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Simultaneous isolation of Rutin and Esculin from plant material and drugs using solid-phase extraction*

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Abstract: Simultaneous isolation of Rutin and Esculin from pharmaceutical materials (plant — *Flos hippocastani* and drugs — *Venescin*®, Venacorn®) using solid-phase extraction (SPE) have been made. For this investigation the Bakerbond SPE columns with different unpolar and polar chemically bonded phases were used. On the basis of isolation investigation the influence of SPE packing materials on the selectivity change and recovery of both extracted substances were studied.

Keywords: *Chemically bonded phase (CBP); solid-phase extraction (SPE); liquid chromatography; packing selectivity; flavonoids; coumarins; isolation from plants and drugs.*

Introduction

The changes in the selectivity of chromatographic separation or isolation of the substances contained in biological matrix by means of solid-phase extraction method (SPE) are made usually by the change in nature or composition of mobile phase [1, 2]. Another approach consists of changing the type or surface properties of the column packing materials. First among these factors relates to the application of typical packings with chemically bonded phase (CBP) containing polar or nonpolar functional groups. The second factor relates to the structure and coverage density of the surface of silica support with alkylsilyl ligands [1–4].

In an earlier paper [5], we have shown that Bakerbond packings used in SPE are characterized by exceptionally high sorption capacity in comparison with other commercial materials, especially in the case of isolation of the substances contained in biological materials (plants, drugs, urine, blood, etc.). Moreover, owing to proper control of the process of chemical modification these materials are characterized by reproducible surface properties [2, 6]. In this connection, such materials were used in our investigations for simultaneous isolation of Rutin and Esculin.

Rutin and Esculin, which represent two groups of the substances (i.e. flavonoides and cumarines, respectively) are among the most important materials used in pharmaceutical industry.

These substances show a similar pharmacological action. They are used as elasticity-

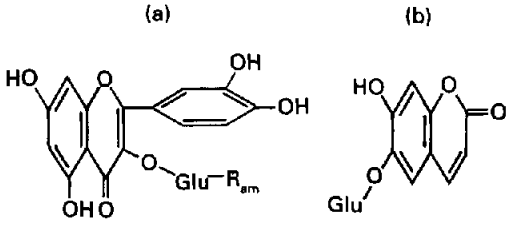

Figure 1
Chemical structure of (a) Rutin and (b) Esculin.

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giving and as sealing materials for blood vascular system [6, 7]. Moreover, Rutin acts as antioxidizer and for this reason, it is used in combination with vitamin C. Simultaneous isolation of both substances from natural materials often create serious difficulties resulting in the differences in their chemical nature, high polarity as well as from the existence of wide family of the substances of very similar properties (flavonoides) [8].

In this paper, the results of the investigations relating to optimization conditions of the simultaneous isolation of Rutin and Esculin from pharmaceutical material utilizing Bekerbond SPE columns packed with different chemically bonded phases were presented. The changes in selectivity of isolation were monitored by the control of dependence recovery on the polarity of organic stationary phase.

Experimental

Materials and reagents

SPE columns with different CBP but the same volume of packing bed (equal to 3 ml) originated from J.T. Baker Inc. (Phillipsburg, NJ, USA). The surface characteristics of these SPE materials are listed in Table 1.

The column for HPLC analysis (100 × 4 mm i.d.) was prepared in our laboratory and packed with a material having high coverage density of mono-C_{18} phase (α_{RP} = 4.2 μmol m^{-2}) obtained from the spherical silica gel with 5 μm particles [9]. As a mobile phase, the MeOH–H_2O/H_3PO_4 (35:65, v/v) composition with pH 3.5 and flow rate 1.5 ml min^{-1} were used.

Analytical-grade chemicals used for SPE and chromatographic investigation were obtained from Merck (Darmstadt, Germany). Water was double distilled in our laboratory.

The test mixture solution consisted of Rutin (0.1–0.75 g l^{-1}) (Merck) and Esculin (0.1–1.0 g l^{-1}) (Merck) were dissolved in water–methanol mixture (7:3, v/v).

Apparatus

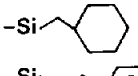
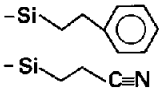
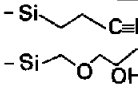
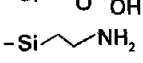
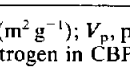
The simultaneous isolation of Rutin and Esculin from standard solution and pharmaceutical materials was carried out on vacuum manifold column processor SPE Model G-12 (J.T. Baker Inc.).

