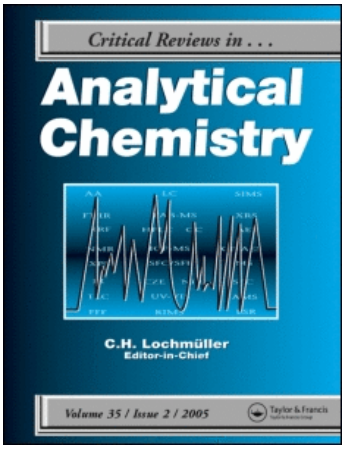

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Analysis of Biologically Active Stilbene Derivatives

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An attempt of synthesis of information concerning chosen stilbene derivatives has been made. These compounds show biological activity that is very important to the whole ecosystem. There are known such derivatives of stilbene that are characterized by a confirmed anti-cancer, antibacterial and fungistatic activity. Some of them have positive influences on the cardiovascular system. Some others have properties of liquid crystals and the ability of complexing. Stilbenes and their various derivatives are still arousing immense interest. High hopes are connected with the possibility of more and wider pharmacological use and curing different diseases.

Keywords (E)-azastilbenes, biological properties, determination, chromatography, capillary electrophoresis

INTRODUCTION

A need of synthesis of more and more new medicines counteracting diseases typical for contemporary civilization (often incurable) leads to necessary development of chemistry. This development includes synthesis of new compounds, testing of their biological activity as well as their determination in different matrices by different techniques.

(E)-azastilbenes belong to a group of compounds widely investigated in the whole world. This fact is confirmed by numerous works published in different papers. Particular interest in these compounds is caused by their biological activity, antibacterial and anti-cancer activity and their use as liquid crystals or complexing agents.

Until now, published reviews concerning chemistry of chosen stilbene derivatives described only methods of synthesis, structures and properties of these compounds (1). Every year the number of papers dedicated to these compounds increased. This confirms rising interest of scientist in this group of molecules. Therefore, this work is an attempt of synthesis of information significantly widened by problems of separation and determination of stilbene derivatives by HPLC (high performance liquid chromatography) and ITP (isotachophoresis) techniques. Special attention was paid to properties and analysis of these compounds. Because they are ionogenic, in the work in addition to typical chromatography, also, electromigrating techniques were included. Optimization of separation and determination processes of (E)-azastilbenes by different techniques certainly will be useful in further research of chemistry of these molecules.

Capillary electromigrating techniques are characterized by reliability, good accuracy and precision of determinations. Capillary ITP, which was used to separate chosen compounds and their isomers (2–4) can serve as an example. This technique is applied more and more often in analytic laboratories. High efficiency of resolution and short time of analysis causes the ITP method to compete with HPLC as well as with other analytical techniques (4).

(E)-azastilbenes are nitrogen-containing derivatives of stilbenes. They have the structure of diphenylethylene. At present, investigations concerning stilbenes as alternatives for antibiotic growth stimulants are carried out in many scientific centers. These compounds can be synthesized in plants from coumaric acid and cinnamic acid. They can also be formed from chalcones and flavonols. They have fungistatic properties (they restrain growth of fungi), fungi toxic (they destroy fungi) and estrogenic properties (5).

Natural stilbenes do not induce tumors and do not have harmful side effects, typical for synthetic stilbestrol. However, they should not be given to healthy men. Their estrogenic and antiandrogenic activity can slow down activity of testosterone. On the other hand, they are recommended to men suffering prostatism. Natural stilbenes, because of estrogenic activity, can be used in the prevention of menopause effects in women, in preventing of degenerative changes of hair glands and hair follicles. They can be also used in the treatment of androgenic acne (6).

Stilbenes occur in coniferous plants (fir tree *Abies*, spruce *Picea*, pine *Pinus*, juniper *Juniperus*, rhubarb *Rheum* and mulberry *Morus*). Natural stilbenes have positive influences on organisms, because they stimulate processes of proteine synthesis,

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increase retention of nitrogen, and accelerate growth and development. These compounds can also be applied as natural anabolics in the breeding of animals. Their estrogenic activity keeps in an organism sodium and water. Moreover, they increase the concentration of alfa lipoprotein and decrease the concentration of cholesterol in blood. They stimulate development of bones and cartilages. They increase blood clotting by blocking of blood clotting inhibitors. They widen blood vessels, improve circulation, and increase penetration through blood vessel walls (7–10).

Phytoalexin and resveratrol (resveratrolum), among others, are natural stilbenes. Resveratrol similar to other stilbenes, has estrogenic, bacteriostatic and fungistatic activity. It prevents prostate tumors in men. Plants rich in resveratrol can be used as growth stimulants in the breeding of animals. Resveratrol is a phytoncide that modifies a qualitative and quantitative composition of micro flora in an alimentary canal, similar to antibiotic growth stimulants used earlier. Well composed natural alternatives of antibiotic stimulants should be characterized by the following properties: they can change composition and topographic placement of microflora and microfauna in an alimentary canal; they will restrain growth of bacteria, such as *Clostridium sp*., *Staphylococus sp*., *Streptococus sp*., *Baccillus subtilis et cereus*, *Escherichia coli*, *Salmonella sp*., and *Shigella sp*.; they can restrain the processes of decay and fermentation in an alimentary canal; they decrease production of ammonia, free aliphatic acids, ketones and aldehydes in an alimentary canal; and they can stimulate excretion of digestive juices (7–9).

IMPORTANCE OF STILBENES FOR THE ECOSYSTEM

Biological Activity

(E)-azastilbenes are known for their biological activity, especially their anti-microbial properties. *Trans*-styrylpyridines, which are effective inhibitors of choline acetyltransferase (11, 12), can serve as an example. It was shown that N-substituted derivatives of (E)-4′ (3′, 2′) hydroxystilbazole-4 are bacteriostatic and fungistatic. Tests were carried out on the following strains: gram-positive cocci (*Staphylococcus aureus* 209P FDA, *Streptococcus 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).

Minimum concentration of an inhibitor (MIC) was determined by treating the investigated micro-organisms with a series of prepared (E)-azastilbenes in DMSO with concentration from 1 to 1000 μg/mL and further incubation for 3–7 days at 25°C. The best results (i. e., the lowest MIC) showed bromides of N-(o-, m-, p-)brombenzyl -(E)-2′-hydroxystilbazole-4 used against *Staphylococcus aureus* with the value of 5 μg/mL and bromide of N-(m-bromobenzyl)-(E)-4′-hydroxystilbazole-4 and chlorides of N-(o-, m-)chlorobenzyl -(E)-2′-hydroxystailbazole-4 used against *Staphylococcus aureus* with the value of MIC equal to 7.5 μg/mL. Other compounds showed MIC value over 100 μg/mL (11).

Significantly better choline acetyltransferase inhibiting properties show stilbazoles with a large, not polar, substituent in position 3′ (3′-CH_3, 3′-Cl and 3′-CH_3O) (6, 12). The methoxy group was introduced to the phenyl ring during synthesis of new derivatives, in order to improve anti-microbial properties of N-substituted haloids of (E)-4′-(3′, 2′)-hydroxystilbazoles. N-substituted haloids of (E)-4′hydroxy-3′-methoxystilbazoles-4 showed high activity against various strains, not only *Staphylococcus aureus*.

