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Effective sample preparation method for extracting biologically active compounds from different matrices by a microwave technique

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ABSTRACT

A method is described for extracting lupin alkaloid (sparteine) and drug metabolites from different matrices (seeds and rat faeces) using microwave energy and for checking the homogeneity of the electric field in the microwave oven. The high-performance liquid chromatographic separation and determination of the extracted compounds showed that the microwave extraction method is more advantageous than other traditional extraction methods with regard to the yield of extraction and the time and cost of the procedure. The potential degradation of the extracted compounds may be considerably reduced.

INTRODUCTION

Sample preparation is a critical step in chemical and biochemical analysis, usually being the slowest step in the analysis. The aim of sample preparation is that the components should be extracted from complex matrices with less time and energy consumption but with the highest efficiency and reproducibility.

A new extraction method has recently been developed using microwave energy for the extraction of various compounds (pyrimidine glucosides, gossypol, crude fat, pesticides) from different sources (fava beans, cottonseed samples, foods and soil samples) [1]. The reproducibility of any technique involving microwave energy is dependent on the homogeneity of the microwave field, determined by the magnetron tube characteristics and by the mode of wave propagation in the microwave cavity. This property, however, might change during the lifetime of the oven causing, "hot spots" and "cold spots" in the electric field of the oven [2], so it is necessary to check the homogeneity of the microwave oven prior to its use.

As the yield of traditional extraction methods is much lower in several instances than those obtained by microwave techniques, the objectives of this work were to study in more detail the efficiency of the microwave method using radiolabelled compounds, and to increase the range of the extracted compounds (alkaloids and drugs).

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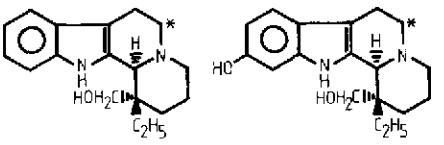
EXPERIMENTAL

Materials

Lupin seeds (*Lupinus mutabilis*) were of commercial origin and the lupin alkaloid sparteine of analytical grade was purchased from Sigma (St. Louis, MO, U.S.A.).

The radiolabelled peripheral blood flow enhancer [6-^{14}C]-(−)-1β-ethyl-1α-(hydroxymethyl)-1,2,3,4,6,7,12,12bα-octahydroindolo[2,3-*a*]quinolizine (RGH 2981) (specific activity 1.85 GBq/mg) was synthesized in our institute (Fig. 1) [3].



1 2

Fig. 1. Structure of (1) RGH 2981 and (2) its metabolite. Asterisks indicate the ^{14}C-labelled atoms.

High-performance liquid chromatographic (HPLC) grade acetonitrile, methanol, 2-propanol, *n*-hexane, dietyl ether and diethylamine used for extractions and chromatography were purchased from Merck (Darmstadt, F.R.G.). Acetic acid, ammonia solution (25%, w/v) and $CuSO_4 \cdot 5H_2O$ were of analytical-reagent grade (Reanal, Budapest, Hungary).

Apparatus

An ER 638 ETD microwave oven (1140 W, 2450 MHz) (Toshiba, Tokyo, Japan) was used for the extraction.

The HPLC equipment consisted of two Model 302 piston pumps, a Model 811 dynamic mixer, a Model 803C manometric module, a Valco C6W injector (Valco Instruments, Houston, TX, U.S.A.) with a 20-μl sample loop and a Model 116 variable-wavelength UV detector (Gilson, Middletown, WI, U.S.A.).

The radioactivity (dpm) of the labelled compound was measured using a Rackbeta 1217 liquid scintillation counter (LKB Bromma, Wallac, Sweden).

Conductometric measurements on the different extraction solvents were performed using a Model OK 102/1 conductometer (Radelkis, Budapest, Hungary).

The microwave extraction was performed using micro-reaction vessels (5 ml) made of glass (Supelco, Bellefonte, PA, U.S.A.).

Methods

Lupine seeds were ground to pass through a 12-mesh sieve (Tyler standard).

Animal treatment. Six male Wistar rats (160–220 g) were treated orally via gavage with the ^{14}C-labelled RGH 2981 in a single dose of 10 mg/kg. The faeces were collected daily for 2 days after drug administration. The daily faeces samples were suspended in 25 ml of methanol and homogenized with an IKKA Ultraturax homogenizer for 10 min and used for further investigations.

Radioactivity of the original faeces sample and homogenates were measured with the combustion technique developed by Gacs *et al.* [4], followed by liquid scintillation counting.

TABLE I

SOLVENT COMPOSITIONS FOR MICROWAVE EXTRACTION

Sample	Solvent composition	
	Components	% (v/v)
Lupin seeds	Methanol–acetic acid	99:1
Rat faeces	(I) Methanol–water–acetic acid	50:47.5:2.5
	(II) Methanol	100
	(III) Methanol–water–ammonia solution	50:48.5:1.5

Shaken flask extraction. A 5-g amount of ground lupin seeds or 1 ml of faeces homogenate were suspended in 10 ml of solvent (see Table I) and shaken in a 100-ml erlenmeyer flask for 20 min. The solvent for lupin seed extraction was taken from ref. 5.

