Permeability Profile Estimation of Flavonoids and other Phenolic Compounds by Biopartitioning Micellar Capillary Chromatography

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This paper points out the usefulness of biopartitioning micellar chromatography (BMC) using capillary columns as a high-throughput primary screening tool providing key information about the oral absorption, skin permeability, and brain–blood distribution coefficients of 15 polyphenols (6 flavones, 2 flavonols, a flavanone, 2 flavan-3-ols, 3 phenolic acids, and a phloroglucinol) in a simple and economical way. For the compounds studied, except vicenin-2, rutin, chlorogenic acid, *p*-hydroxy-cinnamic acid, and 4-hydroxybenzoic acid, maximal oral absorption ($>$90%) can be expected, if there are not solubility problems or metabolic processes. On the other hand, the most retained compounds in BMC, that is, 5-hydroxyflavone, flavone, and flavanone, show the highest brain–blood distribution coefficients and skin permeability coefficients.

KEYWORDS: Biopartitioning micellar capillary chromatography; flavonoids; polyphenolic compounds; absorption percentage; skin permeation; blood–brain permeation

INTRODUCTION

The phenolic acids and flavonoids belonging to the polyphenolic group are secondary plant metabolites. These compounds are present in vegetables and fruits, such as beverages, foods, and some phytomedicines, and their pharmacological activities have received a great deal of attention (*1*). Their beneficial effects are the antiinflammatory, anticarcinogenic, and antimutagenic activities, protective against cardiovascular diseases and antioxidant action, among others (*2*).

Relatively few studies have appeared in the literature about the pharmacokinetic profile of polyphenolic compounds (*3*), but defining their pharmacokinetic characteristics is necessary to interpret in vivo biological data (*4*). For this purpose, predictive models can be an alternative or a complementary tool to conventional assays that can reduce the use of animal experimentation, the cost, and save time. Among the alternative methods to estimate biological or pharmacological properties of compounds without involving animals or volunteers, quan-

titative structure–activity relationships (QSAR) are the most used. For polyphenolic compounds, there are some studies relating distinct substitution patterns to biological or pharmacological activities such as the inhibition of multidrug resistant proteins 1 and 2 for flavonoids (*5*), antioxidant potential (*6–10*), estrogen-like activities (*11, 12*), and union to GABA (A) receptors (*13*). Some studies have been developed using Caco-2 cell lines and rat intestinal tissue to estimate permeability factor (P_{app}) and absorption percentage (%A) for natural products (*3, 14–16*).

Chromatography is a powerful technique for the measurement of physicochemical parameters. Therefore, the chromatographic retention factor (expressed as *k* or its logarithmic form) obtained under adequate experimental conditions has proven to be a good surrogate to log *P* (logarithm of *n*-octanol–water partition coefficient, the most used parameter in QSAR studies) because of its higher accuracy and easier experimental performance. The use of retention as a predictor variable of biological activities has generated the designated quantitative retention–activity relationships (QRAR) (*17*). In this field, our research group has proposed biopartitioning micellar chromatography (BMC), which uses C_{18} stationary phases and polyoxyethylene (23) lauryl ether (Brij35) micellar mobile phases at near physiological conditions. The retention factor (*k* or log *k*) of drugs obtained in BMC has been successfully applied to construct QRAR

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Table 1. Structure, Molecular Weight (MW), Octanol–Water Partition Coefficient (log *P*), Melting Point (MP), Water Solubility (WS), and Acidity Constants (pK_a) of the Compounds Studied

Compounds	N	Structure	MW	logP[a]	MP(°C)[b]	WS (mg/L)[a]	pK$_a$[c]
Flavones							
5-Hydroxyflavone	1		238.25	4.30	151.33	22.2	-
7-Hydroxyflavone	2		238.25	3.62	247	84.6	-
Apigenin	3		270.24	3.02 2.36[d]	347.5	183	-
Flavone	4		222.25	3.56	100	30.5	-
Vicenin-2	5		594.28	-4.31	349.84[a]	1000000	-
Vitexin	6		432.39	0.32	307.42	3919	-
Flavonols							
Quercetin	7		302.24	1.48 1.15[d]	316.5	2472	-
Rutin	8		610.53	-2.02 -1.87[d]	125	27600	-
Flavanone	9		224.26	3.14	77	68	-
Flavan-3-ols							
(+)-Catechin	10		290.28	1.18 0.86[d]	347.5	63000	-
(-)-Epicatechin	11		290.28	1.18 0.86[d]	214	63000	-
Phenolic acids							
Chlorogenic acid	12		354.32	-1.01	247.83	40000	2.66
p-Hydroxycinnamic acid	13		164.16	1.79	211.5	18300	4.64
4-Hydroxybenzoic Acid	14		138.12	1.58	214.5	14500	4.54
Other phenols							
Phloroglucinol	15		126.11	0.1	218.5	264000	8.45

[a,b] Data taken from software EPIWIN (v. 3.12, ©2000, U.S. Environmental Protection Agency version) (*a*, estimated values; *b*, experimental values). [c] PhysProp Database http://www.syrres.com/esc/physdemo.htm. [d] Reference (*36*).

