzyl)-2′-hydroxystilbazole-4 (Figure 1) were dissolved in deionised water (Merck). Standard solutions were prepared with concentrations of $2.5 \times 10^{-3}\,\text{mole} \cdot \text{L}^{-1}$, $5.0 \times 10^{-4}\,\text{mole} \cdot \text{L}^{-1}$, $7.5 \times 10^{-4}\,\text{mole} \cdot \text{L}^{-1}$ in order to draw standard curves. Optimization of conditions of separation and determination was carried out by ITP technique.

The method of optimization of analysis conditions of the above mentioned compounds included preparation of solutions with determined

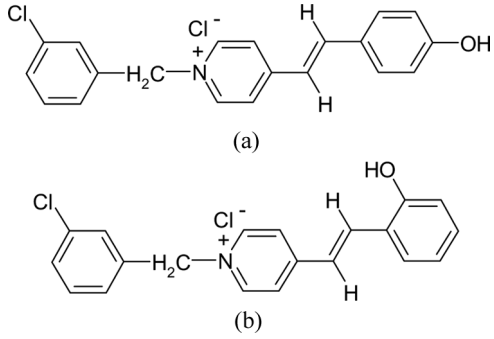

Figure 1. Structures of analyzed compounds (a) chloride of (E)-N-(m-chlorobenzyl)-4′-hydroxystilbazole-4 (**A1**), (b) chloride of (E)-N-(m-chlorobenzyl)-2′-hydroxystilbazole-4 (**A3**).

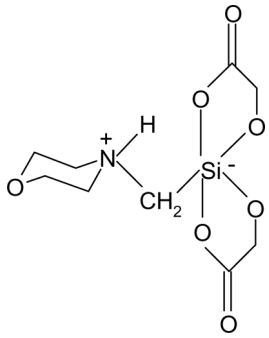

Figure 2. Structure of a compound used in terminating electrolyte: 1-(N-morpholiniomethyl)spirobi(1-sila-2,5-dioxacyclopentan-3-on)ate.

concentration, obtaining of standard curves for investigated compounds, separation and determination of chosen (E)-azastilbenes.

Electrolytes were prepared using reagents of analytical purity, namely: deionised water (Merck), hydrochloric acid (POCh Gliwice), acetic acid (POCh Gliwice), and sodium acetate (POCh Gliwice). Leading electrolytes (Ld-1) and (Ld-2) for an analytic column, exhibiting pH = 3.8, prepared from equal volumes of the following solutions: HCl solution (1×10^{-3} mole $\cdot$ L^{-1}), CH_3COONa solution (2×10^{-3} mole $\cdot$ L^{-1}), CH_3COOH solution (2×10^{-3} mole $\cdot$ L^{-1}). As a terminating electrolyte (Tm), a solution of 1-(N-morpholiniomethyl)spirobi(1-sila-2,5-dioxacyclopentan-3-on)ate (from ES-silanate group) with concentration $3 \cdot 10^{-3}$ mole $\cdot$ L^{-1} was used (Figure 2).

The (E)-azastilbenes were prepared by the method described in the literature.[13] Chosen chemical, physical, and biological data are shown in Tables 1–2.

Apparatus

Analyses were carried out by means of a capillary electrophoresis analysator EA 202 M produced by Villa Labeco s.r.o. in Spisska Nova Ves

Table 1. Chemical and physical data of compounds[13]

Compound	Yield (%)	Mp(°C)	IR (KBr) (cm^{-1}) $\delta_{CH=CH}$	^{1}H-NMR δ(ppm) $-CH_2-{}^+N$
A1	67.0	240–243	985	5.81
A3	87.7	233–236	955	5.93

Table 2. Antimicrobial activity of isomers. Minimal inhibitory concentration (MIC $\mu g \cdot mL^{-1}$)[13]

	Minimal inhibitory concentration (MIC) $\mu g \cdot mL^{-1}$								
Compounds	1	2	3	4	5	6	7	8	9
o-Cl	100	500	500	1000	1000	1000	>500	>500	>500
p-Cl	100	100	500	100	500	1000	>500	>500	>500

1-*Staphylococcus aureus* 209P FDA, 2-*Streptococcus faecalis* ATCC 8040, 3-*Bacillus subtilis* ATCC 1633, 4-*Escherichia coli* PZHO 26B6, 5-*Klebsiella pneumoniae* 231, 6-*Pseudomonas aeruginosa* 5 R1, 7-*Candida albicanus* PCM 1409 PZH, 8-*Microsporum gypseum* K_1, 9-*Aspergillus fumigatus* C1.

