Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 800 (2004) 181–187
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Influence of the extraction mode on the yield of some furanocoumarins from *Pastinaca sativa* fruits

Monika Waksmundzka-Hajnos [a,*], Anna Petruczynik [a], Anna Dragan [a], Dorota Wianowska [b], Andrzej L. Dawidowicz [b], Ireneusz Sowa [a]

[a] *Department of Inorganic and Analytical Chemistry, Medical University, Staszica 6, 20-081 Lublin, Poland*
[b] *Department of Chemical Physics and Physicochemical Separation Methods, Faculty of Chemistry, Maria Curie-Sklodowska University, Pl. MC Skłodowskiej 3, 20-031 Lublin, Poland*

Abstract

Analysis of plant material is an important task in chemotaxonomical investigations, in search of plants with pharmacological activity or in standardisation of plant drugs. The choice of optimal conditions for the analysis of plant material and effect of extraction method on the yield of furanocoumarins from *Pastinaca sativa* fruits were examined. The following extraction methods were used in experiments: exhaustive extraction in Soxhlet apparatus, ultrasonification (USAE) at 25 and 60 °C, microwave-assisted solvent extraction in open and closed system (MASE) and accelerated solvent extraction (ASE). In most cases, the yield of furanocoumarins was highest by use of ASE method as well as by ultrasonification at 60 °C.

Keywords: Solid–liquid extraction; Microwave-assisted solvent extraction; Accelerated solvent extraction; Ultrasonification; Furanocoumarins

1. Introduction

In research of the content of pharmacologically active compounds in medicinal plants, the routine procedure of extraction from plant tissues is usually applied. The extraction from plant material is frequently carried out by means of "classic" solvent-based procedures, in Soxhlet apparatus or more simply in laboratory flask at the temperature of solvent's boiling under reflux [1,2]. The imperfection of these time- and solvent-consuming methods consists of poor penetration of the tissues by the solvent and also possible destruction of the thermolabile compounds. Advantages of conventional extraction methods result from the basic equipment, inexpensive and simple to operate. In Soxhlet extraction, the sample is repeatedly contacted with the fresh portions of the solvent in relatively high temperature and no filtration is required after the leaching step [1,3].

Recently, modern alternative extraction methods, applied in environmental analysis and in phytochemistry, are sometimes reported: (1) ultrasonification (USAE) (maceration in ultrasonic bath at various temperatures) [2–4]; (2) microwave-assisted solvent extraction in closed and open systems (MASE) [2,3]; (3) accelerated solvent extraction (ASE) [2,3,5–7]; and (4) supercritical fluid extraction [2]. The above methods give better penetration of solvents into plant tissues or other solid matrix, are rapid, and solvent saving. ASE apart from this advantage is dynamic, fast and also enables automatization of extraction and analysis procedures.

Our recent investigations [8] indicate the highest yield of furanocoumarins from *Archangelica officinalis* fruits by ASE, using methanol or petroleum ether as the extractant, in comparison to the other leaching methods applied in this target. The dependence of the extraction yield on the extraction conditions and polarity of analyte was ascertained. It was also reported that microwave-assisted solvent extraction in closed system probably caused the change of analytes.

The aim of the work was the investigation of yields of extraction of coumarins from *Pastinaca sativa* fruits, by different methods, to verify our previous conclusions. *P. sativa* roots and fruits were used in medicine as diuretics and sudorifics. *P. sativa* has also nutrimental valuations and has been used as vegetable. *P. sativa* is a rich source of coumarins. Due to their biological activities, coumarins, e.g. furanocoumarins are very interesting compounds and