The introduction of the new substituent caused a significant increase of MIC for bacteria of *Streptococcus faecalis* and *Bacillus subtilis*. The procedure of MIC determination was the same as in the case of N-substituted derivatives without a methoxy group. The same strains of micro-organisms were used in the investigations. The highest activity showed following four compounds: bromide of (E)-N-hexadecyl-4′-hydroxy-3′-metoxystilbazole-4 against *Staphylococcus aureus, Streptococcus faecalis* and *Bacillus subtilis* (MIC = 5 μg/mL); bromide of (E)-N-dodecyl-4′-hydroxy-3′-methoxystilbazole-4 against *Streptococcus faecalis* (MIC = 7.5 μg/mL) and *Staphylococcus aureus* (MIC = 10 μg/mL); bromide of (E)-N-bromodecyl-4′-hydroxy-3′-methoxystilbazole against *Staphylococcus aureus* (MIC = 7.5 μg/mL) and against *Streptococcus faecalis* (MIC = 10 μg/mL); and bromide of (E)-N-decyl-4′-hydroxy-3′-methoxystilbazole against *Staphylococcus aureus* (MIC = 7.5 μg/mL) and against Streptococcus faecalis (MIC = 10 μg/mL) (12).

In cases of other (E)-N-stilbazoles, MIC values were equal or higher than 100 μg/mL [similarly as in the case of N-substituted (E)-4′(3′, 2′)hydroxystilbazoles-4] (11).

Anticancer Activity

(E)-azastilbenes are investigated also considering their anti-cancer activity. It was confirmed that some of them are characterized by such properties. Especially important activities show, first of all, two compounds, stilbazole derivatives of sulfacetamides. Anti-cancer properties of HMN-176 [i.e., (E)-4{[2-N-(4-methoxybenzenesulfonyl)amino]stilbazole}1-oxide] and HMN-214 [(E)-4-2-[2-(N-acetyl-N-[4-methoxy benzenesulfonyl]amino)stilbazole]}1-oxide]. Formulas for the above-mentioned compounds are shown in Fig. 1.

Properties of these compounds were compared with properties of therapeutically used preparations [*cis*-platinum (CDDP), adriamycin (ADM), etoposide (VP-16), taxol and vincristine (VCR)]. Investigations were carried out on 22 groups of human cancer cells isolated from different tissues (two cases of cancer of cervix, two of leukemia, two of prostate, two of pancreas, three of stomach, three of breast, five of lungs, and three of colon). Effective cytoxicity of HMN-176 has been confirmed. Its effectiveness after intravenous and intraperitoneal administration of the solution in 0.9% aqueous sodium chloride was better than that of ADM, VP-16 and CDDP, but lower than of taxol

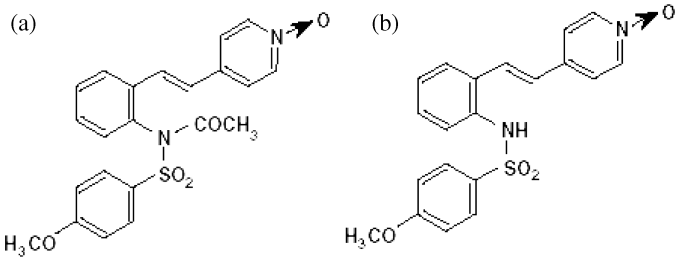


FIG. 1. Structures of stilbazole derivatives of sulfacetamides: a) HMN-214 and b) HMN-176.

or VCR. Lower citotoxicity than HMN-176 showed (E)-4-2-[2-(N-acetyl-N-[methoxybenzenesulfonyl]amino)stilbazole]1-oxide (13).

A series of 43 other stilbene derivatives were investigated concerning anti-cancer activity, which exhibited toxicity towards human lungs carcinoma (A549) (14). They were subjected to a different sort of molecular analysis and modeling of genesis and progress of cancer process.

Influence of Stilbenes on Blood Circulation

Resveratrol (3,5,4′-trihydroxystilbene) belongs to natural stilbenes, which as polyphenol has three hydroxy groups and two aromatic rings (Fig. 2). It appears in plants in a *cis* or *trans* form. Biological activity exhibits only *trans* isomer. Resveratrol can occur also in form of glucoside. Stilbenes are widespread in roots and wood of numerous plants, and also in stems, leaves and fruits. Stilbenes have fungistatic activity and can cause anti-platelet action (15–19). Stilbene influence on blood circulation was most frequently described using resveratrol as an example.

Resveratrol belongs to the group of phenols and occurs in the skin of many fruits, e.g., in red grapes, mulberry, blackcurrant and in peanuts. Resveratrol is a very effective anti-oxidant and not a toxic fungicide (antifungi agent); therefore, it is often used as a food additive. A grand scale is obtained from the dried skin of red grapes (15, 19).

Trans-resveratrol has the ability to activate the SIRT1 gene, which is present in a genome of all mammals (16). *Trans*-resveratrol can be transformed in *cis* isomer by action of UV radiation.

Resveratrol is synthesized by plants using stilbene synthase enzyme. Besides anti-oxidant and anti-fungi properties, it has

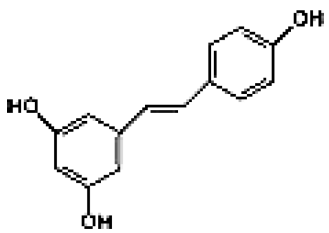


FIG. 2. Structure of *trans*-resveratrol.

not quite explained the ability to prolong the life of mammals. Initially, there was a theory that it can control a speed of metabolism, but investigations did not confirm this assumption. Probably its way of life prolongation is complex and includes several mechanisms, starting from simple anti-oxidant action (similar to action of vitamin C) and activation of SIRT1 gene up to blocking of apoptosis, i.e., genetically programmed cell death (18).

The structure of stilbene was described for the first time in 1829. Then many different stilbene derivatives were synthesized and some of them showed anti-microbial action. Investigations of stilbene properties were started beginning from observations of their antibiotic activity against bacteria and fungi. It was shown by natural stilbene derivatives isolated from various plants (7–9).

Resveratrol occurs in over 70 plants, most of them are edible. Its main sources in the human diet are grapes and wines produced from them, and in less extent peanuts (*Arachidis hipogea*), and fruits of mulberry, apricots and pineapples. It is present in medicinal plants such as: *Cassia quinaquangulata*, *Cassia garrettiana*, *Ficus barteri* (fruits), *Reynoutria japonica*, *Erythroplenum lasiantum* and *Polygonum cuspidatum* (roots) used in Chinese and Japanese folk medicine in diseases of blood circulation. Oxyveratrol occurs as a natural ingredient in *Morus alba* and *Scirupus maritimus*. Isomers of resveratrol were also identified in genus *Gentum* and in *Sophora leachiana* (8–10, 21).

The concentration of resveratrol in grapes is 50–400 μg/g in fresh plants, in red wines produced from grapes 0.92–1.37 mg/mL, white wines 0.04 mg/mL, and in grape juice 0.05 mg/mL (22). The present state of investigations concerning the role of resveratrol in human diet and the mechanism of its action is unsatisfactory and fragmentary. The biological role of resveratrol can be connected with various processes. These problems were a subject of numerous papers (23–27).