Chromatographic measurements were made, using a HP-1050 liquid chromatograph system (Hewlett–Packard, Waldbronn, Germany) with variable UV–vis detector and Vectra QS/16S computer with ChemStation for collection of the data and control of the process.

Sample preparation and SPE procedure

The SPE columns were placed in vacuum manifold processor and then washed with 10 ml of methanol for packings stabilization. Then, the columns were conditioned with 5 ml of methanol–water mixture (2:8, v/v) for non-polar packings (C_{18}, C_8, CH and Ph) and 5 ml of 1,4 dioxane for polar packings (NH_2, CN and DIOL). A 5 ml volume of standard solution contained 0.1 mg ml^{-1} of Rutin and/or

Table 1
Physico-chemical characteristics of Bakerbond SPE column packing material

No.	SPE sorbent and code	Formula of CBP	End-capped	Porosity			Loading (%)	
				S_{BET}	V_p	D	P_c	P_N
—	Silica	–Si-OH	—	351	0.89	5.8		
1	Octadecyl (C_{18})	$-Si-C_{18}H_{37}$	Yes				18	
2	Octyl (C_8)	$-Si-C_8H_{17}$	Yes				14	
3	Cyclohexyl (CH)		Yes				12	
4	Phenyl (Ph)		Yes				10.6	
5	Cyano (CN)		Yes				10.5	2.4
6	Diol (DIOL)		Yes				8.6	
7	Amino (NH_2)		Yes				6.4	2.2

Where: S_{BET}, specific surface area (m^2 g^{-1}); V_p, pore volume (ml g^{-1}); D, mean pore diameter (nm); P_c, percentage of carbon in CBP; P_N, percentage of nitrogen in CBP.

Esculin in methanol–water mixture (2:8, v/v) for nonpolar columns but for polar columns the same standards samples were dissolved in methanol–dixane mixture (2:8, v/v). Before deelution of the isolated standards the columns were washed with: 10 ml of water (nonpolar) and 10 ml of dioxane (polar). Then, adsorbed Rutin and/or Esculin standards were deeluted with 2.5 ml of methanol (nonpolar) and hot water (60°C) (polar packings). All the SPE columns were tested in the above ways and used in the isolation and purification of Rutin and Esculin from plants and drugs.

A 10 g mass of dried *Flos hippocastani* was extracted with excess of hot methanol for 3 days in Soxhlett apparatus. Then, the extract was evaporated to 100 ml, and 1 ml of this extract was mixed with 4 ml of water (for nonpolar packings) or 4 ml of dioxane (for polar packings). A 5 ml volume of these solutions was injected on the individual columns. Isolation and purification procedure were the same as for the standard solution.

The samples utilized for extraction of Rutin and Esculin from drugs: Venescin® and Venacorn® (Herbapol, Poznan, Poland), were prepared in slightly different ways. Extraction was made from one dragee by shake out with 20 ml of methanol–water (20:80, v/v) for nonpolar packings or with 20 ml of methanol–dioxane (20:80, v/v) for polar packing. These mixtures were filtered on the G-4 type of Schott frit. Then, 5 ml of such solutions were injected into the SPE columns. Further procedure was the same as described above.

Results and Discussion

Table 1 shows the important data characterized surface properties of material packings with CBP of used Bakerbond SPE columns. These columns were arranged according to the increase in the bonded stationary phases [1, 2]. It is very interesting that with the increase of CBP polarilty the carbon percentage (P_c) decreases. So high P_c values can suggest that residual silanols groups are sufficiently screened by organosilyl ligands bonded with the surface. This is connected undoubtedly with the fact that all materials listed in Table 1 were additionally deactivated (end-capped). Therefore, the determination of such parameters as coverage density (α_{RP}) and/or homogenity expressed by number of ligands per surface unit is practically impossible. Moreover, it can be expected that on the silica gel surface the 'polymer-linked' CBP structures will be formed. In the case of isolation of the substances with acidic nature, these structures have permitted us to obtain high sorption capacities, recoveries and high reproducibilities of the results [4, 5].