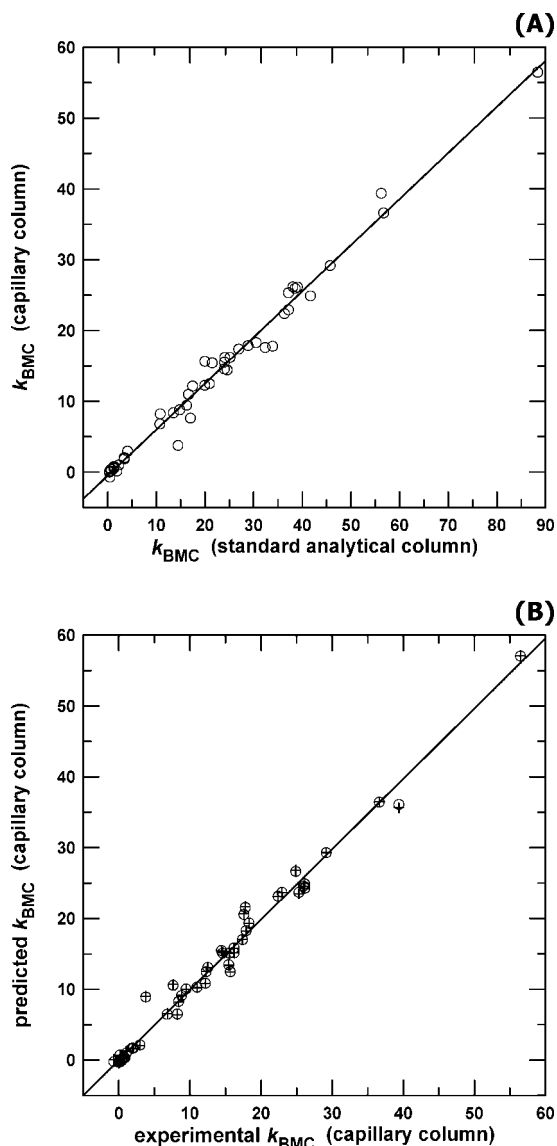**8374** *J. Agric. Food Chem.,* Vol. 55, No. 21, 2007

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Figure 1. (**A**) Retention factors in capillary versus standard analytical column relationship. (**B**) Validation plot of the model: (○) fitted data; (+) cross-validated data. Solid line represents the theoretical line.

models for describing and predicting the drug pharmacokinetic and pharmacodynamic properties of therapeutic families (*18, 19*) and also to obtain general models to evaluate the xenobiotic permeability profile through different biological barriers such as the intestinal tract, skin, ocular tissue, and blood–brain barrier (*20–25*).

The success of BMC for these estimations can be attributed to similarities between the chromatographic system and biological barriers–extracellular fluid (*19*). The retention of a compound in this chromatographic system is mainly governed by its hydrophobic and electronic properties and, to a lesser extent, its steric properties; these features of compounds also determine its passive diffusion across cell membranes. However, BMC has some drawbacks; it may fail when compounds are actively transported or transported by paracellular pathway or when metabolic processes are involved.

The main aim of the present study is to estimate the oral absorption, skin permeability, and brain–blood distribution coefficients of flavonoids and other phenolic compounds from their BMC retention in capillary columns using previously developed and validated QRAR models constructed from BMC retention in standard columns. For this purpose, the adequate

transformation from the experimental capillary retention data to estimated retention data in standard columns is intended.

The polyphenolic compounds studied include the flavones 5-hydroxyflavone, 7-hydroxyflavone, apigenin, flavone, vicenin-2, and vitexin; the flavonols quercetin and rutin; the flavanone; the flavan-3-ols (+)-catechin and (−)-epicatechin; the phenolic acids chlorogenic acid, *p*-hydroxycinnamic acid and 4-hydroxy-benzoic acid; and phloroglucinol.

MATERIALS AND METHODS

Instruments and Measurements. An Agilent 1100 series chromatograph with a binary capillary pump, an UV–visible diode array detector, a column thermostat, and a microautosampler (Palo Alto, CA) was used. Data acquisition and processing were performed with a ChemStation and LC-3D software (Rev. A.10.02[1757] ©Agilent Technologies, 1990–2003). A Kromasil C_{18} capillary column (5 μm, 0.5 × 150 mm i.d., Michrom Bioresources, Inc., Auburn, CA) was used. The mobile phase flow rate was 20 μL min^{-1}, and an injection volume of 1.5 μL was used. Detection was performed in the UV region over the wavelength range of 220–400 nm. All of the assays were carried out at 36.5 °C.