Resveratrol contained in plants is transferred by alimentary canal to different parts of the human organism. Its concentration is small in blood as well as in various organs, but sufficient to have an influence on their action. Distribution of resveratrol in human blood is as follows: plasma-54.8%, erythrocytes-36.0%, leukocytes-4.9% and platelets-1.2% (21). Many investigations

proved its positive influence on the function of blood circulation (15, 18, 24).

Resveratrol, similarly to flavonoids, belong to the most important polyphenols present in wine and possessing anti-oxidant properties. Comparing the action of resveratrol and other polyphenols affirmed that the most important in these compounds is the presence of hydroxy group 4′ in the ring B and metahydroxy structure in the ring A, because they are crucial to the anti-oxidant action of stilbenes (25).

Until now, investigations carried out in vitro and in vivo show that resveratrol restrain some steps of blood platelets activation. It reduces a synthesis of tromboxane A2, a compound increasing reactivity of platelets, and limits a cramp of blood vessels (26–30).

Resveratrol restrains the first step of platelets activation, that is adhesion of platelets to collagen and fibrinogen (28, 29) and restrains aggregation of platelets caused by different agonists (trombine, ADP, collagen) (28, 31). An aggregation of platelets caused by collagen is reduced in 50% in the presence of resveratrol concentration 3.6 μg/L. Red wine containing natural resveratrol in 1.2 μg/L concentration only and other polyphenols (3.6 μg/L) caused inhibition of aggregation induced by collagen in 42%. With an addition of resveratrol in order to obtain a 2.4 μg/L concentration, the inhibition of aggregation was increased to 78%. Executed investigations exhibited interaction between resveratrol and other ingredients of red wine. In the presence of other polyphenols an amount of resveratrol necessary to the inhibition of platelet aggregation can be smaller (15, 31). Resveratrol also decreases a level of reactive forms of oxygen in blood platelets (28).

Reseveratrol has also a positive influence on lipid metabolism. A significant decrease of intracellular concentration of apolipoprotein B occurs as a response to increasing concentration of resveratrol. Moreover, the concentration of esters of cholesterol and triglycerides is decreased in the presence of resveratrol. It suggests a decrease of LDL and VLDL production (32–37).

Properties of Liquid Crystals and Formation of Complexes

(E)-azastilbenes unsubstituted in the N position have on the nitrogen atom a free electron pair. They are bases according to Lewis' theory. The free pair of electrons cause these compounds to be good ligands. These compounds are well-known connections of *trans*-styrylpyridines with transition metals (such as silver, ruthenium, osmium, zinc, wolfram, molybdenum) (38–41).

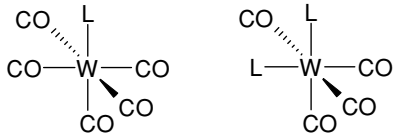


FIG. 3. Structures of complex derivatives of (E)-azastilbene compounds: a) with one azastilbene ligand and b) with two azastilbene ligands.

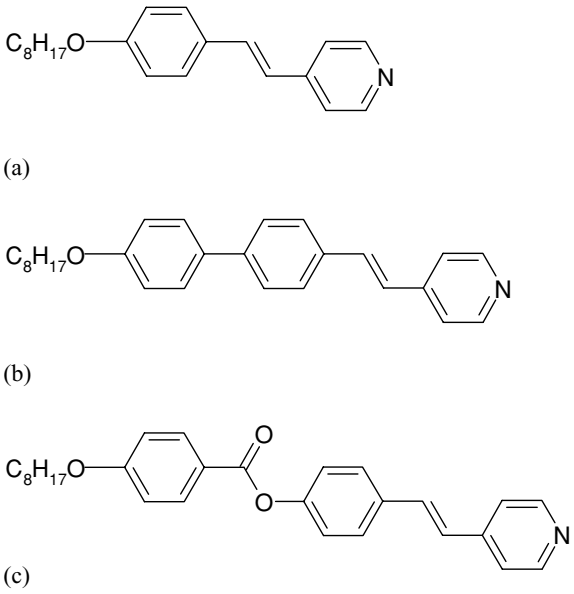


FIG. 4. The structure of ligands [of the derivatives (E)-azastilbenes] used to complex reaction.

Vanadium complexes are hexacoordinated. Azastilbenic ligand occupies one or two places. The rest places are occupied by carbonyl ligands. Three different azastilbene derivatives differing by a substituent in phenyl rings were used for the synthesis of this type of compound. Generally, six complex compounds with various properties were synthesized. Structures of obtained compounds are shown in Fig. 3 (38). Structures of ligands are shown in Fig. 4.

Next metal forming complexes with stilbazoles is molybdenum, which belong to the six group of periodic system. It forms a mixed complex compound, where (E)-azastilbene presents one ligand. Three different azastilbene derivatives differing by

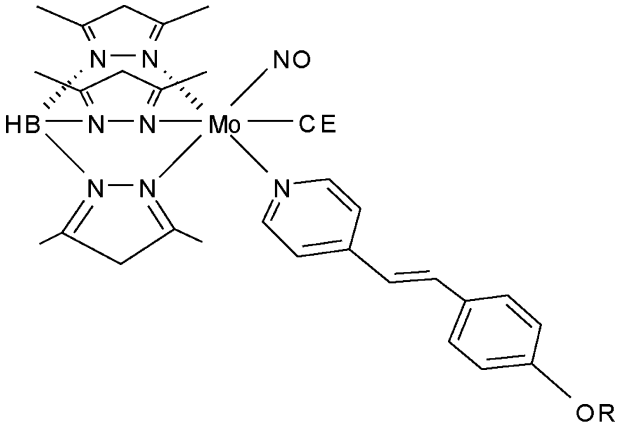


FIG. 5. Structure of molybdenum complex with (E)-azastilbene substituted as a ligand, where R = C_6H_{13}, C_8H_{17}, $C_{11}H_{23}$.

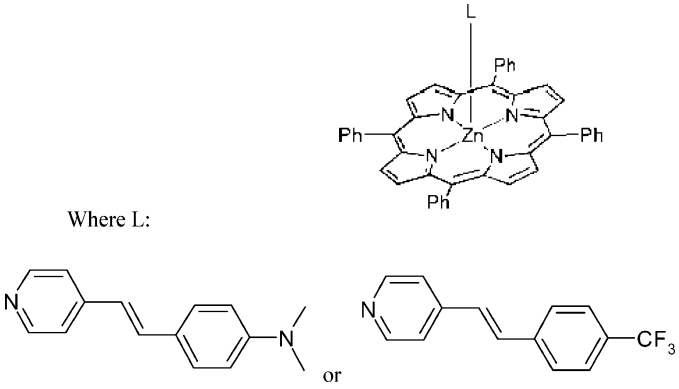


FIG. 6. Scheme of axially substituted metalloporphyrine complexed with zinc and (E)-stilbazole.

a substituent in phenyl rings (39) were used for the synthesis of this type of compound. The structure of this complex is shown in Fig. 5.

Especially interesting complex structures form (E)-stilbazoles as ligands with metalloporphyrines. Porphyrine is axially substituted by phenyl groups and complexed with zinc forming two kinds of complexes with two different azastilbenes (40). The structures of these complexes are shown in Fig. 6.

