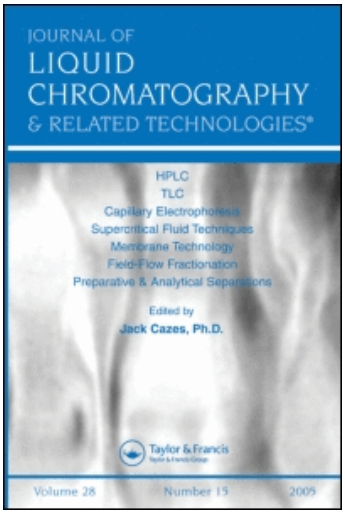

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

NEW METHODOLOGY OF SEPARATION AND DETERMINATION OF BIOLOGICALLY ACTIVE ISOMERS OF NITROBENZYL AZASTILBENE DERIVATIVES

Dorota Prukała[a]; Wiesław Prukała[a]; Konrad Małkiewicz[b]; Magdalena Szymalska[c]; Ewa Witkowska-Krajewska[c]; Mariusz Kluska[d]
[a] Faculty of Chemistry, Adam Mickiewicz University, Poznan, Poland [b] Department of Conservative Dentistry, Medical University of Warsaw, Poland [c] Departments of Environmental Studies, Masurian University in Olecko, Poland [d] Institute of Chemistry, University of Podlasie, Siedlce, Poland

Online publication date: 24 March 2010

To cite this Article Prukała, Dorota , Prukała, Wiesław , Małkiewicz, Konrad , Szymalska, Magdalena , Witkowska-Krajewska, Ewa and Kluska, Mariusz(2010) 'NEW METHODOLOGY OF SEPARATION AND DETERMINATION OF BIOLOGICALLY ACTIVE ISOMERS OF NITROBENZYL AZASTILBENE DERIVATIVES', Journal of Liquid Chromatography & Related Technologies, 33: 6, 761 — 769
To link to this Article: DOI: 10.1080/10826071003684075
URL: http://dx.doi.org/10.1080/10826071003684075

Journal of Liquid Chromatography & Related Technologies, 33:761–769, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826071003684075

NEW METHODOLOGY OF SEPARATION AND DETERMINATION OF BIOLOGICALLY ACTIVE ISOMERS OF NITROBENZYL AZASTILBENE DERIVATIVES

Dorota Prukała,[1] Wiesław Prukała,[1] Konrad Małkiewicz,[2] Magdalena Szymalska,[3] Ewa Witkowska-Krajewska,[3] and Mariusz Kluska[4]

[1]*Faculty of Chemistry, Adam Mickiewicz University, Poznan, Poland*
[2]*Medical University of Warsaw, Department of Conservative Dentistry, Poland*
[3]*Departments of Environmental Studies, Masurian University in Olecko, Poland*
[4]*Institute of Chemistry, University of Podlasie, Siedlce, Poland*

□ *Drivatives of hydroxystilbazole show biological activity in form of antimicrobial and anticancer effect. In this paper are presented optimum conditions of separation and determination of two isomers, derivatives of hydroxystilbazole by a technique of isotachophoresis. Chloride of (E)-N-(o-nitrobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(o-nitrobenzyl)-2′-hydroxystilbazole-4 were subjected to an optimization process. Lengths of analysis steps, electric current intensity and pH of electrolyte solutions and samples were changed during the optimization of conditions. The shortest time of analysis (at about 170 seconds) was obtained during determination of individual isomers. However, in case of mixtures, the time of analysis was lengthened two-fold. It was proved by a process of trial and error that optimum pH for analyzed isomers is 3.7. Linearity of determination of mentioned isomers is between 2 and 35 mg* mL^{-1}.

