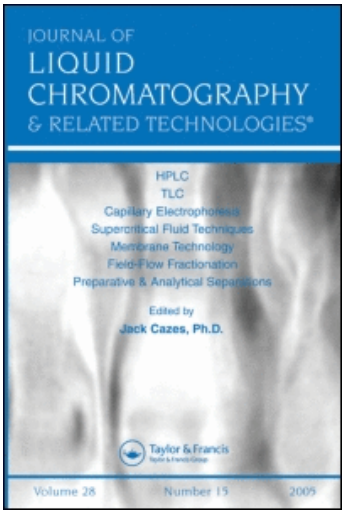

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THE INFLUENCE OF PACKING PROPERTIES ON THE ISOLATION OF RUTIN FROM PLANTS AND DRUGS USING SOLID PHASE EXTRACTION

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ABSTRACT

On the basis of sililca gel with strictly defined porosity the packings with different structure and coverage density of chemically bonded C_{18} phase (CBP) for solid phase extraction (SPE) have been prepared. Home-made packings were compared with five commercially available SPE columns. All packings were applied for isolation of rutin from plant (*Aesculus hippocastanum*) and drug (ESBERIVEN®). Evaluation and choice of optimal SPE columns for isolation and purification of rutin from natural and pharmaceutic products were made by determination of adsorption isoterms and recoveries for standard solution and extract contained rutin.

Part of this work was presented at the VIII Danube Symposium on Chromatography, Warsaw, September 2 - 5, 1991.

INTRODUCTION

Solid phase extraction (SPE) on off-line columns has become the most popular method for purification and isolation of substances present in biological materials, *e.g.* urine, blood, serum, tissue, plants and drugs [1-4]. Development of this method has been made possible by the employement of packing materials with chemically bonded phases (CBP). These packing materials are now widely used in routine biochemical, clinical and/or pharmaceutical analysis [1,4-7].

Many factors influence the recovery rates of substances isolated by SPE from biological materials, and as a consequence, also the reproducibility of the analysis. Among these factors are the packing selectivity, the height the packing bed, and the choice of eluent [1,4, 5,6].

However, little number of works has been published about packing properties for SPE columns [1,5]. It is known that the porosity of the supports, structure and coverage density of CBPs influence significantly elution profiles, sorption capacity and recovery of isolated substances. These parameters are not emphasised in majority of published papers because the lack of precise data characterizing them. On the other side it is known that the materials used as SPE packings differ batch-to-batch [6]. Application of the same isolation conditions and the same elution procedure for the packings produced by different manufacturers may cause the significant differences in recoveries and purification degrees. This can be connected with different effects *e.g.* with interactions between surface - matrix contained isolated substance - mobile phase or/and with inrreversible sorption which can take place during the performance of the process [5]. In this connection the choice of the packing with proper selectivity may be very important, especially in these cases when the biological material is undergone the SPE procedure.

Rutin - flavon glycoside (quercetine 3- rutoside $pK_a = 3.2$) used in medicine is one among many substances contained in plants [8]. This compound being

the component of the drugs is used in the therapy of alergies and phlogistic states. It influences the calcium administration in the organism, making the capillary tubes and decreasing their fragility. This substance inhibits the hematomas, the action of some enzymes of tissue oxidation as well as the action of ascorbic and catecholic oxydases [9,10]. Rutin is present in many plants *e.g. Fagopyrum esculentum, Aesculus hippocastanum*, etc., and is isolated from these plants by the extraction and added to drugs in the pure form, *i.e.* RUTINOSCORBIN^R, ESBERIVEN^R, and/or as a standarized extract *i.e.* VENESCIN^R [8]. Therefore, elaboration of dispensing of drugs containing the rutin requires the control of purity in final products.