* Corresponding author. Tel.: +48-81-5320413; fax: +48-81-5328903.
E-mail address: mwaks@panaceum.am.lublin.pl (M. Waksmundzka-Hajnos).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.07.006

widely investigated. Furanocoumarins play the role of phytoalexins in plants [9], which can be synthetised as a result elicitation by microorganisms, insects, fungi as well as abiotic elicitors such as UV radiation, environment pollutants and mechanical breakage [10]. Defensive activity of furanocoumarins consists in their toxicity against phytopatogens (e.g. retardation of DNA synthesis) [11–13]. Some furanocoumarins have pharmacological activity as Ca-channel blockers [14], anticoagulants [15] cytostatics, antitumoral [16], antiinflammatorial [17] and antifungal [18] drugs. Some substances from this group (especially xanthotoxin and bergapten), having photosensibilitic properties, are important drugs in therapy of leucodermy [16,17,19]. Psoralene derivatives also have the ability to retard DNA synthesis, which is advantageous in the therapy of psoriaris [16,17,19].

The following extraction methods were applied: exhaustive extraction in Soxhlet apparatus, ultrasonification, microwave-assisted solvent extraction and accelerated solvent extraction. The quantitative analysis was performed by RP-HPLC in system C18/methanol + water in gradient elution. The quantitation was performed by calibration curve method and analysed statistically with the 95% confidence level.

2. Experimental

Fruits of *P. sativa* collected in September 2002 were dried, powdered and extracted with different modes.

2.1. Extraction in Soxhlet apparatus

Exhaustive extraction with petroleum ether [16,20] was performed in Soxhlet apparatus, to which exactly weighted portions of plant material were placed in a thimble-holder. Continuous extraction was performed for about 15 h. Then, the extraction of the same plant material was continued with methanol also for about 15 h. The obtained extracts were evaporated to dryness in vacuum evaporator under reduced pressure, dissolved in methanol, transferred into 100 ml volume flasks and filled up to their volume with methanol.

2.2. Ultrasonification

Ultrasound-assisted solvent extraction (USAE) with petroleum ether was performed in ultrasonic bath (Unimasz UM-4, Koszalin, Poland) at ambient temperature (20 °C) three times for 30 min. Extracts were filtered and plant material was afterwards extracted with three portions of methanol. Both extracts, filtered and evaporated to dryness, were dissolved in methanol, transferred into 100 ml volume flasks and filled up to their volume with methanol. The procedure of extraction of plant material was repeated at temperature of 60 °C in the same manner.

2.3. Accelerated solvent extraction

ASE was performed with Dionex ASE 200 instrument additionally equipped with solvent controller for ASE 200 (Dionex, Sunnyvale, CA, USA). The plant material (exactly weighed portion) was mixed with neutral glass and placed into a 22 ml stainless steel extraction cell. The application of a neutral glass, playing the role of dispersion agent, is recommended to reduce the volume of the solvent used for the extraction [21]. All extractions were performed at the same pressure (60 bar). After the extraction process, the extraction cell content was flushed using the same extractant in the amount equal to 60% of the extraction cell volume and purged for 120 s applying pressurised nitrogen (1.034 MPa). The whole volume of collected extracts, which was between 25 and 31 ml depending on packing density of the extraction cells, was evaporated to dryness, dissolved in methanol and transferred into 100 ml volume flasks and filled up to their volume with methanol. Between runs, the ASE system was washed with the extraction solvent.

2.4. Microwave-assisted solvent extraction

MASE was performed with 80% methanol in water using Plazmotronika UniClever BMZ I (Wrocław, Poland) bath using two-step extraction: by 40% generator power during 1 min and by 60% generator power during 30 min in open or closed systems.

All extraction procedures were performed from weighed samples, extracts were evaporated to dryness under reduced pressure, dry residues were dissolved in methanol in measured flasks and analysed quantitatively by RP-HPLC.