Keywords chlorides, hydroxystilbazole, isotachophoresis, nitrobenzyl derivatives

INTRODUCTION

(E)-Azastilbenes are derivatives of stilbenes. They are chemical compounds with a structure of diphenylethylene. At present, in many scientific centers, investigations are carried out concerning stilbenes as alternatives for antibiotic stimulants of growth. These compounds can be synthesized in plants from coumaric acid and cinnamic acid. They can be also formed from chalcones and flavonols. They have fungistatic properties (they

Address correspondence to Mariusz Kluska, Institute of Chemistry, University of Podlasie, 54 3-Maja St., 08-110 Siedlce, Poland. E-mail: kluskam@ap.siedlce.pl

restrain growth of fungi), fungitoxic properties (they destroy fungi) and estrogenic properties.[1]

Appearance of biological activity of (E)-azastilbenes is well known. *Trans*-styrylpyridines are effective inhibitors of choline acetyltransferase.[2–4] It was shown that N-substituted derivatives of (E)-4′-(3′,2′) hydroxystilbazoles-4 exhibited antibacterial and fungistatic activity.

Investigations were carried out testing following microrganisms: Gram-positive cocci (*Staphylococus aureus* 209P FDA, *Streptococus faecalis* ATCC 8040), aerobic 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).[3,4]

Natural stilbenes do not induce tumors and have no harmful side effects, typical for synthetic stilbestrol. They are recommended to men suffering prostatism. Natural stilbenes because of estrogenic activity can be used in prevention of women menopause effects, in preventing of degenerative changes of hair glands and hair follicles. They can be also used in treating of androgenic acne.[2]

Some (E)-azastilbenes, especially stilbazole derivatives of sulfacetamides, show anticancer activity. Anticancer 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-methoxybenzenesulfonyl]amino)stilbazole]}1-oxide) were precisely investigated. Properties of these compounds were compared with properties of therapeutically used preparations (*cis*-platinum (CDDP), adriamycin (AMD), 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, three of colon). Effective cytotoxicity 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 of ADM, VP-16 and CDDP, but lower than of taxol or VCR. Lower cytotoxicity than HMN-176 showed (E)-4-{2-[2-(N-acetyl-N-[methoxybenzenesulfonyl] amino)stilbazole]}1-oxide.[5]

A series of 43 other stilbene derivatives were investigated concerning anticancer activity, which exhibited toxicity towards human lungs carcinoma (A549).[6] They were subjected to different sort of molecular analysis and modeling of genesis and progress of cancer process.

Because of anticancer activity of this class of compounds, all investigations that can help to know their properties are justified. Therefore, the aim of the investigations was elaboration of optimum condition of separation and determination of two biologically active isomers by a technique of electrophoresis (which is often considered as “green chemistry technique”).

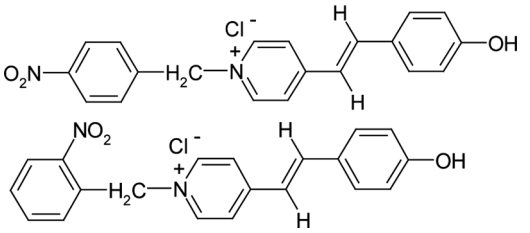

FIGURE 1 Structures of analyzed compounds (a) chloride of (E)-N-(p-nitrobenzyl)-4′-hydroxystilbazole-4 (**A7**) and (b) chloride of (E)-N-(o-nitrobenzyl)-2′-hydroxystilbazole-4 (**A8**).

EXPERIMENTAL

Electrophoretic Analysis of (E)-Azastilbenes

Samples of compounds: Chloride of (E)-N-(p-nitrobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(o-nitrobenzyl)-2′-hydroxystilbazole-4 (Fig. 1) were dissolved in deionised water (Merck). In order to drawing of standard curves were prepared standard solutions and with concentration: 2.5×10^{-3} mole L^{-1}, 7.5×10^{-3} mole L^{-1}, 5.0×10^{-4} mole L^{-1}, 7.5×10^{-4} mole L^{-1}. Optimization of conditions of separation and determination were carried out by ITP technique.

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

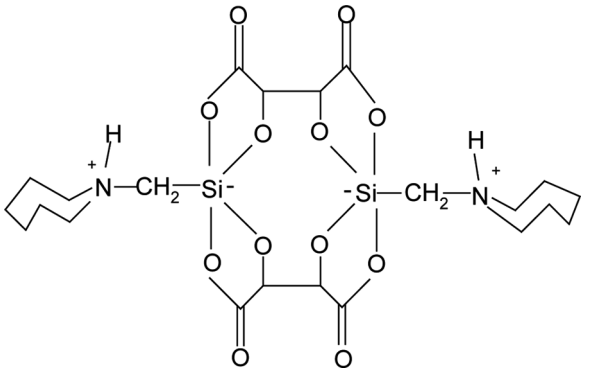

FIGURE 2 Structure of a compound used in terminating electrolyte: 4,4′-bis{1-(perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioksacyklopentan-3-on)]at}.