In this work the SPE method utilizing the packings with chemically bonded C_{18} phases were used for isolation and purification of rutin from plants and drugs. Having at our disposal the home-made packings and SPE columns purchased from five diffrent manufactures, therefore interesting has been determination of physico-chemical properties of material packings on the recovery rates. Moreover, in order of better description of effects accompanying the elution it has examinated the adsorption isotherms utilizing for this purpose the method of breakthrough curves. The effect of pH on the purification degree and isolation conditions were also compared. Standard solutions of rutin as well as their extracts isolated from plants and drugs were investigated by ion-suppresion RP HPLC method with UV detection.

EXPERIMENTAL

Instrumentation

Solid-phase extraction has been made on an vacuum manifold column processor SPE Model G-12 (J.T. Baker Inc., Phillipsburg, NJ, USA).

For determination of rutin, after its previous extraction, a liquid chromatograph Model HP 4001 (Laboratorni pristroje, Prague,

Czechoslovakia) was used. Determinations were made using of UV detector working at constant wavelength 254 nm.

Surface characteristics of bare and modified packings used as material packings of SPE columns have been determined on the basis of physico-chemical measurements *e.g.*: porosimetry, elemental analysis and HPLC. Methodics of these investigations has been described in earlier work [5].

Materials and reagents

Kiesigel Si-60 (SG) (Merck, Darmstad, FRG) was used as the support of CBP in home-made columns. This silica gel was modified with two types of silanes: octadecyldimethylochlorosilane (MC_{18}) and octadecyltrichlorosilane (TC_{18}) purchased from Wacker GmbH. (München, FRG). Moreover, some portion of these two packings was end-capped additionally with trimethylchlorosilane (POCh, Gliwice, Poland). All processes of chemical modification of silica gel support were realized in the presence of activator (morpholine) which sufeguarded controlled dense CBP coverages. Methodics and mechanism of packings preparation were detailly described in earlier papers [5,7,11].

The data characterizing the packings before and after chemical modification are listed in Table 1.

Moreover the following SPE columns were used in our investigations: Supelclean C_{18} (Supelco, Bellefonte, USA), Separcol C-18 (Polymer Institute, Solvak Academy of Science, Bratislava, Czecho-Slovakia), Bakerbond spe C_{18} (J.T. Baker), Bond Elut C_{18} (Analytichem International, Harbor City, CA, USA) and PreSep-1 C_{18} (Laboratorni pristroje). The characteristics of these columns are listed in Table 2.

Extraction procedure

The columns used for SPE and containing the same volume of bed equal to 3 cm^3 were placed in vaccum manifold processor SPE G-12 and then washed

Table 1.
Surface properties of home-made packings for SPE columns; where: d_p - mean particle diameter [μm], D - mean pore diameter [nm], V_p - pore volum [ml/g], S_{BET} - specific surface area [m^2/g], %C - carbon content, c_{RP} - concentration of C_{18} groups on the support surface [$\mu mol/m^2$].

No. of column	Type of packing	d_p	Porosity			Coverage	
			D	V_p	S_{BET}	%C	c_{RP}
-	Bare silica gel(SG)	30-63	5.8	0.89	351	-	-
1.	SG-MC_{18}					19.63	3.12
2.	SG-MC_{18}EC					19.12	-
3.	SG-TC_{18}					21.02	3.95
4.	SG-TC_{18}EC					22.44	4.69

Table 2.
Characterization of commercial SPE columns packings with C_{18} phase

No. of column	Type of SPE column	d_p	Type of phase	End-capped	Content of carbon [%C]
5.	Supelclean	40	T	N	16.10
6.	Separcol	60	T	Y	20.22
7.	Bakerbond	64	T	Y	18.86
8.	Bond Elut	85	T	Y	20.10
9.	Presep 1	60	T	Y	17.01

with 5 cm^3 of methanol in order to stabilization. Then, these columns were conditioned with 5 cm^3 of water. Standard solutions contained 0.1 mg/ml of rutin in methanol-water mixture (30-70 % v/v) were used to testing of these columns (5 cm^3 of standard solution was injected onto the column). Deelution of adsorbed rutin was performed by means of 2.5 cm^3 of methanol. Then the columns were tested in above way and used to isolation and purification of the rutin from leaves and drugs.