Next metalloporphyrines, which form complexes with (E)-azastilbenes, are porphyrines complexed with ruthenium (II) and osmium (II). The metals in these compounds form bonds with the nitrogen atoms of pyrrole rings, with the nitrogen atom in aromatic ring of (E)-azastilbene and with carbonyl group (Fig. 7) (40). (E)-azastilbenes also form complexes with silver ions. Earlier compounds described were mixed complexes, that is (E)-azastilbezole was not only ligand complexing metals. Ag^+ ion is complexed by two molecules of 4-alkoxy-4′-stilbazole (41). The general structure of this complex compounds is shown on Fig. 8. There are also complex silver compounds with 4-alkoxy-3′-stilbazole and 4-alkoxy-2′-stilbazole (41).

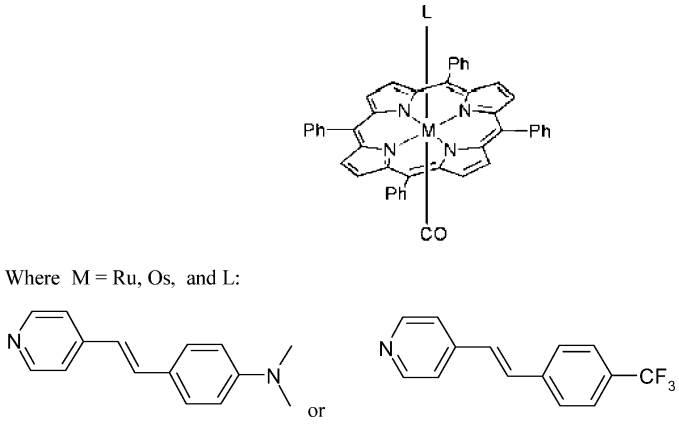


FIG. 7. Axially substituted metalloporphyrine complexed with (E)-azastilbene and carbonyl group.

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

Preparation of Samples for Analysis

Biological activity of stilbenes and their derivatives is the reason for special interest in this group of compounds. This interest also includes (E)-azastilbenes. Their biological activity was proved by Cavallito et al. (42–44) and Arena et al. (45). It is interesting that some of them can be estrogenic or antiestrogenic, depending on a concentration. Because of biological activity and a wide possibility of application, stilbenes and their derivatives are one of the more important group of compounds. Their synthesis and/or extraction from different environmental matrices cause many troubles because of the possibilities of the appearance of various isomers (Fig. 9). Therefore, proper preparation of samples and then their chromatographic analysis is an important task.

Due to immense development of specific packing liquid-solid extraction is the most often used isolation method of compounds from different matrices. However, the most widespread separation and determination technique is still HPLC (46–50). For many stilbene derivatives optimum conditions of determination were elaborated by HPLC and capillary electrophoresis techniques (51, 52); therefore, these methods are reviewed in this paper.

Preparation of samples for an analysis usually requires primary isolation of compounds, often from a very complicated matrix. It especially concerns HPLC analyses. Solution analyses by electrophoresis are easier. This technique does not require concentration, and contents of determined compounds do not need be comparable. Stilbenes and their derivatives described here are hydrolytic stable. Due to this fact, water and water-containing solvents can be used, depending on a need.

Analysis of biologically active substances is of great importance in pharmacy and medicine. For isolation and separation of complex mixtures, an application of selective columns is usually necessary. Stability and reproducibility of columns, extractive as well as chromatographic, is essential for proper analytic procedure. In chromatography and related techniques, the most important is the selection of mobile and stationary phases with reference to determined substances.

In solid phase extraction (SPE) and HPLC column packings with various polarity are applied. Silica stationary phases are

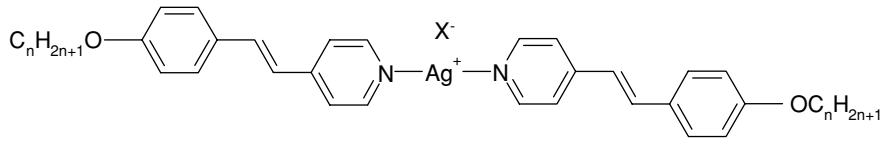


FIG. 8. General structure of complex of (E)-4-stilbazole with silver ion, where $X^- = BF_4$, NO_3, $C_8H_{17}OSO_3$, $C_{12}H_{25}OSO_3$.

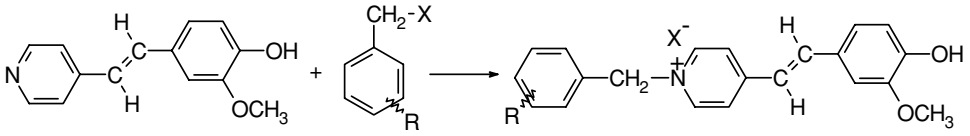


FIG. 9. General reaction scheme of chosen (E)-azastilbene preparation, where X = Br, Cl, I and R = Cl, Br, NO_3.

widespread in extraction as well as in chromatography. They are used mainly in reverse phase systems; the most important are modified sorbents (Fig. 10).

The process of retention in reverse phase chromatography is based on specific and unspecific interactions between stationary phase, mobile phase and chromatographed substance. Optimization is performed by changes of type, composition and character of a mobile phase or changes of type, properties and typography of a stationary phase (53, 54). Effectiveness of these two phases in separation of a mixture depends on thermodynamic properties of the chromatographic system. A column is an essential point of a separation system.

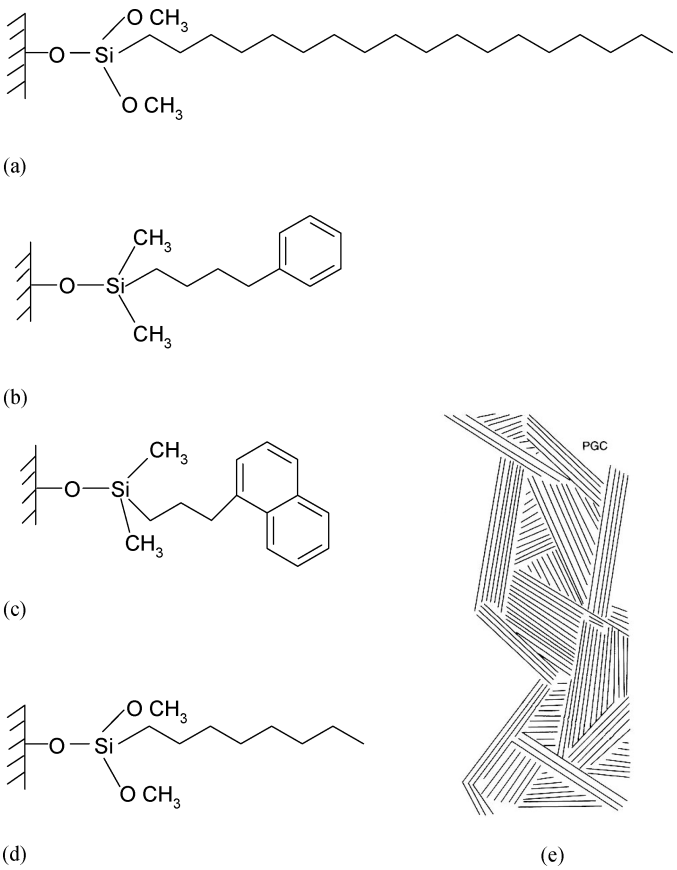


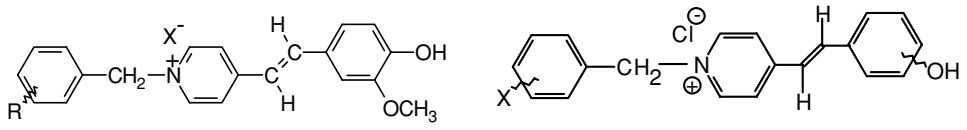
FIG. 10. Scheme of chemically bonded stationary phases: a) octadecyl, b) phenylbutyl, c) naphthylpropyl, d) octyl and e) PGC.