TABLE 1 Chemical and Physical Data of Compounds[3]

Compound	Yield (%)	M.p. (°C)	IR (KBr) (cm^{-1}) $\delta_{CH=CH}$	^{1}H-NMR δ(ppm) $-CH_{2-}{}^{+}N$
(A7)	71.0	240–242	975	5.98
(A8)	79.4	238–241	974	6.15

Ld–1 and Ld–2 (pH = 3.7) were obtained by mixing of adequate volumes of solutions of hydrochloric acid and acetic buffer (from acetic acid and sodium acetate). As a terminating electrolyte (Tm) was used a solution of 4,4′-bis{1-(perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioksacyklopentan-3-on)]at} with concentration 10^{-3} mole $\times$ L^{-1} (Fig. 2).

The (E)-azastilbenes were prepared by the method described in the literature.[3] Chosen chemical, physical and biological data are shown in Tables 1–2.

Apparatus

Optimization of separation and determination of above mentioned derivatives of hydroxystilbazoles was carried out using a capillary electrophoresis analyser EA 202M produced by Villa Labeco (Slovakia), equipped with: injection block with a container for terminating electrolyte, preseparation column (capillary diameter 0.8 mm, length 90 mm), bifurcation block with an electrode block of the preseparational column, analytic column (capillary diameter 0.3 mm, length 160 mm), an electrode block of the analytic column, UV detector, two conductometric detectors with a measurement range between 30 kΩ and 20 MΩ and steering unit – personal computer PC containing converter AD/DA.

^{1}H NMR spectra were recorded on a Bruker–200 in $CDCl_3$, with HMDS as internal standard. The infrared (IR) spectra were recorded on a Nicollet Magna–IR 760 in bromide of potassium.

TABLE 2 Antimicrobial Activity of Isomers. Minimal Inhibitory Concentration (MIC $\mu g \cdot mL^{-1}$)[3]

	Minimal Inhibitory Concentration (MIC) $\mu g \cdot mL^{-1}$								
Compounds	1	2	3	4	5	6	7	8	9
(**A7**)	100	1000	1000	1000	1000	1000	>500	>500	>500
(**A8**)	100	1000	500	1000	1000	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.

RESULTS AND DISCUSSION

Results of investigations are presented in Tables 3–5 and on Figures 3–5. Investigations, which were carried out concerned elaboration of optimum parameters of methods of separation and determination of individual isomers and their mixtures. Analyzed isomers: chloride of (E)-N-(o-nitrobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(o-nitrobenzyl)-2′-hydroxystilbazole-4 were subjected to experiments, in order to separate and determine them in a time as short as possible. In the investigations, isotachophoresis was used.

During optimization of separation and determination of above mentioned hydroxystilbazoles, different conditions of the method were tested. Time of analysis, intensity of the electric current, pH range (starting from zero to seven, because cation forms were analyzed), level of limitation of high voltage, were changed with the use of preseparation column or system of two columns: preseparation and analytic. Analyses were carried out changing the voltage from 9 kV to 15 kV. At the voltage lower than 9 kV, separation was not achieved. Similarly, no effects were obtained at the voltage higher than 12 kV.

During analysis performed by means of isotachophoretic technique, electric field causes ions placed in a system of two electrolytes (leading and terminating) to migrate toward adequate potential. According to the general rule of this technique, ions were moving, in turn, from the highest to the lowest electrophoretic mobility.

Analysis of isomers was carried out in acidic solutions: During the analysis, only cations were determined. Anionic forms were not determined, because chloride was the only anion. The separation was performed on the basis of differences of electrophoretic mobility of analyzed cations. Analyzed compounds showed very similar electrophoretic mobilities.