(Slovakia), equipped with an injection block with a container for terminating electrolyte, preseparation column (capillary diameter 0.8 mm, length 90 mm), bifurcation block with ant electrode block of the preseparational column, analytic column (capillary diameter 0.3 mm, length 160 mm), ant electrode block of the analytic column, UV detector, two conductometric detectors with a measurement range between 30 kΩ and 20 MΩ, steering unit – personal computer PC containing converter AD/DA. After drawing of standard curves for individual compounds optimization of the isotachophoretic separation of the mixture was carried out.

RESULTS AND DISCUSSION

Analyzed (E)-azastilbenes: chloride of (E)-N-(m-chlorobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(m-chlorobenzyl)-2′-hydroxystilbazole were subjected to investigations in order to achieve their separation and determination in a time as short as possible. During ITP determination, the electric field causes ions of the sample when introduced between a system of two electrolytes (a leading and a terminating) to migrate in a direction of adequate potential. Ions migrate in turn from the highest to the lowest mobility.

Optimum parameters of the separation and determination method of individual compounds and their mixtures are shown in Tables 3 and 4. However, isotachophoregrams obtained in optimum conditions are shown in Figures 3–5.

During the optimization of separation and determination of (E)-azastilbenes various conditions of the method were tested. A time of analysis, a value of pH, an intensity of electric current, a level of limitation of voltage were changed, and preseparational or analytical column

Table 3. Conditions of the method of individual determination chloride of (E)-N-(m-chlorobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(m-chlorobenzyl)-2′-hydroxystilbazole-4

Considered parameters				
Stage	Time (s)	Intensity (μA)	Comp (10 mV)	Conductometric detector
1	70	80	0	–
2	50	125	50	–
3	60	50	0	X

were used. Analyses were carried out changing the voltage from 9 kV to 15 kV. At the voltage lower than 9 kV, separation was not achieved. The best results of separation were obtained at the limitation of the voltage to 12 kV (Table 5, Figures 3–5).

Analysis of chosen (E)-azastilbenes was carried out in acidic solutions and during one experiment only cations were determined. Separation was done on the basis of differences of ion electrophoretic mobilities. Analyzed (E)-azastilbenes showed very similar electrophoretic mobility. A new terminating electrolyte (characterized by significantly lower than analyzed isomers) was used for the determinations.

By a process of trial and error a new terminating electrolyte was selected, i.e., a solution of 1-(N-morpholiniomethyl)spirobi (1-sila-2,5-dioxacyclopentan-3-on)ate (Figure 2). This compound belongs to the group of ES-silanates, hypercoordinated organosilicon compounds. Common conditions of determination of analyzed (E)-azastilbenes are shown in Table 3.

By the use of capillary isotachophoresis proper separation and determination of analyzed (E)-azastilbenes were carried out. All analyses were

Table 4. Optimum conditions of isotachophoretic separation of a mixture of chloride of (E)-N-(m-chlorobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(m-chlorobenzyl)-2′-hydroxystilbazole-4

Considered parameters				
Stage	Time (s)	Intensity (μA)	Comp (10 mV)	Conductometric detector
1	100	100	0	–
2	150	250	0	X
3	65	10	0	–
4	10	120	0	–
5	25	100	50	–
6	100	30	0	X