About 10 g of dried flos *Aesculus hippocastanum* was extracted with excess of hot 20 % on aqueous methanol for 3 days in Soxlett apparatus. Then, this solution was evaporated to dry mass which was solved next in methanol-water mixture (30 - 70 % v/v) to 100 cm^3 final volume. 5 cm^3 of this solution were injected on individual columns. In order to washing of impurities the columns were washed with 20 cm^3 of water. The rutin adsorbed on the packing bed was desorbed by means of 2.5 cm^3 of methanol.

The sample utilized for extraction of rutin from ESBERIVEN^R (aqueous extract isolated from *Herba meliloti* with addition of the rutin) was prepared in slightly different way. The content of the vial was transferred quantitatively to 25 cm^3 measuring flash and filled up with water. 5 cm^3 of such solution were then injected onto the column. Further procedure was the same as this described above.

RESULTS AND DISCUSION

Table 1 lists the physico-chemical data characterizing the home-made packings prepared on the basis of Kiesigel Si-60 (SG) with C_{18} CBP. These packings have differed in CBP structure (packings #1 & #3) and deactivation degree of residual silanols (packing #2 & #4). The starting material used for modification was tested earlier in the respect of its porosity (Table 1) which together with elemental analysis data has permitted to precise determination of the surface coverage density of alkylsilyl ligands

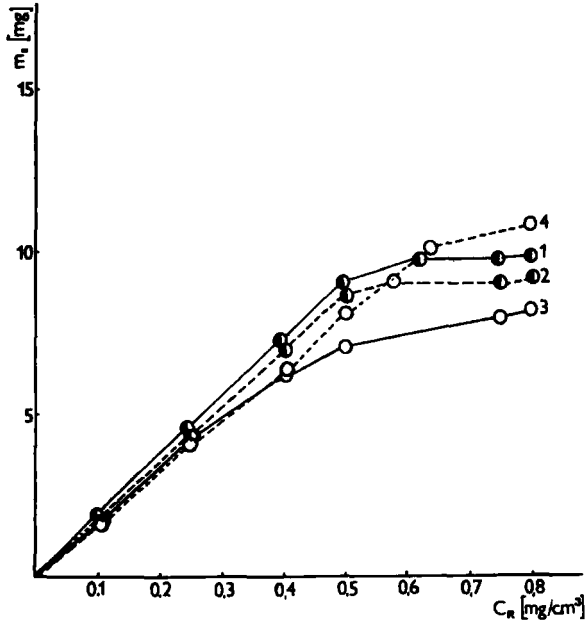

Fig. 1. Adsorption isotherms of rutin from standard solution determined for packings listed in Table 1, where: C_R = concentration of rutin, m_S = mass of adsorbed rutin per 1 g of packing.

(c_{RP}) before and after end-capping. Thus, there was possible the correlation of sorption capacity (SC) and recovery (RS) determined during extraction of rutin (standard solution) with the data characterizing of prepared packings (Fig. 1).

Fig.1. Presents the adsorption isotherms of rutin in the concentration range of 0.1 - 0.8 mg/cm^3 for home-made packings (Table 1). Comparing the courses of the isotherms it can state that the highest SC values (m_S = 10.8 mg/g) were obtained for the materials characterized by "polymeric" structure of CBP which surface was additionally end-capped (packing #4, Table 1). The lowest SC (m_S = 8.0 mg/g) were obtained also for the packing with

"polymeric" structure of CBP, however, the surface of this material was not additionaly deactivated (packing #3). Better SC values obtained for the packing #4 are due probably to higher hydrophobility of the surface of this packing (c_{RP}= 4.69 μmol/m^2, Table 1). It causes the increase of interactions contribution of alkylsilyl ligands during sorption of the rutin. Contribution of residual silanols influences the course of considered profiles, when C_R values are higher than 0.5 mg/cm^3. In consequence, more rapid mass transfer of rutin is possible owing to the increase of accessibility of rutin (pK_a = 3.2) to free silanols [5]. The greatest differences in SC values are ca. 25 % (C_R = 0.75 mg/cm^3).