Separation and Determination of Chosen (E)-azastilbenes by HPLC and ITP Techniques

Among techniques used for the determination of stilbenes and their derivatives capillary electrophoresis is especially important. Effective separation and determination of a series of these compounds were carried out using a capillary electrophoresis analyzer EA 202M produced by Villa Labeco s.r.o. in Spisska Nova Ves (Slovakia) (Fig. 11).

The separation of the compounds was performed on the base of differences of analyzed ion mobility. The difference between ion mobility in the analyzed mixture was only slight; therefore, isotachophoretic determinations caused numerous troubles. In order to do this it was necessary to have an elaboration of suitable terminating electrolytes. Two ionic compounds were used in the investigations: 1-(N-morpholiniomethyl)spirobi(1-sila-2,5-dioxacyclopentan-3-on)ate and 4,4′-bis{1-(perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentan-3-on)]ate} (Fig. 12) (55, 56). These compounds belong to the ES -silanate group. They are hypercoordinated organosilicon compounds, which are characterized by substantiated biological activity (56–59). Optimum conditions for a method of separation and determination of a mixture chloride of (E)-N-(m-chlorobenzyl)-4′-hydroxystilbazole -4 (A1) and chloride of (E)-N-(m-chlorobenzyl)-2′-hydroxystilbazole-4 (A3) are shown in Table 1. The final result of the elaboration of optimum conditions is confirmed by obtained isotachophoregram (Fig. 13). Good separation of two isomers was obtained in less than 7 minutes (60).

During determinations carried out by ITP techniques, the applied electric field causes that ion of samples introduced between a system of two electrolytes—leading and terminating—to migrate to adequate potential. Ions move in turn from the highest mobility to the lowest.



(a) (b)

FIG. 11. Examples of structures of chosen (E)-azastilbenes, subjected to optimization of chromatographic separation and determination conditions: a) bromides of (E)-N-o-(m- or p-)nitrobenzyl-4′-hydroxy-3′-methoxystilbazoles, where X = Br, R = o-NO_2, m-NO_2, p-NO_2, b) chlorides of (E)-N-o(m- or p-)chlorobenzyl-γ-azastilbeneols-2′(3′ or 4′), where X = o-Cl, m-Cl, p-Cl.

During the optimization of the separation and determination process of the above-mentioned (E)-azastilbenes different conditions of methods were tested. The time of analysis, pH range (from 0–7, because cationic forms were analyzed), the intensity of electric current, the level of limitation of high voltage, and the kind of columns (pre-separational column or pre-separational column with analytic column) were changed. Analyses were carried out changing the voltage from 9,000 to 15,000 V. At the voltage lower than 9,000 V separation was not achieved. The best results of separation were obtained at limitation of the voltage to 12,000 V.

Analyses were carried out in acidic medium. During one analysis, only ions of the same sign were determined, in this case cations. The separation was performed on the base of differences in electrophoretic mobility of analyzed ions.

By the use of the capillary ITP method proper separation and determination of analyzed (E)-stilbenes have been performed. All analyses were performed applying two-dimensional analysis with switching the column. Qualitative analysis was carried out on the base of zone height on obtained isotachophoregrams. These heights of zones were compared with those obtained on isotachophoregrams of standard solutions.

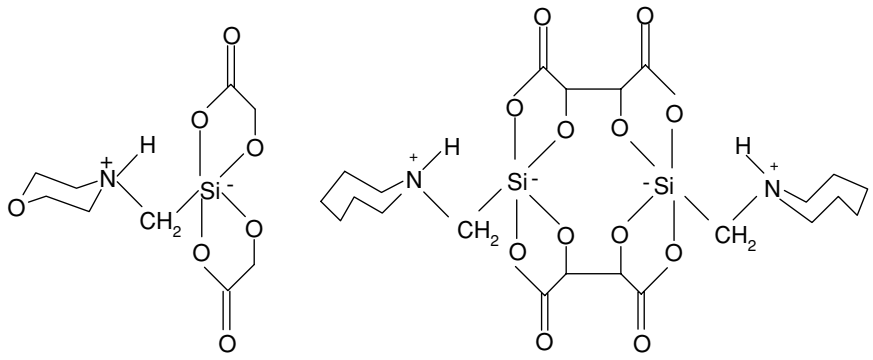
Separation of (E)-azastilbene isomers was very difficult, because these compounds have only different positions of the substituents in the ring and because of that are characterized by similar mobility. During the process of optimization, the intensity of the electric current was changed (it was justified by the mobility dependence on the electric field intensity), along with 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 a pH close to neutral, isotachophoregrams were characterized by steep zones for investigated mixtures and a blurred zone for the terminating electrolyte.

At a pH of 3.8, it was necessary to select adequate intensity and time for individual steps of analysis. All things considered, selection of these three parameters allowed the optimum separation of analyzed isomers to be obtained (Fig. 13).

Time of analysis was variable and depended on a number of compounds in analyzed samples. The shortest times were obtained during determination of the individual compound. However, times of analysis of mixtures were twice as long.

The applied technique was characterized by high precision and accuracy of obtained results (Table 2). Linearity was between 2 and 30 mg/L and the detection limit was 1 mg/L. The precision and accuracy of results obtained by the capillary ITP method is better than in classic methods.

High efficiency of separation by capillary ITP is comparable with electrophoresis methods, showing that ITP can, in many points, compete not only with HPLC but also with other techniques.



(a) (b)

FIG. 12. Scheme of structures of compounds used as terminating electrolytes to determinations by ITP method: a) 1-(N-morpholiniomethyl)spirobi(1-sila-2,5-dioxacyclopentan-3-on)ate and b) 4,4′-bis1-(perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentan-3-on)]ate.

TABLE 1
Optimum conditions of determination of a mixture chloride of (E)-N-(m-chlorobenzylo)-4′-hydroksystilbazolu-4 (A1) and chloride of (E)-N-(m-chlorobenzylo)-2′-hydroksystilbazolu-4 (A3)

Optimum parameters					
Step	Time (s)	Intensity (μA)	Composition (10 mV)	Column	Conductometric determination
1	100	100	0	Upper	
2	150	250	0	Upper	X
3	65	10	0	Lower	
4	10	120	0	Lower	
5	25	100	50	Lower	
6	100	30	0	Lower	X

ITP enables good separation and determination of stilbene derivatives. The main advantage of ITP is the possibility of simultaneous determination of micro and macro ingredients in times under 20 minutes. No complicated preparation of samples is needed before analysis. It is convenient for routine analytical procedures. No toxic reagents and solvents are needed; therefore, ITP can be numbered among green chemistry techniques.

The next technique commonly used in different analyses is HPLC. Analysis of three isomers (E)-N-(o-, m- and p-) nitrobenzyl-4′-hydroxy-3′-methoxystilbazoles-4 were carried out at wavelength 412 nm. Only anhydrous mobile phases were used—acetonitrile, methanol and dichloromethane with various flow. Because of long retention times and bad separation water containing solvent mixtures were not considered. Five different stationary phases were tested—octadecyl, octyl, phenylbutyl, naphthylpropyl and porous graphitized coal (PGC) (51–54).