2.5. HPLC

The analysis was carried out using liquid chromatograph LC-10 AT_{VP} Shimadzu (Kyoto, Japan) equipped with SUPELCOSIL™ LC-18 150 mm × 4.6 mm column (Supelco, Bellefonte, PA, USA) $d_p = 5\,\mu m$, UV-Vis SPD-$10AV_{VP}$ Shimadzu detector and Rheodyne 20 μl injector. Quantitative analysis was performed using calibration curve method for every standard. Gradient elution: 0–10 min, 45% MeOH; 10–20 min, 45–55% MeOH; 20–30 min, 55–70% MeOH; and 30–40 min, 70% MeOH in water (bidistilled). Components of extracts were identified chromatographically and by comparing their UV spectra and spectra of adequate standards using stop-flow method. Typical chromatogram is presented in Fig. 1.

Quantitation of investigated furanocoumarins (listed in Table 1) was performed with the external standard by the calibration curve method. Regression coefficients of all calibration curves were $r > 0.999$. Six measurements for calibration curve and three measurements of every peak area for extract components were performed.

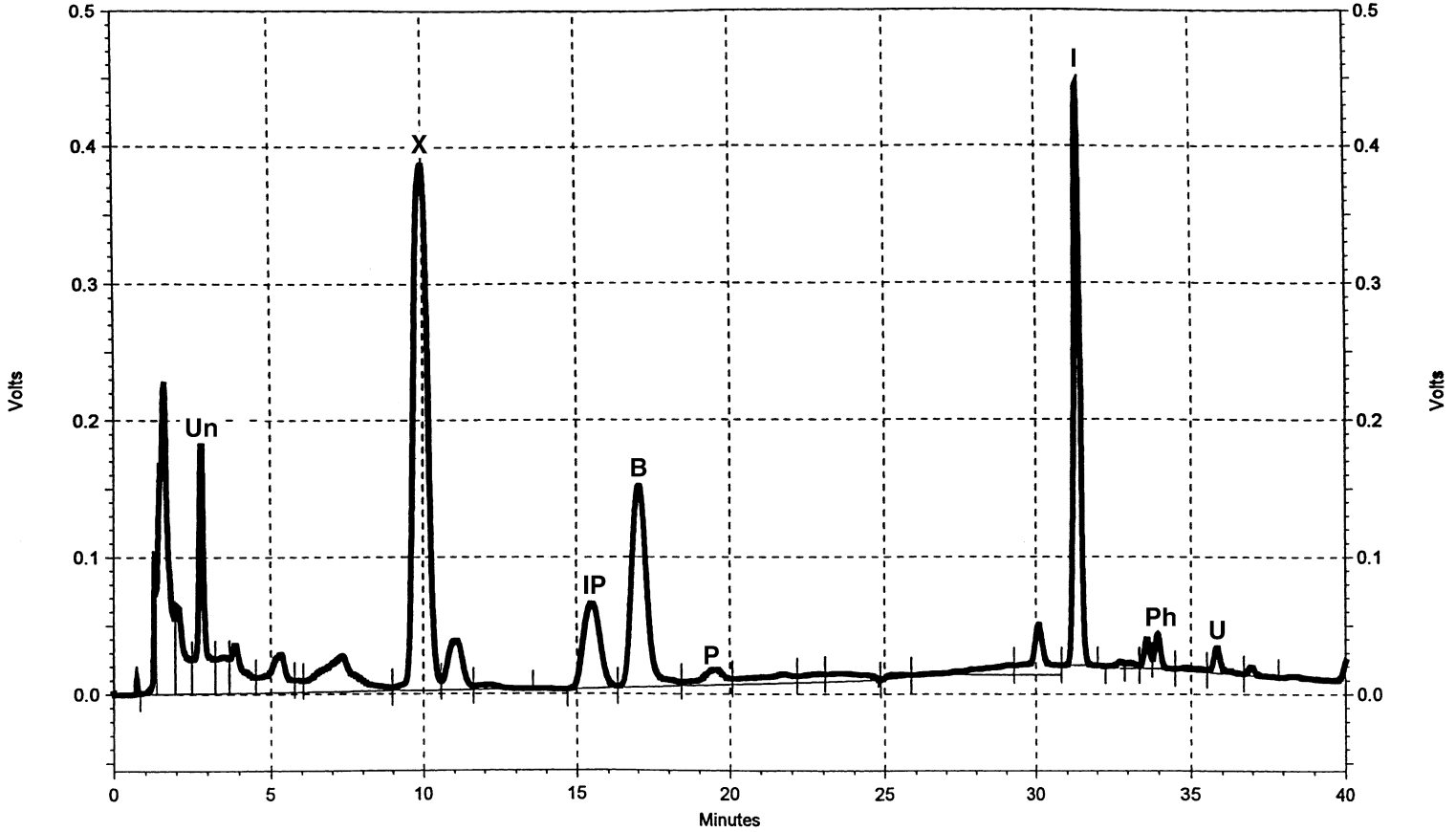
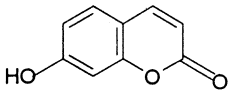
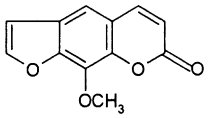
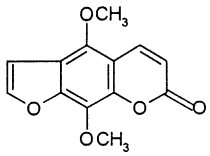
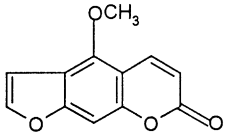
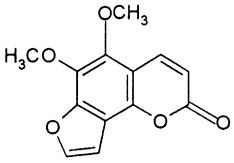
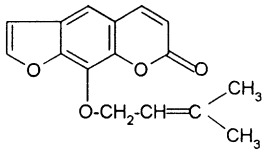
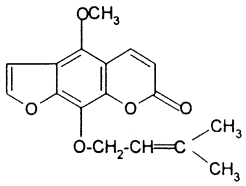
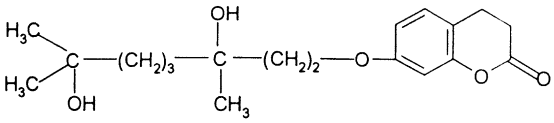