Comparing the SC values (Fig. 1) obtained for the packing with "monomer" structure of CBP before and after end-capping (packings #1 & #2, Table 1) it observe an opposite course of considered m_s *vs* C_R dependence. Higher SC values of about 7-10 % are obtained for nonend-capped packing (packing # 1). This is resonable, considering that in results of secondary silanization the carbon content (% C, Table 1) were deminished by 3 - 5 % w/w, and, in this connection the exact calculations of c_{RP} values were not possible. This effect was mentioned earlier and has consisted among other in the creation of additional silanols owing to destruction of CBP [11]. The convergence of the results obtained on "monomer" phase *e.g.* for indols (pK_a = 4.7) [5] and for rutin (pK_a = 3.2) is very interesting. In the range of C_R values from 0.1 mg/cm^3 to 0.6 mg/cm^3 the highest SC values are observed which can betoken a hydrophobic nature of the surface and better mass transfer than in the case of the packing #4. This effect may be better illustrated by breakthrough curves determined for tested adsorbents presented in Fig. 2.

Breakthrough curves presented in Fig. 2 confirm univocally the effect of the structure and coverage density of CBP on the sorption capacity. Nummerical values of adsorbed rutin mass, calculated on the basis of these curves, show a good agreement with data calculated from adsorption isotherms.

There was interesting also the examination of SPE columns purchased from different producers (columns with different physico-chemical properties of

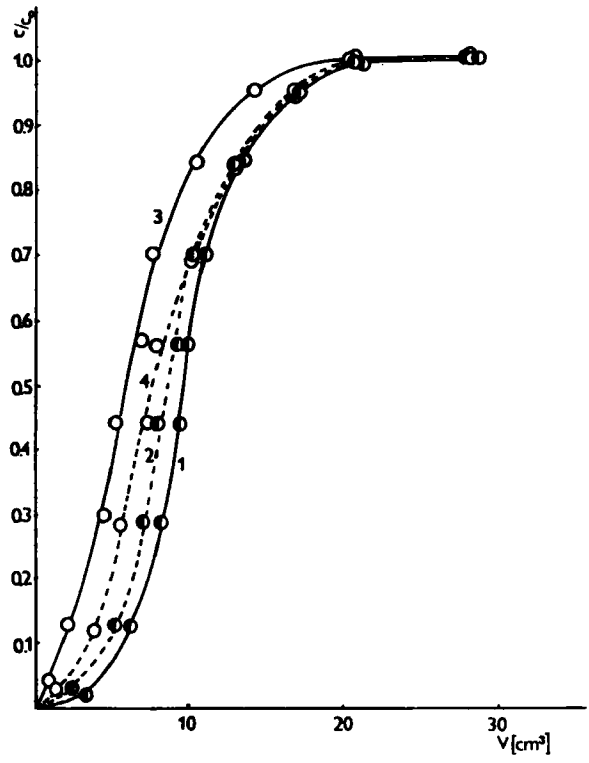

Fig. 2. Breakthrough curves for rutin (C_R = 0.5 mg/cm^3) obtained on home-made packing listed in Table 1, where: V = volume of injected samples, c/c_0 - retio of eluate to initial concentration.

material packings) as well as comparison of recoveries obtained on the packings prepared in our laboratory. Hence, Table 2 lists the data characterizing the commercial SPE packings (purchased from producers), and Fig. 3 presents the adsorption isotherms obtained on these columns in analogical manner as on home-made materials.

