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ORIGINAL

Potential of High-Performance Liquid Chromatography for Distribution Coefficient Determination of 3-Hydroxyquinolin-4(1H)-one Derivatives

Tereza Volná[1] · Kamil Motyka[1] · Jan Hlaváč[2]

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Abstract The potential of reversed-phase HPLC for the determination of distribution coefficient $D_{7.4}$ of selected 3-hydroxyquinolin-4(1H)-ones (3HQs) as compounds with significant biological activity was studied. Various stationary phases with C18 as well as hexyl-phenyl modification reflect current trends in RP-HPLC development such as higher sorbent silanophilicity, core–shell technology, hybrid and/or charged surface particles. Because of significant peak tailing of 3HQs at physiological pH on reversed-phase sorbents the separations at pH 3 were performed as well. Surprisingly, the pH change did not affect significantly the partition coefficients of 3HQs. Very affordable and common standards such as anisole, acetophenone, benzyl alcohol, brombenzene, ethylbenzoate and trichlorethylene were applied in the described methodology. The best linearity (R^2 0.9895) of the correlation between log P and log k_w for standards was obtained for hexyl-phenyl sorbent, but this stationary phase was shown to be unsuitable for HPLC separation of 3HQs. The highest linearity (R^2 0.9499) of the relationship between log $D_{7.4}$ determined by the classic shake-flask method and log D determined by means of HPLC for 3HQs was attained with Cortecs C18+ column at pH 7.4. The described methodology with Cortecs C18+ as stationary phase offers fast and accurate estimation of log $D_{7.4}$ of the tested 3HQs. In an effort to increase the throughput of the HPLC method for log $D_{7.4}$ determination, we evaluated almost aqueous mobile phase that contained only 3 % of acetonitrile. Although a worse correlation between log $D_{7.4}$ determined by shake-flask method and HPLC with almost aqueous mobile phase was observed, the described procedure offers a very simple and high-throughput alternative for the estimation of log $D_{7.4}$.

Keywords Liquid chromatography · Distribution coefficient · Lipophilicity · 3-Hydroxyquinolin-4(1H)-ones

Introduction

3-Hydroxyquinolin-4(1H)-ones (3HQs) are heterocyclic compounds that are studied for their relevant biological activity. 3HQs exhibit anti-cancer, anti-inflammatory, diuretic, anti-viral and anti-hypertensive effects [1]. Recently, the fluorescence properties of 3HQs were studied for their potential as fluorescent labels [2–5]. Moreover, 3HQs were studied as the quorum-sensing signaling molecules which play an important role in intercellular communication to coordinate bacterial behavior such as secondary metabolite production, biofilm development, swimming and swarming motility and virulence of some bacteria [6, 7].

Lipophilicity, expressed by the logarithm of octanol/water partition coefficient log P (or distribution coefficient log D for ionizable compounds), is one of important parameters in description of absorption, distribution, metabolism and elimination (ADME) properties, as well as, in the pharmacodynamic and toxicological profile of drugs [8, 9]. The lipophilicity is essential for the penetration across biological membranes and hydrophobic interactions with receptors, high log P/log D values are associated with undesired drug features, like extensive and unpredictable metabolism, high plasma protein binding, or accumulation to tissues [10]. For

✉ Kamil Motyka
motyka@orgchem.upol.cz

[1] Department of Organic Chemistry, Faculty of Science, Palacký University in Olomouc, 17. listopadu 12, Olomouc 77146, Czech Republic

[2] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University in Olomouc, Hněvotínská 1333/5, Olomouc 77900, Czech Republic

lipophilicity assessment, chromatographic techniques and particularly reversed-phase HPLC offer several practical advantages compared to the traditional shake-flask method, including speed, reproducibility, broader dynamic range, online detection, insensitivity to impurities or degradation products and reduced sample handling and sample sizes [11, 12].