Fig. 1. Chromatogram of *P. sativa* fruit extract obtained in Soxhlet extractor with methanol as extractant (after petroleum ether). System: C-18/gradient MeOH + H_2O (see Section 2). Symbols as in Table 1.

Table 1
List of compounds investigated

Number	Name of compound	Abbreviation	Structure	Melting point (°C)
1	Umbelliferone	Un		225–228
2	Xanthotoxin	X		145–146
3	Isopimpinellin	iP		148–151
4	Bergapten	B		188–191
5	Pimpinellin	P		117–119
6	Imperatorin	I		102–105
7	Phellopterin	Ph		102
8	Umbelliprenin	U		61–63

2.6. Solvents and standards

All solvents for extraction were purchased from Polish Reagents (POCh, Gliwice, Poland). For HPLC experiments, methanol, gradient grade (E. Merck, Darmstadt, Germany), was used. Bidistilled water was used for extraction and chromatographic experiments. Standards of solutes: umbelliferon, bergapten and xanthotoxin were purchased from Fluka (Buchs, Switzerland) and others from various suppliers.

3. Results and discussion

Petroleum ether is the extractant usually used in selective extraction of furanocoumarin fraction from plant tissues [16], whereas more polar coumarins—hydroxyderivatives are extracted with methanol. Therefore, petroleum ether was chosen as extractant of furanocoumarin fraction in our experiments. Methanol, used after petroleum ether on the same plant material, extracted more hydrophylic coumarins, but also the rest of furanocoumarins. Consistently, the yield of extraction of individual quantified furanocoumarins was presented as the sum of extraction yield with petroleum ether and methanol. Obviously, there was not any possibility to use petroleum ether (nonpolar solvent) in the microwave-assisted extraction. Volume heating in this method is only possible using solvents of the high dielectric constant. Therefore, 80% methanol in water mixture was applied in our experiment.