Comparing the isotherms presented in Fig 3. and considering the data listed in Table 2 it can state that the highest SC values were obtained for the

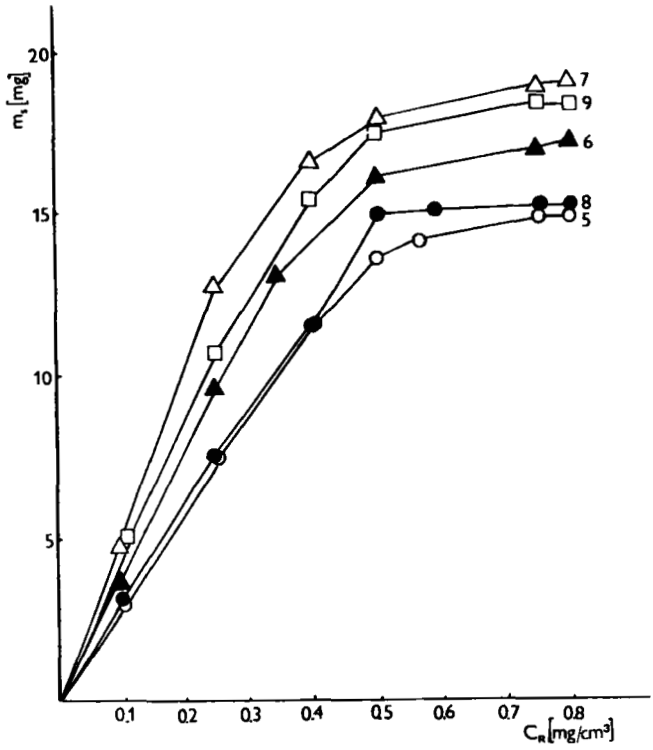

Fig. 3. Adsorption isotherm of rutin from standard solution obtained for packings listed in Table 2. Abbreviation see Fig. 1.

packing #7 (Bakerbond spe C_{18})(SC = 18.9 mg/g) and the lowest for the packings #5 & #8 (Supelclean C_{18} & Bond Elut C_{18}) (SC = 13.8 and 15.2 mg/g respectively). The highestSC values were obtained probably owing to highest coverage density, however, the precise determination of c_{RP} values was not possible because the lack of the data characterizing porosity of starting silica gels (especially S_{BET}). For this reason we not agree with far-reaching suggestions of Marko et al. [6] relating to the changes of the conformation type of alkylsilyl chains (to small differences in carbon content) during solid phase isolation of pentacaine and stobadin. More precise

Table 3.
Recovery [%] and standard deviation (SD) for rutin standard solution (RS) and isolated from leafs of *Aesculum hippocastanum* (RA) and drug ESBERIVEN^{R} (RE) obtained on home-made and commercial SPE columns.

No. of column	RS	SD	RA	SD	RE	SD
1.	96.1	± 0.4	94.7	± 0.5	93.8	± 0.5
2.	89.6	± 0.6	89.1	± 0.8	89.4	± 0.8
3.	90.1	± 0.6	89.3	± 0.8	89.6	± 0.8
4.	91.0	± 0.6	89.7	± 0.7	89.4	± 0.7
5.	90.9	± 0.6	89.4	± 0.7	89.8	± 0.6
6.	97.4	± 0.3	97.0	± 0.5	97.1	± 0.5
7.	98.7	± 0.2	97.9	± 0.5	98.1	± 0.5
8.	92.2	± 0.4	90.8	± 0.6	91.8	± 0.6
9.	90.0	± 0.4	89.4	± 0.6	89.8	± 0.6

conclusions may be drawn on the basis of data obtained by means of solid state NMR [12,13] completed with full physico-chemical data.

The comparison of SC values obtained for home-made (Fig.1, Table 1) and commercial (Fig.3, Table 2) packings is surprising. In general, SC values obtained for commercial packings were ca. 40 % higher than those obtained for home-made packings. This was due probably to different porous structure of silica gel used by us as a support of CBP. There is very interesting the comparison of rutin recoveries from standard solution and from purified natural samples (Table 3).