Mobile phase solutions were degassed in an ultrasonic bath (JP Selecta, Barcelona, Spain). A Crison Micro pH 2000 pH-meter from Crison Instruments (Barcelona, Spain) was employed.

Reagents and Standards. The chromatographic data of compounds were obtained using micellar mobile phases of 0.04 mol L^{-1} polyoxyethylene (*23*)lauryl ether (Brij35, Acros, Geel, Belgium) prepared in 0.05 M citrate buffer at pH 4.0, 5.0, 5.5, 6.5 and 7.4. Sodium citrate (analytical reagent, Guinama S.L., Valencia, Spain) and hydrochloric acid (for analysis, Merck, Darmstadt, Germany) were used to adjust the mobile phase pH. To reproduce the osmotic pressure of biological fluids, NaCl (purissimum, Panreac, Montplet & Esteban S.A., Barcelona, Spain) was added to the micellar mobile phase (9.2 g L^{-1}) and sodium azide (0.2 g L^{-1}, synthesis grade, Scharlab, Barcelona, Spain) for antifungal activity.

The 47 compounds used for the comparison between retention data obtained with standard and capillary columns were cimetidine, famotidine, 17β-estradiol, and estrone (all from Guinama); amobarbital, butabarbital, chlorpheniramine, cortisone, deoxycorticosterone, hydrocortisone, metoprolol, nadolol, sulpiride, testosterone, and terbutaline (Sigma, St. Louis, MO); atenolol and propranolol (ICI-Farma, Barcelona, Spain); quazepan (Quiedorm, Laboratorios Menarini, Barcelona, Spain); acetanilide, propiophenone, methanol, *n*-butanol, urea, toluene, resorcine, and benzene (Scharlab); acemetacin and ibuproxan (Laboratorios Fher, Barcelona, Spain); cortexolone and phenobarbital (Bayer, Barcelona Spain); phenol, 2,4-dimethylphenol, 2-nitrophenol, 4-nitrophenol, and 2,4-dinitrophenol (Dr. Ehrenstorfer, Augsburg Germany); 17α-hydroxyprogesterone and corticosterone (Fluka, Buchs, Switzerland); salicylic acid (Panreac); testosterone (Schering, Madrid, Spain); naproxen (Syntex Latino, Madrid, Spain); thiourea (UCB, Brus, Belgium); fenbufen (Cincopal, Cynamid Iberica S.A., Madrid, Spain); fentiazac (Donorest100, Wyeth-Orfi, Barcelona, Spain); flurbiprofen (Froben50, Laboratorios Knoll, Madrid, Spain); ketoprofen (Rhône-Poulenc Rorer, Madrid, Spain); tolmetin (Laboratorio Estedi, Barcelona, Spain); diclofenac (Novartis, Barcelona Spain); indomethacin (Laboratorios Llorens, Barcelona, Spain); lidocaine (Seid, Barcelona, Spain); and piketoprofen (Laboratorios Farmacéuticos Almirall, Barcelona, Spain).

The commercial sources of polyphenolic compounds studied were flavone, flavanone, and 7-hydroxyflavone (Acros); 5-hydroxyflavone, *p*-hydroxycinnamic acid, 4-hydroxybenzoic acid, and phoroglucinol (Sigma); rutin (Carl Roth Gmbh, Karlsruhe); (–)-epicatechin, vitexin, and apigenin (Fluka); chlorogenic acid and (+)-catechin (Cayman Chemical Co.); and vicenin-2, isolated according to the method of Gobbo-Neto (*26*).