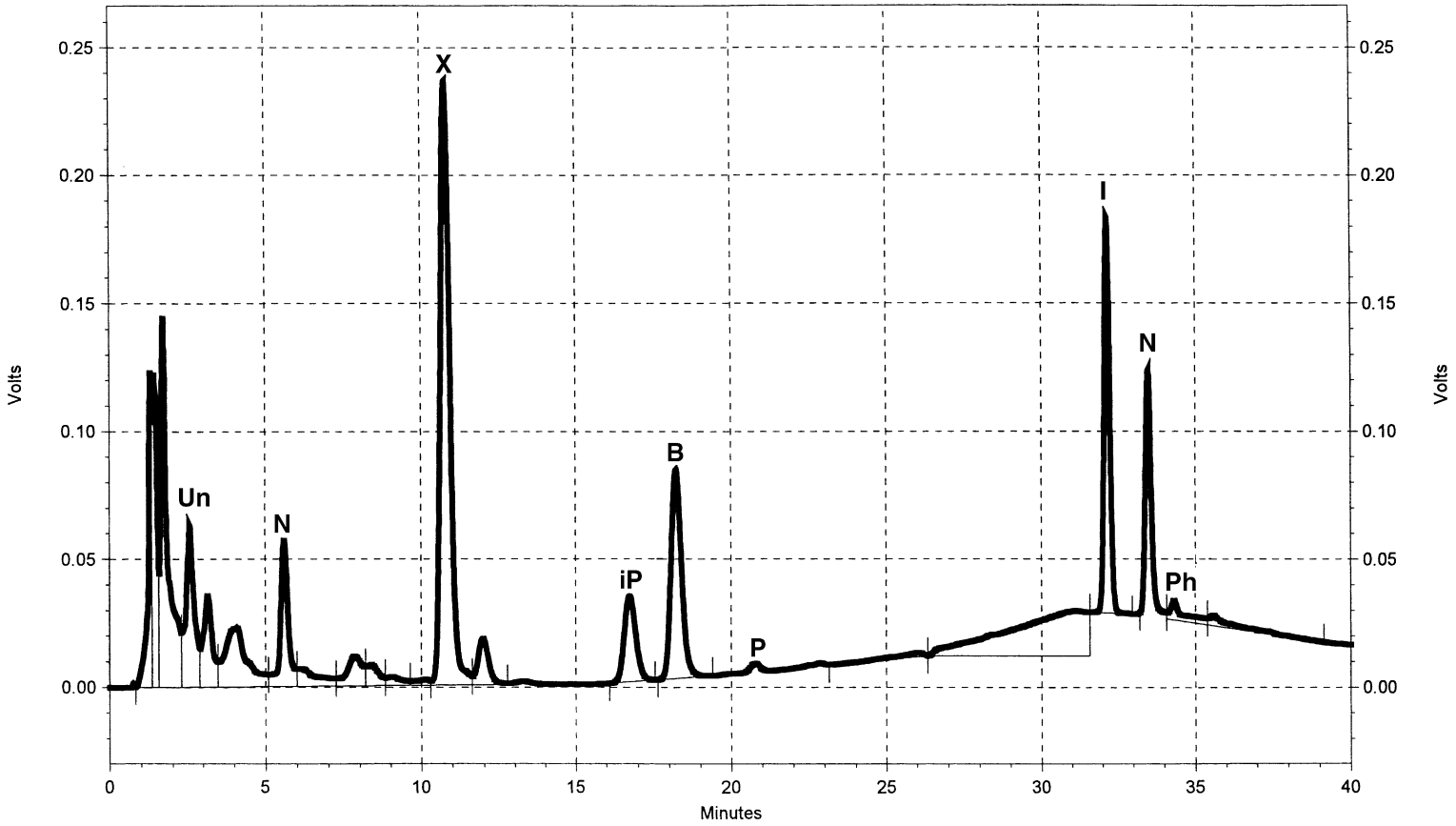


Fig. 2. Chromatogram of *P. sativa* fruit extract obtained in closed microwave extractor (MASE pressurides) with 80% MeOH in water. System: C-18/gradient MeOH + H_2O (see Section 2). Symbols as in Table 1, N: unidentified peaks.