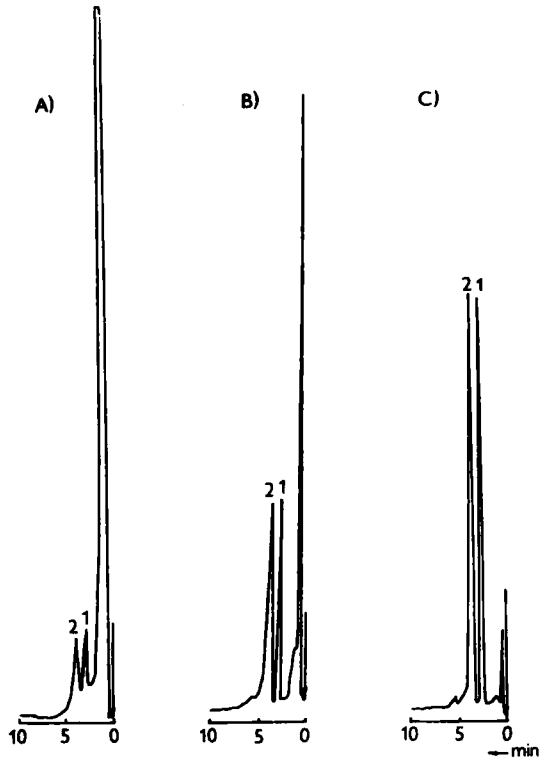

Fig. 4. Separation of rutin from ESBERIVEN^{R}; a) directly injection, b) after purification and pre-concentration according J.T Baker procedure, c) after puricfication and pre-concentration according ours method. *Chromatographic conditions*: columns (100 x 4.0 mm I.D.) - LiChrosorb RP-18, d_p-10 μm, mobile phase: 35 - 65% v/v MeOH-H_2O with 10^{-3} M H_3PO_4, flow rate: 2 cm^3/min, detector: UV-254 nm.

Analysing the recoveries obtained for standard solution (RS) it can be seen that also in this case the highest RS value is obtained for the column #7 (RS = 98.7% ± 0.2). Slightly lower RS values are obtained for the columns #1 & #6 (96.1% ± 0.4 & 97.4% ± 0.3 respectively). Other RS values were ca. 90 ± 2%.

Similar results were obtained for rutin isolated from biological material (Table 3). The differences in RA and RE values in comparison to RS were due undoubtebly to by effect of ballast substances (impurities). This may suggest (considering also SD values) the effectivity of rutin purification from materials characterized by relatively high content of flavonoides *e.g. Flos hippocastni*, VENESCIN^R, etc. Some difficulties appearing during the purification of rutin contained in ESBERIVEN^R in spite of relatively high RE values were due to greater content of impurities in investigated material.

Fig. 4 presents the chromatograms illustrating the effectivity of rutin purification and concentration contained in ESBERIVEN^R.

Analysing the chromatograms presented in Fig. 4a-c it can be seen that direct injection of diluted ESBERIVENU^R (Fig 4 a) not permits to precise determination of rutin because: 1) overloading of the sample, 2) the presence of great amount of impurities having the retention time near to this of rutin, 3) poor resolution. Therefore, the procedure proposed by J.T. Baker Inc. [14] (Fig. 4b) was used in further investigations. Analytical conditions were significantly improved owing to more effective method of purification. In comparison to nonpurified sample the preconcentration was two time higher. This merthod not permits, however, to complete removal of impurities. For this reason a new method permitting to more exact of rutin purification with simultanous maintenance of high recovery was elaborated (Fig. 4c). owing to ion-suppresion effect this method makes possible the application of mobile phase of higher elution strengh which causes that the effectivity of removal of impurities as well as the concentration degree of rutin are significantly (ca. 5 times) higher.

Proposed procedure

SPE column was stabilized with 5 cm^3 of methanol and then conditioned with 5 cm^3 water containing 10^{-3} M H_3PO_4. Then 5 cm^3 of tested sample (ESBERIVEN^R) were injected onto the column and the sorption was performed. In order to washing of impurities by 5 cm^3 of mobile phase (30-

70 % v/v MeOH-H_2O, 10^{-3} M H_3PO_4) were used. Rutine was desorbed from the packing bed with 1 cm^3 of MeOH. The recoveries obtained form standard solution (RS) and ESBERIVEN^R (RE) were 98.2% for SD = ± 0.3.

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