Stock standard solutions of 1000 mg L^{-1} were prepared by dissolving 10 mg of compound in 10 mL of methanol (Multisolvent, Scharlab). Working solutions of 40 mg L^{-1} were prepared by dilution of the stock
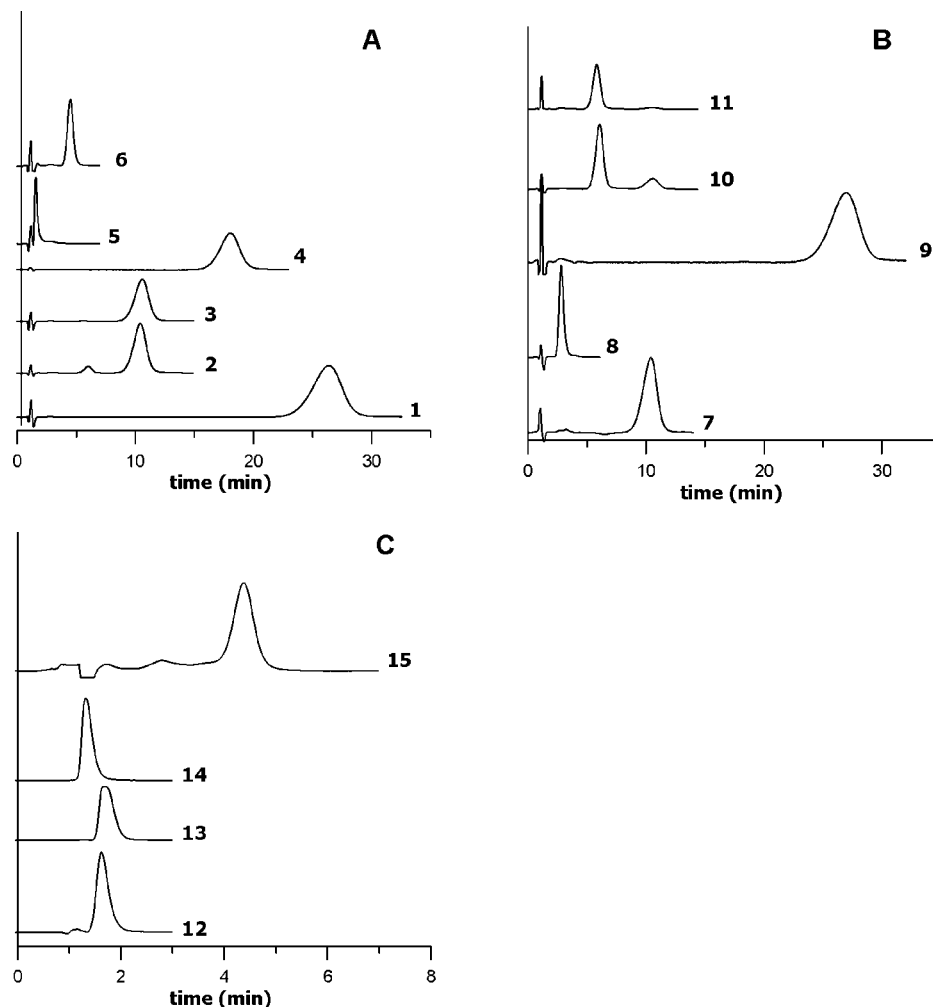
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Figure 2. Chromatograms obtained in BMC using a capillary column and 0.04 mol L^{-1} Brij35 at pH 7.4 mobile phase: (**A**) flavones, 5-hydroxyflavone (1), 7-hydroxyflavone (2), apigenin (3), flavone (4), vicenin-2 (5), and vitexin (6); (**B**) flavanone, flavonols, and flavan-3-ols, quercetin (7), rutin (8), flavanone (9), (+)-catechin (10), and (−)-epicatechin (11); (**C**) phenolic acids and other phenols, chlorogenic acid (12), *p*-hydroxycinnamic acid (13), *p*-hydroxybenzoic acid (14), and phloroglucinol (15).

standard solutions with the mobile phase solution. The solutions were stored in the refrigerator at 4 °C.

Barnstead E-pure, deionized water (Sybron, Boston, MA) was used throughout. The mobile phases and the solutions injected into the chromatograph were vacuum-filtered through 0.45 µm nylon membranes (Micron Separations, Westboro, MA).

BMC Data. The retention factors of compounds were estimated according to the approach previously proposed (*27, 28*)

$$k'^2 = \frac{k_2(t_R^g - t_{R1}^g) + k_1(t_{R2}^g - t_R^g)}{t_{R2}^g - t_{R1}^g} \quad (1)$$

where k'^2 is an estimate of k for the test compound, which besides its gross retention time, t_R^g, uses the gross retention times of the references R1 (acetanilide) and R2 (propiophenone) (t_{R1}^g and t_{R2}^g) injected during the working session at regular time intervals (~1.5 h). k_1 and k_2 are the retention factors of references, previously established for the experimental conditions assayed (surfactant concentration and temperature), and were considered to be constant. The use of this approach provides retention factor estimations more stable along time than the classical estimations based on the measurement of the dead time (actually, the gross hold-up time). Retention factor values were averages of at least triplicate determinations.

Software and Data Processing. The values of the physical properties melting point (MP), water solubility (WS), molecular weight (MW), and logarithm of octanol–water partition coefficient (log *P*) are from EPI Suite software (v. 3.12, ©2000, U.S. Environmental Protection

Agency version). This software estimates physical properties of compounds but also contains a database with experimental values. In this paper, experimental data are used when available (see **Table 1**).

Microsoft Excel 2000 software (Microsoft Corp.) and STAT-GRAPHICS (V. 2.1, Statistical Graphics Corp.) were used to perform the statistical analysis of the regressions.