Table 2
Yield of extraction of investigated furanocoumarins from *P. sativa* fruits by different methods

Extraction mode and conditions	Extractant	Yield of the investigated furanocoumarins (mg/g) ± S.D.				
		Xanthotoxin	Isopimpinellin	Bergapten	Imperatorin	Phellopterin
Soxhlet[a]	Petrol	4.432 ± 0.198	1.655 ± 0.028	1.149 ± 0.058	7.396 ± 0.330	0.331 ± 0.076
	MeOH	2.576 ± 0.145	0.530 ± 0.034	2.386 ± 0.174	1.269 ± 0.125	0.027 ± 0.008
	Total	7.008	2.185	3.535	8.665	0.358
USAE[a] (25 °C)	Petrol	0.275 ± 0.097	–	–	1.890 ± 0.246	0.306 ± 0.037
	MeOH	–	–	–	0.161 ± 0.003	0.185 ± 0.003
	Total	0.275	–	–	2.051	0.491
USAE[a] (60 °C)	Petrol	6.648 ± 0.248	2.490 ± 0.116	2.280 ± 0.056	11.153 ± 0.344	0.241 ± 0.08
	MeOH	4.579 ± 0.160	2.287 ± 0.032	1.683 ± 0.060	3.291 ± 0.576	0.023 ± 0.001
	Total	11.227	4.777	3.963	14.444	0.264
ASE (100 °C, 60 bar)	Petrol	2.501 ± 0.286	1.485 ± 0.122	1.602 ± 0.112	11.258 ± 0.205	–
	MeOH	3.123 ± 0.586	1.872 ± 0.387	6.196 ± 0.842	3.864 ± 0.666	0.922 ± 0.120
	Total	5.624	3.357	7.798	15.122	0.922
MASE[a] (25 °C)	80% MeOH	2.734 ± 0.688	0.661 ± 0.117	2.816 ± 0.606	2.258 ± 0.911	0.324 ± 0.030
MASE closed	80% MeOH	8.588 ± 0.120	4.248 ± 0.128	4.170 ± 0.126	1.580 ± 0.220	–

[a] Atmospheric pressure.

The results of extractions are presented in Table 2 and they compare the yield of extraction of the furanocoumarins listed in Table 1. It is clearly seen from the table that the extraction yield with petroleum ether + methanol after petroleum ether depends on the mode of extraction. In most cases, exhaustive extraction in Soxhlet apparatus does not give the highest yield. For example, the use of ultrasonification at 60 °C give in most cases higher yield than exhaustive Soxhlet method. In some cases, this method gives the highest yield of extraction (for xanthotoxin and for isopimpinellin) in comparison to all methods used in experiment. Also, the use of pressurised solvent extraction (accelerated solvent extraction) gives in most cases higher yield than Soxhlet extraction (compare yield of extraction of isopimpinellin, bergapten, imperatorin and phellopterin, Table 2). In case of bergapten, imperatorin, and phellopterin the yield of extraction by ASE is the highest in comparison to all extraction methods used in experiments (see Table 2). Microwave-assisted solvent extraction give fair extraction yield for more polar furanocoumarins, probably because of necessity of use more polar extractant (80% MeOH in water). The comparison of MASE in open and closed (pressurised) systems gives similar conclusions as in our previous experiments [8]. MASE in closed system probably cause changes of analytes. From the data presented in Table 2, it is seen that the extraction yield of phellopterin and imperatorin in pressurised MASE is distinctly lower than in open system. When chromatogram of extract obtained by pressurised MASE and chromatograms of extracts obtained by other methods were compared, qualitative differences were noticed. Similar as previously for *A. officinalis* fruit extracts, apart from peaks of furanocoumarins presented in each extract the other peaks in pressurised MASE extracts appeared (compare Figs. 1 and 2). It shows that in a closed system, the extracted compounds were changed by microwaves. Hence, pressurised MASE cannot be recommended as a leaching method of furanocoumarin fraction.