The determinations used a solution of terminating electrolyte characterized by significantly lower mobility, than analyzed isomers. The terminating electrolyte consisting of aqueous solution of 4,4′-bis{1-(perhydroaminiomethyl)[spirobi(1-sila-2,5-dioxacyclopentan-3-on)]at} was

TABLE 3 Conditions of the Method of Individual Determination Chloride of (E)-N-(P-Nitrobenzyl)-4′-Hydroxystilbazole-4 (**A7**)

Considered parameters				
Stage	Time [s]	Intensity [μA]	Comp [10 mV]	Conductometric detector
1	100	85	0	–
2	60	80	50	–
3	45	50	0	X

TABLE 4 Conditions of the Method of Individual Determination Chloride of (E)-N-(o-Nitrobenzyl)-2′-hydroxystilbazole-4 (**A8**)

Considered parameters				
Stage	Time [s]	Intensity [μA]	Comp [10 mV]	Conductometric detector
1	100	85	0	–
2	65	80	50	–
3	40	55	0	X

repeatedly, with success, used in analyses. This compound is a hypercoordinated organosilicon compound. Common conditions of analyzed heptacoordinated derivatives are shown in Table 5.

The separation of the mixture was difficult, because the compounds differed only by the position of the substituent in the ring and showed similarly mobility. During optimization process time of analysis, intensity of the electric current, pH of solutions of leading electrolytes and samples were changed. By a process of trial and error, it was proven that the optimum pH for analyzed isomers was 3.7. A time of analysis was changed depending on the number of compounds in the analyzed samples. The shortest optimum time of analysis was obtained for one derivative (Table 3, Figs. 3 and 4). On the other hand, optimum time of analysis of was extended over two times (Table 5, Fig. 5).

To choose a proper pH, it was necessary to choose adequate current intensity and time for individual steps of analysis. Finally, selection of these three parameters enabled optimum resolution of analyzed isomers (Fig. 5).

The ITP method was characterized by the high precision and accuracy of obtained results (Table 6). Linearity of the method was between 3 and 35 $mg\,mL^{-1}$, detection limit was 1 $mg\,mL^{-1}$.

So, optimum separation and determination of analyzed isomers has been achieved using isotachophoretic technique. Investigations of isomer

TABLE 5 Optimum Conditions of Isotachophoretic Separation of Chloride of (E)-N-(P-Nitrobenzyl)-4′-hydroxystilbazole-4 (**A7**), and Chloride of (E)-N-(o-Nitrobenzyl)-2′-hydroxystilbazole-4 (**A8**)

Considered parameters				
Stage	Time [s]	Intensity [μA]	Comp [10 mV]	Conductometric detector
1	105	100	0	–
2	200	250	0	X
3	65	10	0	–
4	15	140	0	–
5	30	110	50	–
6	20	35	0	X

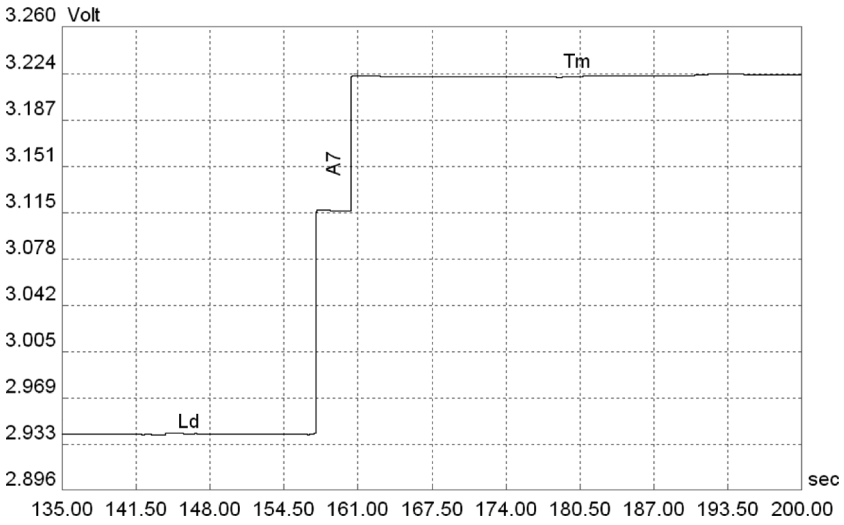

FIGURE 3 Isotachophoregram of chloride of (E)-N-(p-nitrobenzyl)-4′-hydroxystilbazole-4 (**A7**).

mixture by ITP technique were carried out using two-dimensional analysis with switching of a column. A qualitative analysis was performed on the basis of height of the zones. Obtained results were compared with isotachophoregrams of standard solutions.