Descriptive and Predictive Ability of the Models. To evaluate the predictive ability of the models in terms of cross-validated data, the comparison between the fit error (e.g., the root-mean-square error of calibration, rmsEC) and the prediction error based on cross-validation (e.g., root-mean-square error of cross-validation, rmsECV) was used. The rmsEC value provides the average deviation of the model from the data

$$\mathrm{RMSEC} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}} \quad (2)$$

where $\hat{y}_i$ is the predicted dependent variable value when all of the n molecules are included in the model construction. In contrast, the rmsECV value is a measure of the model ability to predict the dependent variable of new compounds. rmsECV is defined as rmsEC in eq 2 except that now, $\hat{y}_i$ values are predictions for other compounds not included in the model formulation (e.g., randomly omitting an arbitrary number of molecules from the calibration set and then predicting their dependent variable value in the model built with the remaining molecules; this procedure is repeated until all molecules have been excluded from the
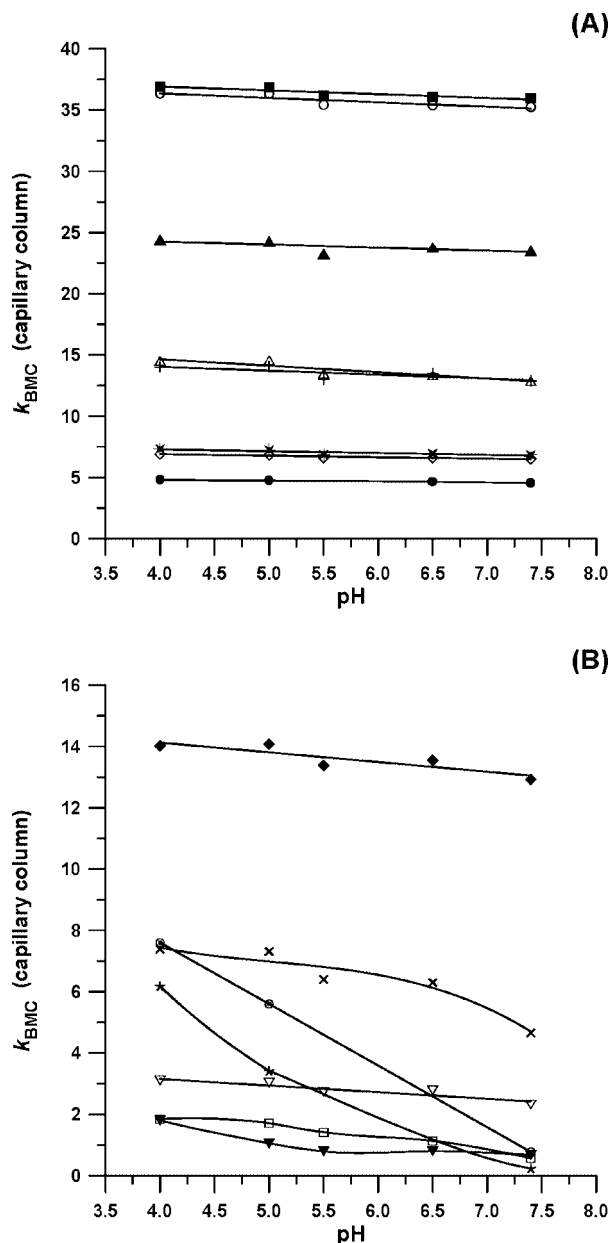
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Figure 3. Retention behavior of polyphenolic compounds in BMC as a function of mobile phase pH using a capillary column and a 0.04 mol L^{-1} Brij35 mobile phase: (**A**) (△) apigenin, (★) catechin, (◇) epicatechin, (■) flavanone, (▲) flavone, (○) 5-hydroxyflavone, (●) phloroglucinol, and (+) quercetin; (**B**) (▼) chlorogenic acid, (∗) *p*-coumaric acid, (⊙) *p*-hydroxycinnamic acid, (□) vicenin-2, (×) vitexin, and (▽) rutin.

model; this methodology is referred to as the Venetian blind cross-validation).

From a qualitative point of view, the more differences between rmsEC and rmsECV that exist, the lower is the robustness of the models obtained and, therefore, more caution must be taken in future predictions.

RESULTS AND DISCUSSION

Comparison between Standard Analytical and Capillary Columns in BMC. As has been indicated in the Introduction, BMC has proven to be a useful high-throughput technique to obtain reliable models for the estimation of the permeability and pharmacokinetic profile of xenobiotics. To increase the high-throughput capability of the technique, capillary instead of standard analytical columns were used. First of all, a comparison

between retention in BMC using standard, Kromasil C$_{18}$ (5 μm, 4.6 × 150 mm i.d.) (*23, 24*), and capillary columns, Kromasil C$_{18}$ (5 μm, 0.5 × 150 mm i.d.), was made in order to use the previously reported BMC-QRAR models obtained from retention data in a standard column.