The suspensions were centrifuged for 10 min at 11 000 *g* and the extraction was repeated twice. The supernatants were collected and analysed.

Microwave extraction. The homegeneity of the microwave field was checked using $CuSO_4 \cdot 5H_2O$ crystals. A petri dish was filled with crystals of $CuSO_4$ in a layer thickness of *ca.* 5 mm and then microwave irradiated for 12 min. Owing to the absorption of the microwave energy, the $CuSO_4$ crystals partially lose their water content. The decoloration pattern of the crystals indicates the homogeneity or inhomogeneity of the electric field. The decoloration pattern was taken into consideration when positioning the samples in the microwave oven.

In each instance 0.5 g of ground seeds or 1 ml of faeces homogenate were suspended in a screw-capped vial with 3 ml of solvent. The solvents were the same as in the shaken flask extraction. The samples were irradiated in the microwave oven; to avoid boiling of the solvent the irradiation was interrupted after 30 s. The samples were cooled to room temperature in 2 8 min and then irradiated and cooled again when necessary. Following the extraction the samples were centrifuged at 11 000 *g* for 10 min. The yield of the extraction was calculated from the radioactivity data for the supernatant and the sediment.

For lupin seeds the supernatant was analysed directly by HPLC. The supernatant of the rat faeces samples was further cleaned by concentration on a Rotavapor, dilution with water and subsequent extraction with diethyl ether as described in detail in ref. 6. The diethyl ether was evaporated, the residue was dissolved in the eluent and the solution was injected onto the HPLC column.

Chromatography. The sparteine content of the lupin seeds was determined by HPLC with a BST (Bioseparation Company, Budapest, Hungary), Si-60 (7 μm) column (250 × 4 mm I.D.) with *n*-hexane–2-propanol (90:10, v/v) as eluent at a flow-rate of 1 ml/min and detection at 210 nm.

The drug content of the rat faeces was determined on a Hypersil (Shandon, Runcorn U.K.) ODS (5 μm) column (250 × 4.6 mm I.D.) with a Hypersil ODS precolumn (50 × 4.6 mm I.D.) with acetonitrile–water (60:40, v/v; pH adjusted to 8.5 with diethylamine) as eluent at a flow-rate of 1 ml/min and detection 284 nm.

Each extraction and chromatographic step was repeated in triplicate.

Fig. 2. Discoloration pattern of $CuSO_4 \cdot 5H_2O$ crystals. The dark shade represents darker blue and the light shade pale blue to white.

RESULTS AND DISCUSSION

Extraction

Fig. 2 shows the decoloration pattern of the $CuSO_4$ surface used for the indication of the inhomogeneities in the electrical field. The samples were put onto the locations having the same colour within the oven.

TABLE II

EFFECT OF SOLVENT COMPOSITION ON THE EXTRACTION EFFICIENCY FOR RAT FAECES SAMPLES

Eluent[a]	Yield (%)[b]	ε[c]	Conductivity (μS)
I	80 ± 3	50.5	174.0
II	12 ± 3	32.6	2.9
III	56 ± 2	51.9	91.4

[a] See Table I.

[b] The extraction yield was calculated from 100 · dpm supernatant/(dpm supernatant + dpm sediment).

[c] The dielectric constants of the solvents were calculated from the specific ε^0 data taken from ref. 7.

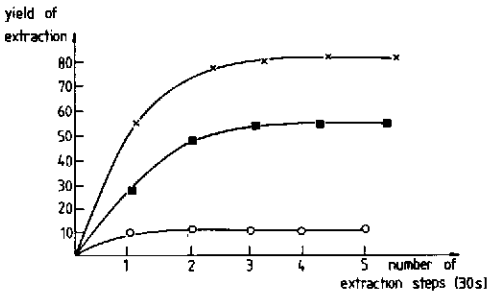


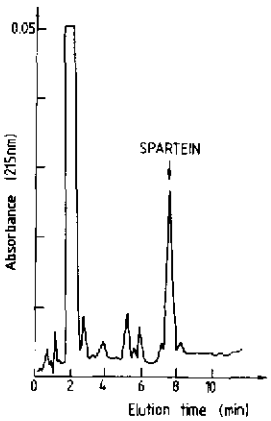
Fig. 3. Efficiency of the microwave extraction (yield in %) depending on the extraction time in the case of drug extraction (faeces samples). Solvent: × = I; ○ = II; ■ = III. For solvent compositions, see Table I.

Under our experimental conditions the shaken flask extraction of sparteine from lupin seeds proved to be 20% less efficient than the microwave technique. Using the microwave extraction considerable decreases in time and solvent consumption could also be achieved.