To sum up, the aim of our investigation has been fully executed. Optimum conditions of isotachophoretic separation and determination

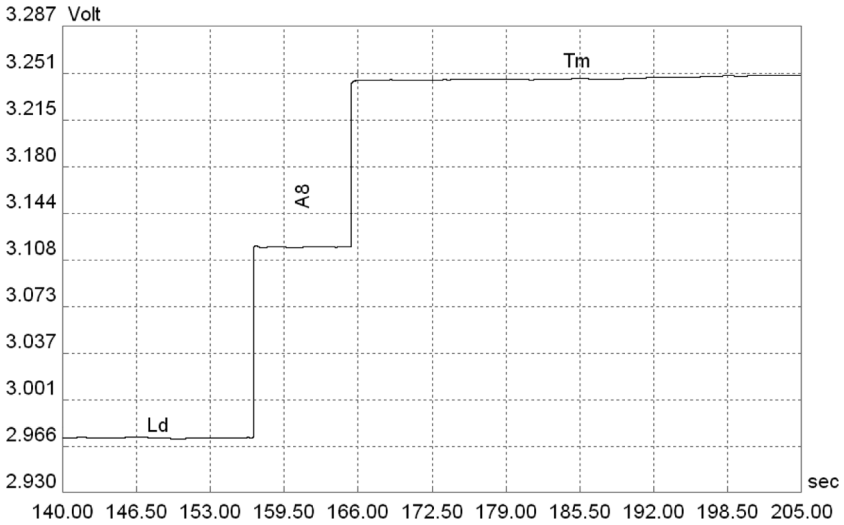

FIGURE 4 Isotachophoregram of chloride of (E)-N-(o-nitrobenzyl)-2′-hydroxystilbazole-4 (**A8**).

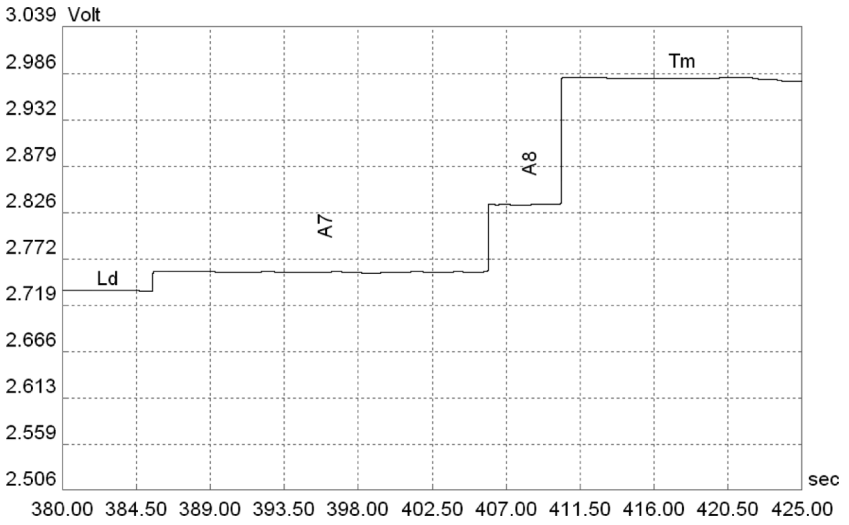

FIGURE 5 Isotachophoregram of chloride of (E)-N-(p-nitrobenzyl)-4′-hydroxystilbazole-4 (**A7**), and chloride of (E)-N-(o-nitrobenzyl)-2′-hydroxystilbazole-4 (**A8**).

of two isomers: chloride of (E)-N-(o-nitrobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(o-nitrobenzyl)-2′-hydroxystilbazole-4 were elaborated. Chromatographic investigation concerning this group of compounds and their derivatives was connected with numerous difficulties, independently on selection of analytical technique.[7–14] However, for reasons of biological activity of these compounds and wide possibilities of their applications, research in this area seems to be justified. These investigations are especially important because of antimicrobial and anticancer activity of hydroxystilbazoles. An increasing number of papers concerning this group of compounds confirms its importance.