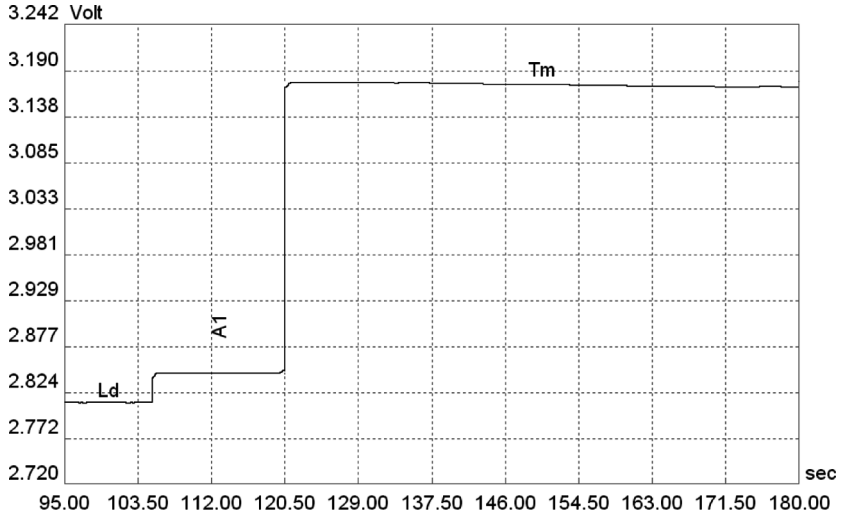

Figure 3. Isotachophoregram of chloride of (E)-N-(m-chlorobenzyl)-4′-hydroxy-stilbazole-4 (**A1**).

performed applying two dimensional analysis with switching the column. Qualitative analysis was carried out on the basis of zone height on obtained isotachophoregrams. The heights of zones were compared with these obtained on isotachophoregrams of standard solutions.

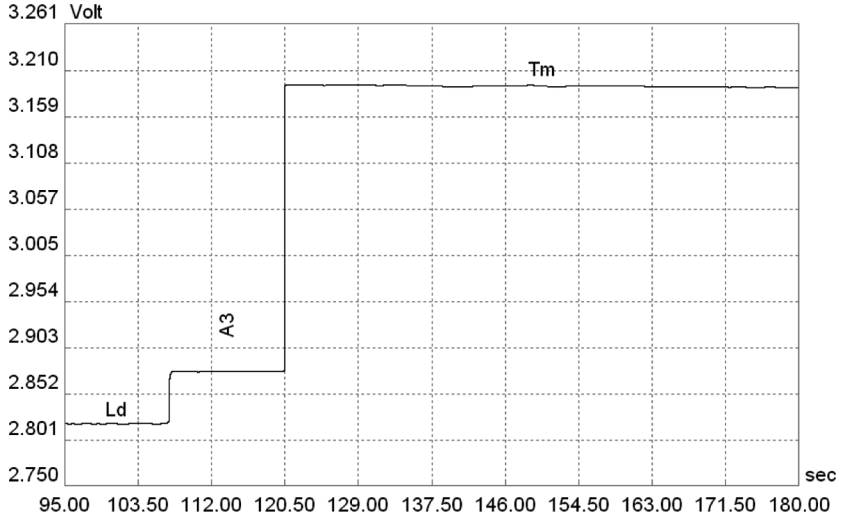

Figure 4. Isotachophoregram of chloride of (E)-N-(m-chlorobenzyl)-2′-hydroxy-stilbazole-4 (**A3**).