4. Conclusions

Pressurised solvent extraction gives, in most cases, higher yield of extraction of furanocoumarins from *P. sativa* fruits, as exhaustive extraction in Soxhlet apparatus. In case of more hydrophobic furanocoumarins (bergapten, imperatorin and phellopterin), it is the most capacitive extraction method from all extraction methods examined.

Ultrasound-assisted solvent extraction (USAE) at 60 °C, simple and widely available method, also gives in most cases, higher yield of extraction of furanocoumarins from *P. sativa* fruits, as exhaustive extraction in Soxhlet apparatus. In case of more hydrophilic furanocoumarins (xanthotoxin and isopimpinellin), it is the most capacitive extraction method from all extraction methods examined.

Despite of the fact that pressurised MASE gives high yield of extraction of more polar furanocoumarins, it cannot be recommended as a leaching method of furanocoumarin fraction because of the probable change of analytes during the leaching process.

References

[1] M.D. Luque de Castro, M.P. da Silva, Trends Anal. Chem. 16 (1997) 16.
[2] N. Saim, J.R. Dean, Md.P. Abdullah, Z. Zakaria, J. Chromatogr. A. 791 (1997) 361.
[3] M.D. Luque de Castro, L.E. Garcia-Ayuso, Anal. Chem. Acta 369 (1998) 1.
[4] W.A. Court, J.G. Hendel, J. Elmi, J. Chromatogr. A. 755 (1996) 11.

[5] M. Papagiannopoulos, B. Zimmermann, A. Mellenthin, M. Krappe, G. Maio, R. Galensa, J. Chromatogr. A. 958 (2002) 9.
[6] E.S. Ong, S.O. Woo, Y.L. Yong, J. Chromatogr. 313 (2000) 57.
[7] E. Boselli, V. Velazco, M.F. Caboni, G. Lercker, J. Chromatogr. A. 917 (2001) 239.
[8] M. Waksmundzka-Hajnos, A. Petruczynik, A. Dragan, D. Wianowska, A.L. Dawidowicz, manuscript in preparation.
[9] A. Szakiel, Postepy Biochemii 37 (1991) 104.
[10] A.E. Osbourn, Fungal Gent. Biol. 26 (1999) 163.
[11] S. Sardari, Y. Mori, K. Horita, R.G. Micetich, S. Nishibe, M. Daneshtalab, Bioorg. Med. Chem. 7 (1999) 1933.
[12] W.L. Fowlks, D.G. Griffith, E.L. Oginsky, Nature 181 (1958) 571.
[13] G.H.N. Towers, Planta Med. 53 (1987) 536.
[14] J. Summanen, P. Vuorela, J.P. Rauha, P. Tammela, K. Marjomaki, M. Pasternak, K. Tornquist, H. Vuorela, Eur. J. Pharmacol. 414 (2001) 125.
[15] J.S. Chen, C.T. Chang, W.S. Sheen, C.E. Teng, I.L. Tsai, C.Y. Duh, F.N. Ko, Phytochemistry 41 (1996) 525.
[16] K. Glowniak, Investigation and isolation of coumarine derivatives from polish plant material, Dissertation, Medical University, Lublin, Poland, 1988 (in Polish).
[17] W. Cisowski, Herba Pol. 29 (1983) 301.
[18] T. Wolski, Z. Gliñski, K. Buczek, A. Wolska, Herba Pol. 42 (1996) 168.
[19] O. Ceska, S.K. Chaudhary, P.J. Warrington, M.J. Ashwood-Smith, Phytochemistry 26 (1987) 165.
[20] F. Hadacek, C. Müller, A. Werner, H. Greger, P. Proksch, J. Chem. Ecol. 20 (1994) 2035.
[21] ASE 200 Accelerated Solvent Extraction Operator's Manual, Document no. 031149, revision 01, Dionex, Sunyvale, CA, 1995 (Sections 3–5).