In order to compare the selectivities of the examined CBP packings in relation to isolated substances which has determined the sorption capacity (S_c) for Rutin and Esculin based on the breakthrough curves (isotherm) [5, 10] [Fig. 2(a) and (b)]. In each case, the measurements conditions were the same. From the comparison of these isotherms, it can be seen that for Rutin a highest sorption capacity (S_c) is obtained on nonpolar packings (C_{18}, C_8, CH and Ph) particularly in the packing with C_{18} phase. For Esculin the highest S_c value is obtained on the Ph packing. This is fully reasonable considering the dimensions and polarity of Esculin molecules (Fig. 1) and the stereospecific interactions can expect the higher recovery values. This supposition confirmed the results obtained on the polar packings CN, DIOL and NH_2 [Fig. 2(b)], in spite of the differences in sorption mechanism. The highest S_c values were obtained for strongly polar NH_2 phase, in spite of high elution power of the mobile phase ($MeOH–H_2O$; 3:7, v/v).

The change in the sequence of sorption capacity for both tested substances with the change of polarity of CBP is very interesting. Either, in the case of nonpolar and/or polar phases the sequence mentioned above is: $C_{18} > CH > C_8 \geq Ph$ for Rutin $Ph > CH > C_{18} > C_8$ for Esculin and $NH_2 > CN \geq DIOL$ for both analytes, respectively. These differences result probably from the different interactions between isolated substances, mobile phase and sorbent surface as well as from different rates of mass transfer between long and short organosilyl ligands [4, 5, 11, 12].

Considering the recoveries obtained from standard solution (Table 2, Fig. 3) it can be seen that the highest RR_S and RE_S values are obtained from C_{18} phase. It is noteworthy, that for nonpolar phases the above values correlate with the decrease of hydrophobicity of the stationary phase. Moreover, in each of the four considered cases (packings C_{18}, C_8, CH and Ph) the recoveries obtained for Esculin were higher. From analysis of RR_S and RE_S values obtained for the packings CN, DIOL and NH_2 it can be stated that, the polarity of the

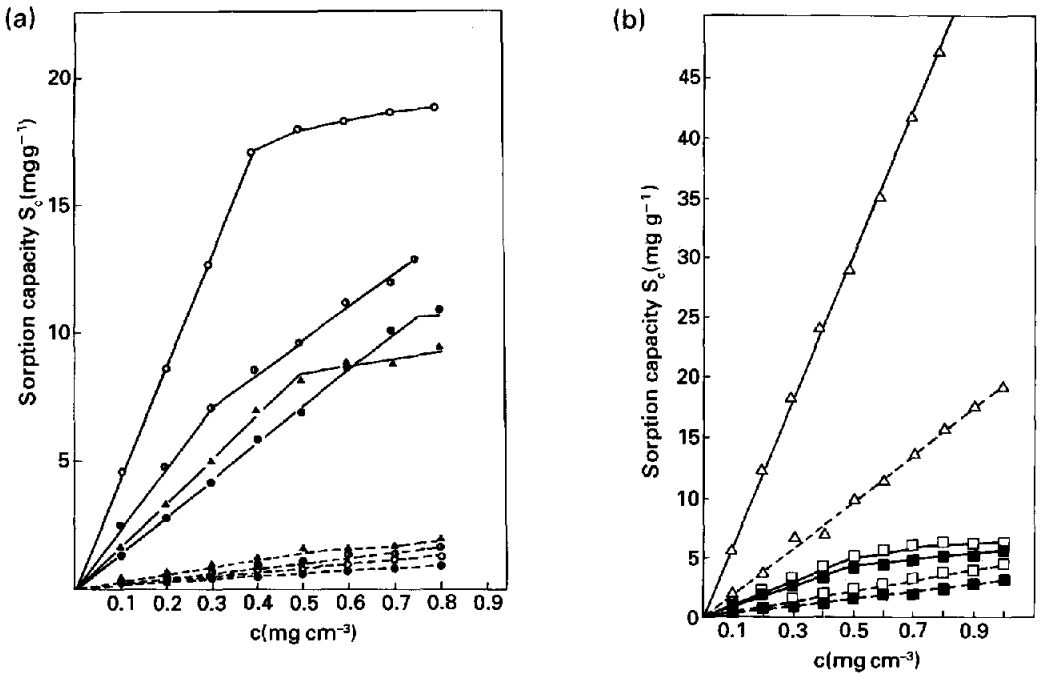


Figure 2
Sorption isotherms for Esculin (---) and Rutin (——) obtained on the (a) nonpolar and (b) polar packings from Table 1. Packings: (○), C_{18}; (●), C_8; (◐), CH; (▲), Ph; (□), CN; (■), DIOL; (△), NH_2. Isolation conditions: mobile phase: methanol–water (3:7, v/v); flow rate: 0.5 ml min^{-1}; temperature: 20°C.