For this purpose, the retention factors of a heterogeneous group of 47 compounds, selected to cover a wide range of hydrophobicity and acid–base properties (see Materials and Methods), in standard analytical columns and in capillary columns were related. The equation of the fitted model (eq 33), coefficient confidence intervals (at 95% confidence level), and statistical features were

$$k_{BMC}(capillary column) = (-0.5 \pm 0.7) +$$
$$(0.65 \pm 0.03)k_{BMC}(standard\ column) \quad (3)$$

$$n = 47;\ r^2 = 0.98;\ SE = 1.6;\ F = 2716;\ p < 0.0001$$

The model explains 98% of variance in the data. The slope and the model were statistically significant at the 99% confidence level ($p < 0.01$). The intercept was not statistically significant ($p > 0.05$), and its elimination from the model did not change the slope value:

$$k_{BMC}(capillary column) =$$
$$(0.637 \pm 0.016)k_{BMC}(standard\ column) \quad (4)$$

$$n = 47;\ r^2 = 0.992;\ SE = 1.6;\ F = 6185;\ p < 0.0001$$

The adequate statistical features of models indicate that eq 4 can be used to switch capillary–standard retention data. The use of capillary columns in the conditions used in this paper allows a reduction of approximately 40% in standard retention data (**Figure 1**).

The predictive ability of the models was evaluated by means of the rmsEC and rmsECV statistics based on the Venetian blind cross-validation. The rmsEC and rmsECV values, 1.53 and 1.59, respectively, were similar, suggesting the robustness and the predictive ability of the model are adequate. **Figure 1B** shows the predicted versus experimental (fitted and cross-validated) values of retention data, obtained from eq 4. As can be observed, the fitted and cross-validated point distributions agree with the theoretical straight line slope and intercept equal to 1 and 0, respectively.

Chromatographic Behavior of Phenolic Compounds in BMC Using Capillary Columns. Table 1 shows the chemical structures of the 15 polyphenolic compounds considered in this paper, which include 6 flavones, 2 flavonols, a flavanone, 2 flavan-3-ols, 3 phenolic acids, and a phloroglucinol. As can be observed in **Table 1**, the compounds considered present a wide range of hydrophobicity (octanol–water partition coefficients, log *P*, ranging between −4.3 and 3.6) and acid character. The retention of compounds considered in this study was obtained in BMC conditions using a capillary column to save time, reagents, and samples and to improve efficiency and sensitivity. **Figure 2** shows the chromatograms of the polyphenolic compounds obtained in a 0.04 mol L^{-1} Brij35 mobile phase at pH 7.4 and the Kromasil C$_{18}$ capillary column. As can be observed, compounds eluted between 1 and 30 min, and their chromatographic peaks showed adequate symmetry. On the other hand, as expected, the most hydrophobic compounds, that is, 5-hydroxyflavone (1), flavone (4), and flavanone (9), were the most retained, whereas the retention of the most hydrophilic polyphenolic compounds, that is, vicenin-2 (5), chlorogenic acid (12), and rutin (8), was the lowest. In **Figure 2**, the retention of *p*-hydroxycinnamic (13) and *p*-hydroxybenzoic acid (14) was

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Table 2. QRAR Models To Estimate Transport of Xenobiotics through Different Biological Barriers Obtained Using Retention in BMC with a Standard Analytical Column [a]

biological barrier	pharmacological parameter	mobile phase pH [b]	model [c]	ref
gastrointestinal tract	percentage of oral absorption in humans, %A	6.5 (average pH of the small intestine)	$\%A = k_{BMC} / (0.7 \pm 0.2) + (1.02 \pm 0.03)k_{BMC} \times 100$ $n = 74$; $r^2 = 0.72$; $F = 3185$; SE = 9.8; $p < 0.0001$	(21)
		7.4 (plasmatic pH)	$\%A = k_{BMC} / (1.0 \pm 0.3) + (1.00 \pm 0.03)k_{BMC} \times 100$ $n = 74$; $r^2 = 0.72$; $F = 3174$; SE = 9.8; $p < 0.0001$	
blood–brain barrier	brain–blood distribution coefficient in rats, BB	7.4 (plasmatic pH)	$\log BB = (-0.84 \pm 0.12) + (0.76 \pm 0.08) \log k_{BMC} + (0.26 \pm 0.11)\alpha$ $n = 42$; $r^2 = 0.75$; $F = 60$; SE = 0.39; $p < 0.0001$ rmsEC = 0.379; rmsECV = 0.421	(22)
skin	skin permeability coefficient in humans, K_p (cm h^{-1})	5.5 (skin pH)	$\log K_p = (-3.3 \pm 0.3) + (1.3 \pm 0.2) \log k_{BMC} - (0.0080 \pm 0.014)MP$ $n = 40$; $r^2 = 0.83$; $F = 93$; SE = 0.51; $p < 0.0001$ rmsEC = 0.498; rmsECV = 0.537	23, 24

[a] C$_{18}$ Kromasil (internal diameter = 4.6 mm). [b] 0.04 mol L^{-1} Brij35 at indicated pH values. [c] Statistical parameters and variables: *n*, number of molecules included in the model; *r*², determination coefficient; *F*, F ratio (residual to modeled variance ratio)' SE standard error of estimate of the model; *p*, probability (measure of significance of the model); rmsEC, root mean square error of calibration; rmsECV, root mean square error of cross-validation (Venetian blind cross-validation); k_{BMC}, retention factor in BMC; MP, melting point (°C); α, total molar charge.