TABLE 6 Characteristic of Used Analytical Method

Parameter	Unit	For examined ion
Precision[a]	%	$1.5-3$
Recovery[b]	%	93 ± 5
Linearity[c]	$mg\cdot mL^{-1}$	$2-35$
Limit of identification[d]	$mg\cdot mL^{-1}$	1

[a]n = 6, the samples were analyzed twice.
[b]The sample was enriched with 2 mL of a solution containing $1\,mg\cdot mL^{-1}$ of examined ion, n = 6.
[c]Correlation coefficient above 0.98.
[d]Calculated from the limit of identification and coefficients of the calibration curve.

CONCLUSIONS

Optimum conditions of isotachophoretic separation and determination of two isomers: chloride of (E)-N-(o-nitrobenzyl)-4′-hydroxystilbazole-4 and chloride of (E)-N-(o-nitrobenzyl)-2′-hydroxystilbazole-4, have been elaborated by an ITP technique. The elaborated method is characterized by a high precision and recovery. Elaboration of optimum conditions of separation and determination by the isotachophoresis technique allows to widen the possibility of investigations of azastilbenes, an important group of biologically active compounds.

ACKNOWLEDGMENT

The authors are cordially indebted to prof. N. Erchak for making available the main component of the terminating electrolyte.

REFERENCES

1. Wyrzykiewicz, E.; Prukała, W.; Kędzia, B. Il Farmaco. **1994**, *49*, 127.
2. Baker, B.R.; Gibson, R.E. J. Med. Chem. **1972**, *15*(6), 639.
3. Wyrzykiewicz, E.; Prukała, W. Il Farmaco. **1995**, *50*, 779.
4. Prukała, W.; Kędzia, B. Il Farmaco. **1999**, *54*, 584.
5. Takagi, M.; Honmura, T.; Watanabe, S.; Yamaguchi, R.; Nogawa, M.; Nishimura, I.; Katoh, F.; Matsuda, M.; Hidaka, H. In vivo antitumor activity of a novel sulfonamide HMN–214, against human tumor xenografts in mice and the spectrum of cytotoxicity of its active metabolite HMN–176. Invest. New Drugs. **2003**, *21*, 387.
6. Kim, S.; Min, S.Y.; Lee, S.K.; Cho, W.J. Chem. Pharm. Bull. **2003**, *51*(5), 516.
7. Kluska, M.; Pypowski, K.; Chrząścik, I.; Koval, T.; Erchak, N. J. Liq. Chromatogr. & Rel. Technol. **2009**, *32*, 896.
8. Kluska, M.; Pypowski, K.; Chrząścik, I.; Koval, T.; Erchak, N. J. Liq. Chromatogr. & Rel. Technol. **2009**, *32*, 2001.
9. Chrząścik, I. Crit. Rev. Anal. Chem. **2009**, *39*, 70.
10. Kluska, M.; Pypowski, K.; Chrząścik, I.; Erchak, N. J. Liq. Chromatogr. & Rel. Technol. **2009**, *32*, 2396.
11. Prukała, W.; Prukała, D.; Pypowski, K.; Chrząścik, I.; Kluska, M. J. Liq. Chromatogr. & Rel. Technol. **2008**, *31*, 578.
12. Prukała, W.; Prukała, D.; Pypowski, K.; Chrząścik, I.; Kluska, M. J. Liq. Chromatogr. & Rel. Technol. **2008**, *31*, 2612.
13. Prukała, W.; Prukała, D.; Pypowski, K.; Chrząścik, I.; Kluska, M. J. Liq. Chromatogr. & Rel. Technol. **2008**, *31*, 2784.
14. Prukała, D.; Chrząścik, I.; Prukała, W.; Pypowski, K.; Szymalska, M.; Kluska, M. J. Liq. Chromatogr. & Rel. Technol. **2009**, *32*, 2193.