The effect of solvent composition on the extraction efficiency in the case of faeces extraction is shown in Table II. The yield of the traditional extraction method was 52 $\pm$ 3% using extraction solvent I, while the microwave extraction with the same solvent system yielded 80 $\pm$ 3%. Using solvent II, less than 15% of the total drug content could be extracted from the faeces samples by both the microwave and shaken flask extraction methods. With solvent III, the microwave extraction proved to be 15% more efficient than the traditional technique. Using solvent I, a high extraction yield was obtained. The solvent had relatively high dielectric constant (ε) and conductivity values and we found that the drug and its metabolite were fairly soluble in this solvent. The low extraction yield with solvent II can be explained by both the poor solubility and the low ε and conductivity values. With solvent III, an intermediate extraction yield was obtained. In this instance, the drug and its metabolite were also fairly soluble in the solvent, but the conductivity of the solvent was much lower than that of solvent I. This may lead to the relatively large difference observed in the extraction yield.

Fig. 3. shows the yield of the drug extraction from faeces samples as a function of the extraction time using different extraction solvents. It can be seen that after the third irradiation step the extraction was complete in each instance. However, the maximum yield depended considerably on the solvent compostion. It should be noted that the number of extraction steps needed for the maximum extraction yield may depend on the matrix and also on the compounds to be extracted [1].

According to our previous experiments, we assume that the ε and conductivity values of the solvent influence the energy absorption and distribution of the energy within the sample, hence they are of considerable importance in the design of efficient microwave extraction processes. In commercial microwave ovens the frequency of the microwaves varies between 2000 and 3000 MHz (in our case 2450 MHz). This means that a dipole molecule placed in the oven rotates $2 \cdot 10^9$ $3 \cdot 10^9$ cycles/s (in our case $2.45 \cdot 10^9$ cycles) as the polarity of the energy source changes. According to our measurements, the more polar the solute and the solvent, the better was the extraction efficiency achieved using the microwave extraction technique. Owing to their water,



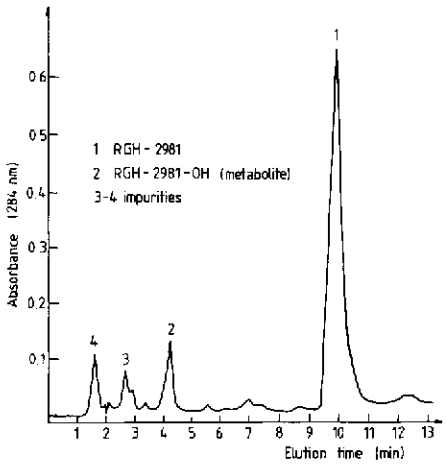


Fig. 4. Chromatogram of sparteine extracted by the microwave technique from *Lupinus mutabilis*. For chromatographic conditions, see text.

Fig. 5. Chromatogram of RGH 2981 and its metabolite, extracted from rat faeces. For chromatographic conditions, see text. Peaks: 1 = RGH 2981; 2 = RGH 2981 OH (metabolite); 3,4 = impurities.

salt and other polar component contents, the samples absorb a significant fraction of the energy from the microwave field [8]. The disruption of weak hydrogen bonds resulting from dipole rotation of the molecules and the migration of the dissolved ions facilitate the penetration of the solvent molecules into the matrix and the solvation of the component to be extracted. Owing to the physico-chemical phenomena mentioned above, the extraction time decreases dramatically.

Chromatography

Fig. 4 shows a chromatogram of the lupin extract obtained after the microwave extraction. The retention time of sparteine is 7.6 min and the peak is well separated from all impurities.

A typical chromatogram of RGH 2981 and its metabolite obtained after the microwave extraction is shown in Fig. 5. The separation proved to be sufficiently selective ($\alpha_{12} = 3$) to monitor the drug and metabolite ratio in a further metabolism study.

REFERENCES

1 K. Ganzler, A. Salgo and K. Valko, *J. Chromatogr.*, 371 (1986) 299.
2 L. Dehne, W. Bogl and D. Grossklaus, *Fleischwirtschaft*, 63 (1983) 231.
3 Gy. Kalaus, N. Malkiech, I. Katona, M. Kajtar-Peredy, T. Koritsanszky, A. Kalman, L.Szabo and Cs. Szantai, *J. Org. Chem.*, 50 (1985) 3760.
4 I. Gacs, Z. Vargay, E. Dobis, S. Dombi, K. Payer and L. Otvos, *J. Radioanal. Chem.*, 68 (1982) 93.
5 D. Vagujfalvi, *Planta Med.*, 8 (1960) 34.
6 M. Ledniczky, I. Szinai, K. Ujszaszi, S. Holly, V. Kemeny, Gy. Mady and L. Otvos, *Arzneim.-Forsch./Drug Res.*, 28 (1978) 673.
7 C. D. Hodgman, R. C. Weast and S. M. Selby (Editors), *Handbook of Chemistry and Physics*, Chemical Rubber Publishing, Cleveland, OH, 43rd ed., 1961, p. 2550.
8 R. E. Mudget, *Food Technol.*, 36 (1982) 110.