The octadecyl column was used as a reference column. The best results of separation were obtained using naphthylpropyl as the chemically bonded phase and acetonitrile (100%) as the mobile phase. Optimum time of separation and determination in these conditions did not exceed 6 minutes (Fig. 14).

However, the same solvent system and PGC yielded slightly shorter retention time, but did not allow for satisfactory separation. The phenylbutyl column was characterized by better selectivity, but times of retention were few minutes longer.

The octyl phase was characterized by relatively weak interactions between analyzed compounds and the stationary phase; independently of flow intensity separation was not observed. The use of methanol and dichloromethane gave results comparable with results obtained applying acetonitrile.

The PGC column was characterized by other kinds of surfaces than the remaining columns (Fig. 10). It yielded shorter times of retention but worse selectivity.

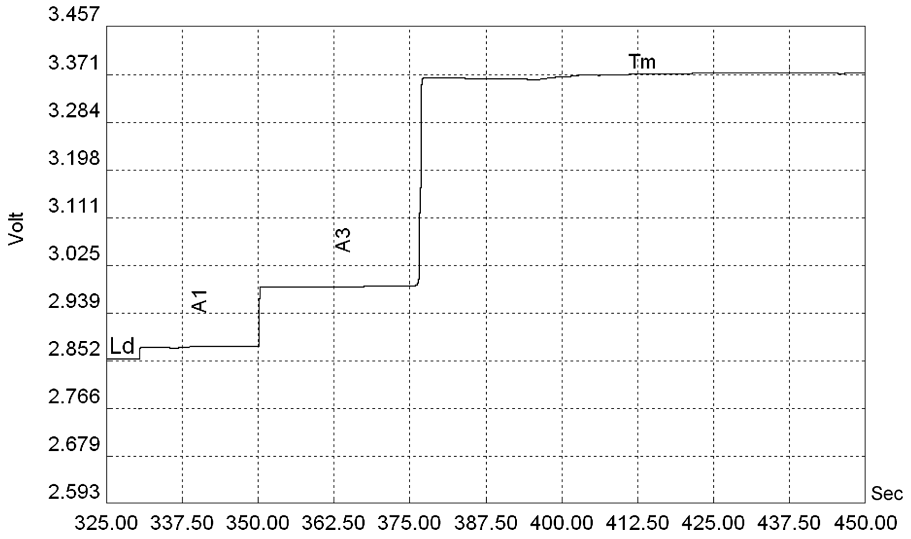


FIG. 13. Isotachophoregram of a mixture chloride of (E)-N-(m-chlorobenzylo)-4′-hydroksystilbazolu-4 (A1) and chloride of (E)-N-(m-chlorobenzylo)-2′-hydroksystilbazolu-4 (A3).

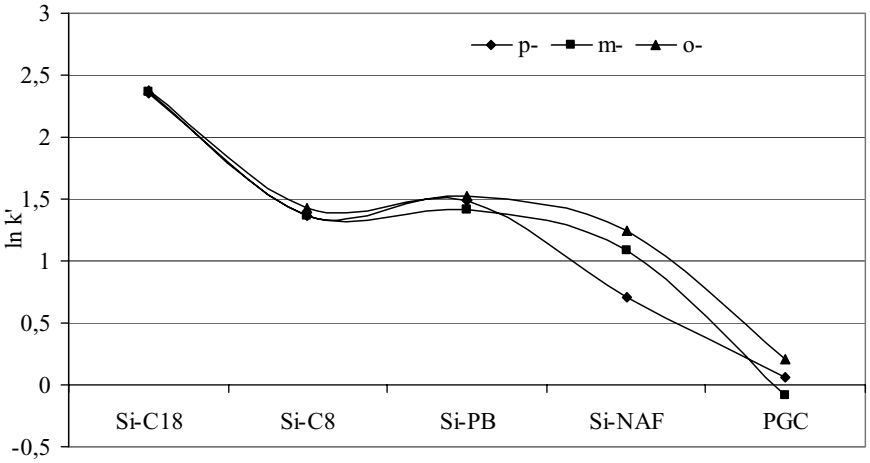


FIG. 14. Results of separation chlorides of (E)-N-(o-, m- or p-)nitrobenzyl-4′-hydroxy-3′-methoxystilbazoles-4′ with the use of stationary phases: Si–C_{18}(octadecyl), Si–C_8 (octyl), Si–PB (phenylbutyl), Si–NAF (naphthylpropyl), and PGC. The mobile phase: acetonitrile (100%).

SUMMARY

In summary, one can say that chromatographic investigations concerning this group of compounds, especially separation of isomers, is connected with numerous difficulties, independent of the analytical technique used. However, these investigations leading to better knowledge of this group of compounds are advisable because of biological activity and the wide possibilities of application. In particular, anti-cancer and anti-microbial activity is a convincing argument for this research. Therefore, stilbenes and their derivatives are investigated by many scientists all around the world.

For determinations of these types of compounds, HPLC as well as capillary electrophoresis can be used. Many mixtures of (E)-azastilbenes have been successfully separated. Optimum conditions have been elaborated using ITP with conductometric detection, in spite of small differences of electrophoretic mobility of analyzed compounds. New terminating electrolytes were used: solutions of 1-(N-morpholiniomethyl)spirobi(1-sila-2,5-dioxacyclopentan-3-on)ate and 4,4′-bis{1-(perhydroazepiniomethyl)spirobi(1-sila-2,5-dioxacyclopentan-3-on)]ate}.

TABLE 2
Characteristic of used analytical method

Parameter	Unit	For examined ion
Precision[1]	%	2–3.5
Recovery[2]	%	92 ± 4
Linearity[3]	mg/L	2–30
Limit of identification[4]	mg/L	1

[1]n = 5, the samples were analyzed twice
[2]The sample was enriched with 1.5 mL of a solution containing 1 mg/mL of examined ion, n = 5
[3]Correlation coefficient above 0.97
[4]Calculated from the limit of identification and coefficients of the calibration curve

Optimum conditions of separation and determination of these compounds have been elaborated with success also using the HPLC technique. Independent of the kind of mobile phase, the highest selectivity exhibited naphthylpropyl stationary phase. In addition to high selectivity, this phase was characterized by the shortest retention times of analyzed compounds. The main reason of this behavior was the presence of additional interactions of π electrons between stationary phase and analyzed compounds. Then, the PGC phase was characterized by the shortest times of retention of analyzed compounds but separation was unsatisfactory. These differences can be explained by differences of the structure of surfaces. The phenylbutyl phase exhibited even worse separation and longer times of retention of analyzed (E)-azastilbenes. The longest retention times were obtained using alkyl phases.

Optimization of conditions of determination of this group of compounds by HPLC and capillary electrophoresis certainly will contribute to the development of the chemistry of stilbenes and their derivatives. This work is an attempt of synthesis of information concerning biologically active stilbene derivatives and would enable further progress in the chemistry of stilbenes.

REFERENCES

1. B. Yin, F. Niemeyer, J. A. Gareth, W. Ji Jiang, A. Boucekkine, L. Toupet, H. Le Bozec, and V. Guerchais, Synthesis, structure, and photophysical properties of luminescent platinum (II) complexes containing cyclometalated 4-styryl-functionalized 2-phenylpyridine ligands. *Inorganic Chemistry* 45 (2006):8584−8596.