Table 3. Retention Factors of Polyphenolic Compounds in BMC Using a Capillary Column and 0.04 mol L^{-1} Brij35 Mobile Phases at pH 7.4, 6.5, and 5.5 (k_{BMC}), Predicted Percentage of Oral Absorption (*A*), Predicted Brain–Blood Distribution Coefficients (log BB), and Predicted Skin Permeability Coefficients (K_p)

compound	N	k_{BMC} (pH 7.4)	k_{BMC} (pH 6.5)	k_{BMC} (pH 5.5)	A^a (%)	log BB	log K_p (cm h^{-1})
flavones							
5-hydroxyflavone	1	32.25 ± 0.12	35.39 ± 0.07	35.43 ± 0.10	97.5 ± 1.0	0.484	−2.242
7-hydroxyflavone	2	12.93 ± 0.06	13.559 ± 0.007	13.4 ± 0.4	95.1 ± 0.2	0.153	−3.557
apigenin	3	12.9 ± 0.2	13.34 ± 0.02	13.5 ± 0.6	95.1 ± 0.3	0.152	−4.357
flavone	4	23.528 ± 0.013	23.78 ± 0.04	23.3 ± 0.5	96.8 ± 0.8	0.351	−2.069
vicenin-2	5	0.566 ± 0.003	1.1360 ± 0.0001	1.70 ± 0.07	59 ± 17	−1	−5.544
vitexin	6	4.66 ± 0.02	6.439 ± 0.008	7.06 ± 0.3	90 ± 3	−0.2	−4.402
flavonols							
quercetin	7	12.89 ± 0.08	13.485 ± 0.003	12.99 ± 0.04	95.1 ± 0.2	0.152	−4.130
rutin	8	2.318 ± 0.008	2.780 ± 0.003	2.7 ± 0.4	82 ± 4	−0.4	−3.479
flavanone	9	35.960 ± 0.011	36.06 ± 0.03	36.23 ± 0.16	98 ± 1.0	0.491	−1.635
flavan-3-ols							
(+)-catechin	10	6.83 ± 0.02	6.939 ± 0.002	6.9 ± 0.3	91.8 ± 0.5	−0.057	−4.738
(-)-epicatechin	11	6.45 ± 0.02	6.592 ± 0.003	6.60 ± 0.13	91.5 ± 0.6	−0.074	−3.692
phenolic acids							
chlorogenic acid	12	0.67 ± 0.14	0.80 ± 0.02	0.8 ± 0.2	57 ± 9	−1.082	−5.161
p-hydroxycinnamic acid	13	0.773 ± 0.016			55 [b]	−1.036	
4-hydroxybenzoic acid	14	0.233 ± 0.003			27 [b]	−1.431	
other phenols							
phloroglucin	15	4.529 ± 0.006	4.6319 ± 0.0009		89.6 ± 1.4	−0.214	

[a] Average value of estimates obtained at pH 7.4 and 6.5. [b] Oral absorption estimated at pH 7.4.

lower than expected with respect to their log *P* values (1.79 and 1.58, respectively) because they are highly ionized at pH 7.4.

To evaluate the mobile phase pH effect on the BMC retention behavior of 15 polyphenolic compounds, 0.04 mol L^{-1} Brij35 mobile phases were prepared at different pH values in the range of 4.0–7.4. **Figure 3** shows the results obtained. As can be expected from the bibliographic pK_a values, the retention of carboxylic compounds, chlorogenic acid, *p*-hydroxycinnamic acid, and 4-hydrobenzoic acid clearly decreases as pH increases as a consequence of ionization of the carboxylic group. For vicenin-2, vitexin, and rutin decreases of 69, 36, and 25%, respectively, in the retention factors were observed. This behavior indicates that the compounds show an acidic character, probably due to the presence of sugar moieties in their molecules (*29, 30*). On the other hand, the retention of the rest of flavones, flavonols, flavanone, flavan-3-ols, and phoroglucinol does not change significantly as mobile phase pH increases.

Permeability Profile Estimation of Polyphenolic Compounds. Table 2 summarizes previously developed and vali-

dated QRAR models based on BMC retention in standard analytical columns to estimate passive transport of xenobiotics through different biological barriers: the gastrointestinal tract, blood–brain barrier, and skin (*21–24*). From the BMC experimental retention data of polyphenolic compounds at different pH values in the capillary column (**Table 3**), their BMC retentions in a standard column were calculated using eq 4 and used to estimate the permeability profile of unaltered products (**Table 3**).