Table 2
Comparison of recoveries (%) and relative standard deviation (RSD) for Rutin and Esculin isolated from standard solution and pharmaceutical materials

Type of CBP	Standard solution		Pharmaceutical materials					
			Flos hippocastani		Venescin®		Venacorn®	
	Rutin RR_S RSD	Esculin RE_S RSD	RR_1 RSD	RE_1 RSD	RR_2 RSD	RE_2 RSD	RR_3 RSD	RE_3 RSD
C_{18}	97.4 ± 0.3	97.6 ± 0.5	97.1 ± 0.8	97.3 ± 0.3	97.3 ± 0.8	97.5 ± 0.4	97.1 ± 0.7	97.2 ± 0.3
C_8	92.4 ± 0.3	93.2 ± 0.4	92.0 ± 0.8	92.8 ± 0.4	92.2 ± 0.8	93.0 ± 0.5	92.2 ± 0.7	93.1 ± 0.4
CH	92.4 ± 0.4	93.7 ± 0.4	92.1 ± 0.8	93.5 ± 0.3	92.3 ± 0.7	93.6 ± 0.4	92.3 ± 0.6	93.6 ± 0.4
Ph	88.2 ± 0.5	89.3 ± 0.5	88.1 ± 0.8	89.1 ± 0.6	88.2 ± 0.7	89.2 ± 0.5	88.2 ± 0.5	89.2 ± 0.5
CN	95.8 ± 0.5	96.3 ± 0.4	95.2 ± 0.6	96.0 ± 0.7	95.4 ± 0.5	96.2 ± 0.6	95.7 ± 0.5	96.2 ± 0.4
DIOL	94.3 ± 0.6	92.8 ± 0.5	93.8 ± 0.7	92.4 ± 0.7	93.7 ± 0.8	92.6 ± 0.7	94.0 ± 0.7	92.7 ± 0.5
NH_2	90.3 ± 0.8	91.5 ± 0.7	90.1 ± 0.8	91.2 ± 0.6	90.2 ± 0.8	91.0 ± 0.6	90.3 ± 0.7	91.4 ± 0.7

Where: RR_S, recovery for standard solution of Rutin; RE_S, recovery for standard solution of Esculin; RR_1, recovery for Rutin from *Flos hippocastani*; RE_1, recovery for Esculin from *Flos hippocastani*; RR_2, recovery for Rutin from Venescin; RE_2, recovery for Esculin from Venescin; RR_3, recovery for Rutin from venacorn; RR_3, recovery for Esculin from Venacorn.

orgnosilyl phase influences significantly the isolation selectivity. The selectivity in relation to polarity can be arranged in the following sequence: CN < DIOL < NH_2. In the case of DIOL phase only, the recovery for Esculin was lower than for Rutin. This is due probably to the effect of competitive interactions of polar OH groups and hydrophobic alkyl chain with polar and nonpolar substituents of Esculin. The differences in the recovery between polar and nonpolar phases can result also from different courses of sorption–desorption cycle observed for both types of the phases.

Table 2 lists also the recovery values obtained for Rutin and Esculin simultaneously isolated from pharmaceutical materials. Considering these values it can be seen that in all cases RR and RE values show good agreement. The recoveries level was high (above 90%) and only for the Ph phase were slightly

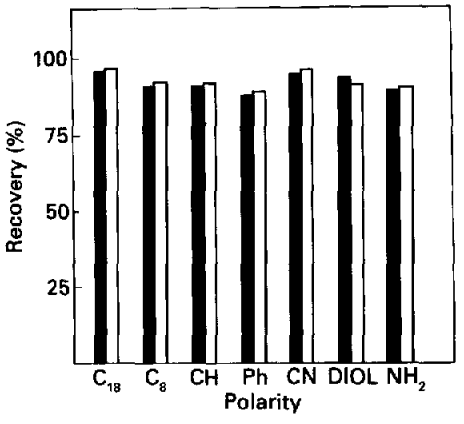


Figure 3
Comparison of recovery values versus polarity of Bakerbond SPE columns for Rutin (■) and Esculin (□) from standard solution (0.1 mg ml^{-1}).

lower recovery values observed (88.1–89.3%). Standard deviation (SD) never exceeds 1%. This fact confirms a high reproducibility of isolation and purification conditions using Bakerbond SPE columns with CBP of different polarity.

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