2. M. Hutta, D. Kaniansky, E. Śimuniéova, V. Zelenska, V. Madajova, and A. Śiśkova, Solid phase extraction for sample preparation trace analysis of ionogenic compounds by capillary isotachophoresis. *Journal of Radioanalytical and Nuclear Chemistry* 1, 163 (1992):87–98.
3. F. M. Everaerts, J. Beckers, and L. Verheggen, Isotachophoresis. Theory, instrumentation and applications. *Journal of Chromatography Library (JournalSeek)* 6 (1976):418–428.
4. J. Østergaard, Application of retention factors in affinity electrokinetic chromatography and capillary electrophoresis. *Analytical Sciences* 23 (2007):489–492.
5. E. Wyrzykiewicz, W. Prukała, and B. K¸edzia, Synthesis and antimicrobial properties of N-substituted derivatives of (E)-alpha-(or gamma)-azastilbenols. *Ill Farmaco* 49 (1994):127–131.
6. B. R. Baker and R. E. Gibson, Irreversible enzymne inhibitors. Inhibition of choline acetyltrasferase. Mode of binding by 4-stilbazoles. *Journal of Medicinal Chemistry* 15, 6 (1972):639–648.
7. V. Bangani, N. R. Crouch, and D. A. Mulholland, Homoisoflavanoes and stilbenoids from Scilla nervosa. *Phytochemistry* 51 (1999):947–951.
8. O. Daniel, M. S. Meier, J. Schlatter, and P. Frischhnecht, Selected phenolic compounds in cultivated plants: Ecologic functions, health implications, and modulation by pesticides. *Environmental Health Perspectives* 107 (1999):109–114.
9. J. Bais, P. J. Murphy, and I. B. Dry, The molecular regulation of stilbene phytoalexin biosynthesis in Vitis vinifers during grape berry development. *Australian Journal of Plant Physiology* 27 (2000):425–433.
10. J. D. Hipskind and N. L. Paiva, Constitutive accumulation of a resveratrol-glucoside in transgenic alfaincrases resistance to Phoma medicaginis. *Molecular Plant-Microbe Interactions* 13 (2000):551–562.
11. E. Wyrzykiewicz and W. Prukała, Synthesis and antimicrobial properties of N-substituted halides of (E)-azastilbenols. *Il Farmaco* 50 (1995):779–782.
12. W. Prukała and B. Kędzia, Antimicrobial activity of some N-alkyl and N-nitrobenzyl substituted halides of (E)-4′-hydroxy-3′-methoxystilbazoles-4. *Il Farmaco* 54 (1999):584–587.
13. M. Takagi, T. Honmura, S. Watanabe, R. Yamaguchi, M. Nogawa, I. Nishimura, F. Katoh, M. Matsuda, and H. Hidaka, In vivo antitumor activity of a novel sulfonamide HMN–214, against human tumor xenografts in miceand the spectrum of cytotoxicity of its active metabolite HMN–176. *Investigational New Drugs* 21 (2003):387–399.
14. S. Kim, S. Y. Min, S. K. Lee, and W. J. Cho, Comparative molecular field analysis study of stilbene derivatives active against A549 lung carcinoma. *Chemical & Pharmaceutical Bulletin* 51,5 (2003):516–521.
15. A. Bertelli, L. Giovannini, D. Z. Giannessi, M. Migliori, W. Bernini, M. Fregoni, and A. Bertelli, Anti-platelet activity of synthetic and natural resveratrol in red wine. *International Journal of Tissue Reaction* 17 (1995):1–3.
16. A. Bertelli, L. Giovannini, W. Bernini, M. Migliori, M. Fregoni, L. Baveresco, and A. Bertelli, Anti-platelet activity of cis-resveratrol. *Drugs Under Experimental and Clinical Research (Geneva)* 22 (1996):61–63.
17. T. A. Aburjai, Anti-platelet stilbenes from aerial parts of Rheum palaestinum. *Phytochemistry* 55 (2000):407–410.
18. L. M. Hung, J. K. Chen, S. S. Huang, R. S. Lee, and M. J. Su, Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovascular Research - Elsevier* 47 (2000):549–555.
19. G. O. Armstrong, M. G. Lamberchts, E. P. G. Mansvelt, D. P. Van Velden, and J. S. Pretorius, Wine and health. *South African Journal of Marine Science* 97 (2001):279–282.
20. M. I. Chung, C. M. Teng, K. L. Cheng, and F. N. Ko, An antiplatelet principle of Veratrum Formosanum. *Planta Medice* 58 (1992):274–276.
21. S. Kobayashi, C. K. Ding, Y. Nakamura, I. Nakajima, and R. Matsumoto, Kiwifruits (actinida deliciosa) transfered with a Vitis stilbene synthase gene produce piceid (resveratrol-glukoside). *Plant Cell Reports* 19 (2000):904–910.
22. R. Pace-Asciak, S. Hahn, E. P. Diamandis, G. Soleas, and D. M. Goldberg, The red wine phenolics, trans-resveratrol and quercetin block human platelet aggregation and eicosanoids synthesis: Implication for protection against coronary heart disease. *Clinica Chimica Acta* 23 (1995):207–219.
23. A. Olas, B. Wachowicz, J. Saluk-Juszczak, J. Szewczuk, and W. Kaca, The effect of resveratrol on the platelet secretory process induced by endotoxin and thrombin. *Microbios* 105 (2001):7–13.
24. M. H. Azizi, R. Kumar, and N. Ahmad, Cancer chemoprevention by resveratrol: In vitro and in vivo studies and the underlying mechanisms (Review). *International Journal of Oncology* 23 (2003):17–28.
25. J. Burns, P. Gardner, and T. O'Neal, Relationship among antioxidant activity, vasodilatation capacity, and phenolics content of red wines. *Journal of Agricultural and Food Chemistry* 48 (2000):220–230.
26. S. Yehuda, S. Rabinovitz, and D. I. Mostofsky, Essential fatty acids are mediators of brain biochemistry and cognitive functions. *Journal of Neuroscience Research* 56 (1999):565–570.
27. J. Martinez and J. J. Moreno, Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. *Biochemical Pharmacology - Elsevier* 59 (2000):865–870.
28. B. Olas, H. M. Bikowska, B. Wachowicz, T. Krajewski, A. Buczyński, and A. Magnuszewska, Inhibitory effect of resveratrol on free radical generation in blood platelets. *Acta Biochimica Polonica* 46 (1999):991–996.
29. B. Olas, B. Wachowicz, J. Saluk-Juszczak, T. Zieliński, W. Kaca, and A. Buczyński, Antioxidant activity of resveratrol in endotoxin stimulated blood platelets. *Cell Biol. Toxicol*. 17 (2001b):10–19.
30. C. M. A. Nieuwenhouys, A. H. Feijge, R. F. G. Offermans, A. D. M. Kester, and G. Homstra, Modulation of rat platelet activation by vessel wall-derived prostaglandin and platelet-derived thromboxane: Thromboxane-prostaglandin balance. *Artherosclerosis* 154 (2001):355–366.
31. L. Fremont, Biological effects of resveratrol. *Life Science - Elsevier* 66 (2000):663–673.
32. N. Frankel, J. Kanner, J. B. German, E. Parks, and J. E. Kinsella, Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *The Lancet* 341 (1993):454–457.
33. L. Fremont, L. Belguendouz, and S. Delpal, Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. *Life Science - Elsevier* 64 (1999):2511–2521.