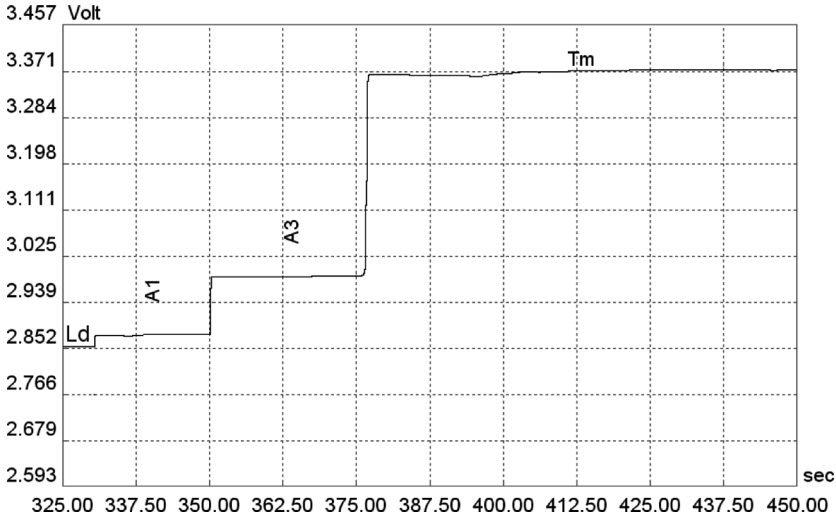

Figure 5. Isotachophoregram of the mixture of chloride of (E)-N-(m-chlorobenzyl)-4′-hydroxystilbazole-4 (**A1**) and chloride of (E)-N-(m-chlorobenzyl)-2′-hydroxystilbazole-4 (**A3**).

Separation of (E)-azastilbenes isomers was very difficult because these compounds have only different positions of the substituents in the ring and because they are characterized by similar mobility. During the process of optimization intensity of the electric current was changed (it was justified by the mobility dependence on the electric field intensity) and pH of solutions of electrolytes and samples. By a process of trial and errors it was proven that the optimum pH for analyzed isomers was 3.8. At pH close to neutral, isotachophoregrams were characterized by steep zones for the investigated mixture and blurred zones for the terminating electrolyte.

Apart from keeping pH equal 3.8, it was necessary to select adequate intensity and time for individual steps of analysis. All things considered,

Table 5. Common parameters of optimal **A1** and **A3** separation and determination by isotachophoresis method

Parameters of the method	
UV filter [nm]	200
High voltage limit [kV]	12
Sample rate [smp/s]	50
Polarity	+ cations

Table 6. Characteristic of used analytical method

Parameter	Unit	For examined ion
Precision[a]	%	2–3.5
Recovery[b]	%	92 ± 4
Linearity[c]	$mg \cdot mL^{-1}$	2–30
Limit of identification[d]	$mg \cdot mL^{-1}$	1

[a]$n = 5$, the samples were analyzed twice.
[b]The sample was enriched with 1.5 mL of a solution containing $1\,mg \cdot mL^{-1}$ of examined ion, $n = 5$.
[c]Correlation coefficient above 0.97.
[d]Calculated from the limit of identification and coefficients of the calibration curve.

selection of these three parameters allowed obtaining optimum separation of analyzed isomers (Figure 5).

The time of analysis depended on a number of compounds in an analyzed sample. The shortest time was obtained for individual isomers (Table 3, Figures 3 and 4). However, in the case of a mixture of isomers, optimum time of analysis was more than twofold (Table 4, Figure 5).

The elaborated technique was characterized by the high precision and accuracy of obtained results of analyses (Table 6). Linearity was between 2 and $30\,mg \cdot L^{-1}$, detection limit was $1\,mg \cdot L^{-1}$. Precision and accuracy of results obtained by the capillary isotachophoretic technique is higher than in classic methods.

In summary, the intended aim of investigations was fully executed. Optimum conditions of isotachophoretic separation and determination of chosen (E)-azastilbenes has been elaborated. Research by chromatographic methods concerning this group of compounds caused many troubles regardless of the used analytical techniques.[14] However, because of biological activity of these compounds and the wide possibility of their application, investigations leading to better knowledge of (E)-azastilbenes are purposeful. A continuation of investigations are especially advisable because of their antimicrobial and anticancer activities.

CONCLUSIONS

A method of separation of a mixture consisting of chloride of (E)-N-(m-chlorobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(m-chlorobenzyl)-2′-hydroxystilbazole has been elaborated upon. For the determinations a new terminating electrolyte was used, i.e., a solution of 1-(N-morpholiniomethyl)spirobi(1-sila-2,5-dioxacyclopentan-3-on)ate.

(E)-azastilbenes can be successfully analyzed by capillary electrophoresis. A new, elaborated method of separation and determination opens wide possibilities of investigation of the biological activities of these kinds of substances.

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