The major absorption barrier to orally administered drugs is the intestinal mucosa where drugs are generally absorbed by a passive diffusion mechanism. In a previous paper, the usefulness of BMC in predicting oral drug absorption in humans was evaluated (*21*). A univariate hyperbolic model based on the BMC retention in 0.04 M Brij35 at pH 6.5 (the average pH of the small intestine) and 7.4 (the plasmatic pH value) of compounds was proposed as a fast primary screening tool for predicting the extension of oral drug absorption. As with other reported methods, that is, Caco-2 cell lines and PAMPA, the initial steep slope of the model limits the prediction accuracy

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for low to medium absorption drugs. The models fail when factors that decrease the absorption of drugs exist, that is, poor dissolution of the compound, chemical and bacterial decomposition at the absorption site, and first-pass metabolism in the intestinal cells and the liver.

If passive diffusion is the mechanism responsible for absorption, from the retention data in BMC of compounds two groups of polyphenols are observed:

Group 1 consists of compounds with retention factors higher than 3 at pH 6.5 in capillary columns; maximal oral absorption (>90%) is predicted. This is the case of flavones (except vicenin-2), flavonols (except rutin), flavanone, flavan-3-ols, and phloroglucinol. For the compounds 5-hydroxyflavone, 7-hydroxyflavone, apigenin, flavone, and flavanone, which present low solubility, the dissolution in the gastrointestinal tract could limit the absorption processes, and differences in presentation forms and physiological variables could influence their absorption.

On the other hand, for xenobiotics with maximal absorption and high solubility [i.e., vitexin, quercetin, (+)-catechin, (−)-epicatechin, and phloroglucinol; **Table 1**], their bioavailability could only be reduced due to presystemic metabolism and/or first pass metabolism. In this sense, reported values for bioavailability of (−)-epicatechin, quercetin, and (+)-catechin are 39% (*4*), 24% (*31*), and 10% (*32*), respectively. The reduced bioavailability of (+)-catechin and (−)-epicatechin could be attributed to a chemical decomposition with the pH; in fact, at pH 6.5 and 7.4 another peak appeared in the chromatogram with a larger retention time. No chemical decomposition with pH for the rest of the compounds of group 1 was observed.

Group 2 consists of compounds with retention factors $k < 3$ at pH 6.5 (vicenin-2, rutin, and phenolic acids); low and variable extents of absorption should be expected. For the intake of chlorogenic acid, an oral absorption in humans of $33 \pm 17\%$ has been reported (*33*), which agrees with the predicted value in BMC. The bibliographic results found for rutin indicate a bioavailability value of 17% (*31*). For this compound chemical degradation at pH 6.5 and 7.4 was observed, evidenced by the presence of another peak in the chromatogram.

One of the most interesting properties of polyphenolic compounds, from a therapeutic point of view, is their antioxidant activity. This property is of special importance for the prevention of neurological diseases, that is, Alzheimer's, Parkinson's, and cerebrovascular diseases (*34*). Therefore, prediction of passage across the blood–brain barrier (BBB) of polyphenolic compounds is of great importance. In a previous paper (*22*), a multiple linear regression model (see **Table 2**) that relates the brain–blood distribution coefficients (BB) data with BMC retention data and total molar charge of compounds was proposed. The analysis of the coefficients of the proposed model implies that the passage of neutral or cationic and hydrophobic compounds through the BBB is favored. By interpolation of the retention data of considered compounds into the log BB model, polyphenols can be classified as poor (BBB−) or easy (BBB+) brain penetrators. As can be observed in **Table 3**, the use of a cutoff of 0 implies that xenobiotics with log BB > 0 accumulate preferably in the brain (>50%). Using this criterion, 5-hydroxyflavone, 7-hydroxyflavone, apigenin, flavone, quercetin, and flavanone can cross the BBB. If a more restrictive cutoff is chosen to define BBB+ compounds (log BB > 0.5), only 5-hydroxyflavone, flavone, and flavanone can be considered as easy brain penetrators.

Another area of growing interest in polyphenolic compound research is their use in cosmetics and cosmeceutics. The penetration of xenobiotics through the skin may be expressed either as percent of absorption or as permeability coefficient, K_p.

Martínez-Pla et al. (*23, 24*) developed a QRAR model based on BMC retention using a 0.04 mol L^{-1} Brij35 at pH 5.5 mobile phase to describe and predict skin permeability (**Table 2**). In this model the retention of compounds in BMC expressed as log k_{BMC} and melting point (MP), a physicochemical property strongly dependent on molecular weight and hydrogen bonding, was used as a predictive variable. By interpolating the retention of polyphenolic compounds obtained at pH 5.5 and their corresponding melting points, the estimated log K_p values (**Table 3**) were obtained. As can be observed, flavanone, flavone, and 5-hydroxyflavone are the compounds that can more quickly cross the skin barrier. Vicenin-2, chlorogenic acid, and (+)-catechin show the lowest skin permeability. The only log K_p value found in the literature was for (+)-catechin (log K_p = −5.924) (*35*), which agrees with the low permeability predicted in BMC.

This paper points out the usefulness of BMC using capillary columns as a high-throughput primary screening tool that can provide key information about the potential transport properties of polyphenols in a simple and economical way.

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