34. D. Blache, L. Gesquiere, N. Loreau, and P. Durand, Oxidant stress: The role of nutrients in cell-lipoprotein interactions. *The Journal of Nutritional Biochemistry* 58 (1999):559–563.
35. B. Halliwell, Lipid peroxidation, antioxidants and cardiovascular disease: How should we move forward. *Cardiovascular Research - Elsevier* 47 (2000):410–418.
36. T. M. Bray, Dietary antioxidants and assessment of oxidative stress. *Nutrition* 16 (2000):578–581.
37. M. Mutanen and R. Freese, Fats lipids and blood coagulation. *Current Opinion Lipidology* 12 (2001):25–29.
38. W. Ducan, J. Bruce, and P. Rourke, The synthesis and characterization of potentially mesomorfic carbonyl complexes of tungsten (0) with substituted pyridines. *Polyhedron* 14, 13–14 (1995):1915–1922.
39. T. James, C. J. Jones, J. A. McCleverty, D. W. Bruce, and M. G. Hutchings, The synthesis of some paramagnetic stilbazole complexes and an evaluation of their redox and mesogenic properties. *Polyhedron* 14, 17–18 (1995):2499–2504.
40. Annoni, M. Pizzotti, R. Ugo, S. Quici, T. Morotti, N. Casati, and P. Macchi, The effect on E-stilbazoles second order NLO response by axial interaction with M(II) 5,10,15,20-tetraphenyl porphyrinates (M = Zn, Ru, Os); a new crystalline packing with very large holes. *Inorganica Chimica Acta* 359 (2006):3029–3041.
41. D. M. Huck, H. L. Nguyen, S. J. Coles, M. B. Hursthouse, B. Donnio, and D. W. Bruce, Mesomorfic silver (I) complexes of 4-alkoxy-2′-stilbazoles and 4-alkoxy-3′-stilbazoles. Crystal and molecular structure of 4-methoxy-2′-stilbazole. *Journal of Materials Chemistry Articles* 12 (2002):2879–2886.
42. J. C. Smith, C. J. Cavallito, and F. F. Foldes, Choline acetyltransferase inhibitors: a group of styrylpyridine analogs. *Biochemical Pharmacology* 16 (1967):2438.
43. C. J. Cavallito, H. S. Yun, J. C. Smith, and F. F. Foldes, Choline acetyltransferase inhibitors. Configurational and electronic features of styrylpyridine analogs. *Journal of Medicinal Chemistry* 12 (1969):134–138.
44. C. J. Cavallito, H. S. Yun, T. Kaplan, J. C. Smith, and F. F. Foldes, Choline acetyltransferase inhibitors. Dimensional and substituent effects among styrylpyridine analogs. *Journal of Medicinal Chemistry* 13 (1970):221–224.
45. Arena, F. Manna, C. Pizza, M. L. Stein, and M. Grifantini, Structure–activity relations in reactirators of organophosphorus-inhibited acetylcholinesterase. 10. Hydroxyiminomethylarylethenylpyridine methiodides. *Journal of Medicinal Chemistry* 18 (1975):1147–1151.
46. B. Buszewski, R. Gadzała-Kopciuch, and M. Jaroniec, Chromatographic properties of mixed chemically bonded phases with alkylamide and aminopropyl ligands. *Journal of Liquid Chromatography & Related Technologies* 20 (1997):2313–2325.
47. B. Buszewski, R. Gadzała-Kopciuch, M. Markuszewski, and R. Kaliszan, Chemically bonded silica stationary phases: Synthesis, physicochemical characterization, and molecular mechanism of reversed-phase HPLC retention. *Analytical Chemistry* 69 (1997):3277–3284.
48. B. Buszewski, R. Gadzała-Kopciuch, R. Kaliszan, M. Markuszewski, M. T. Matyska, and J. J. Pesek, Polyfunctional chemically bonded stationary phases for reversed phase high performance liquid chromatography. *Chromatographia* 48 (1998):615–622.
49. B. Buszewski, K. Krupczyńska, R. Gadzała-Kopciuch, G. Rychlicki, and R. Kaliszan, Evaluation of HPLC columns: A study on surface homogeneity of chemically bonded stationary phases. *Journal of Separation Science* 26 (2003):313–321.
50. R. Gadzała-Kopciuch, M. Kluska, M. Wełniak, and B. Buszewski, Silicon dioxide surfaces with aryl interaction sits for chromatographic applications. *Materials Chemistry and Physics* 89 (2005):228–237.
51. W. Prukała, K. Pypowski, I. Chrząścik, and M. Kluska, Separation of biologically active isomers of (E)-N-meta- and paranitroazastilbenes by the HPLC technique. *Journal of Liquid Chromatography & Related Technologies* 31 (2008):578–585.
52. W. Prukała, D. Prukała, K. Pypowski, I. Chrząścik, and M. Kluska, Chromatography of biologically active chlorides of (E)-N-o-(m- or p-)chlorobenzyl-γ-azastilbenols-2′(3′ or 4′). *Journal of Liquid Chromatography & Related Technologies* 31 (2008):2612–2620.
53. M. Kluska, Some aspects of the analysis of biologically active organogermanium substances, *Critical Reviews in Analytical Chemistry* 38 (2008):85–93.
54. M. Kluska, K. Pypowski, I. Chrząścik, T. Koval, and N. Erchak, Separation and determination of chosen λ^5-silanates by the isotachophoresis technique. *Journal of Liquid Chromatography & Related Technologies* 32 (2009):896–907.
55. R. Gadzała-Kopciuch and B. Buszewski, Comparative study of hydrophobicity of octadecyl and alkylamide bonded phases using the methylene selectivity. *Journal of Separation Science* 26 (2003):1273–1283.
56. M. Kluska, E. Liepinsh, K. Pypowski, and N. Erchak, Separation of azepinio-methyl derivatives of ES-silanates by the use of aryl stationary phases in high performance liquid chromatography. *Journal of Liquid Chromatography & Related Technologies* 31 (2008):675–682.
57. M. Kluska, Analytical techniques in determination of biologically active organosilicons of the ES-silanate group. *Critical Reviews in Analytical Chemistry* 38 (2008):216–226.
58. M. Kluska, K. Pypowski, I. Chrząścik, R. Gadzała-Kopciuch, M. Michel, E. Liepinsh, and N. Erchak, High performance liquid chromatography of benzyl derivatives of ES-silanates. *Journal of Liquid Chromatography & Related Technologies* 31 (2008):1195–1203.
59. M. Kluska, E. Liepinsh, K. Pypowski, I. Chrząścik, M. Michel, and N. Erchak, Separation of chosen derivatives of hoszczawa-silanates with taking advantage of π–π interactions. *Journal of Liquid Chromatography & Related Technologies* 31 (2008): 2812–2820.
60. D. Prukała, I. Chrząścik, W. Prukała, K. Pypowski, M. Szymalska, and M. Kluska, Isotachophoresis of chosen biologically active (E)-azastilbenes. *Journal of Liquid Chromatography & Related Technologies* (2009), (in press).