Silica gel modified by C18 groups is the most widely used packing material for the reversed-phase columns that are appropriate for lipophilicity determination. However, the interference of silanophilic interactions in the partition mechanism was recognized long ago as a serious drawback, especially in the case of basic drugs [13, 14]. The problem is faced by the addition of a masking agent in the mobile phase and the selection of a stationary phase with reduced free silanol sites [15, 16]. End-capping of the silanol residues by trimethylchlorosilane (TMCS) or hexamethyldisilazane (HMDS) is usually performed during the manufacturing process, leading to a higher degree of silanization [17, 18]. The end-capped base deactivated silica (BDS) column is a better choice than octadecyl silica, since in its manufacture a higher degree of silanization is achieved using small alkyls to react with remaining silanol sites. Recently the LC-ABZ and the discovery-RP-amide-C-16 phases which are considered to be further deprived of silanophilic effects were successfully applied for log *P* determination. These columns contain an amide functional group which provides electrostatical shielding to the silanol sites, while a high degree of orientation of the alkyl chains is achieved [19, 20]. Another alternative, the polymer-based octadecylpolyvinyl (ODP) stationary phase, which is completely devoid of reactive silanol groups, has also been used for lipophilicity measurements [21]. However, it seems that the retention mechanism on ODP stationary phase and octanol–water partitioning are controlled by a different balance of structural properties. Thus, the data produced may be not suitable to reproduce the classical log *D* values [22]. The proposed methodologies require the addition of *n*-octanol or *n*-decylamine in the mobile phase in order to improve the simulation of the chromatographic conditions with the octanol–water system. Recently the hydrophilic interaction chromatography (HILIC) was applied for log *P* measurement for polar neutral compounds as well [23]. The criticism towards octanol as an isotropic medium with only a superficial similarity to biomembranes and the difficulties associated with the use of liposomes as more representative models have triggered the development of immobilized artificial membrane (IAM) stationary phases for use in HPLC. IAM chromatography has unfolded new perspectives in the application of HPLC as a tool to mimic specific interactions with phospholipids [24]. Recently, amino-P-C18 and IAM.PC.DD2 stationary phases have been compared. Retention parameters (log k_w) calculated

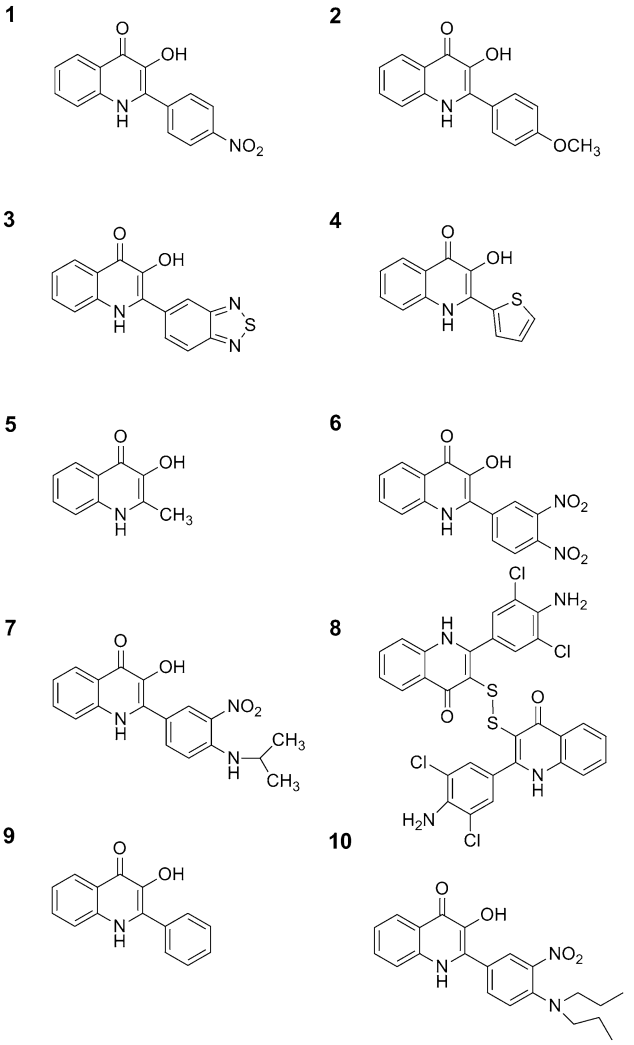

Fig. 1 Structures of investigated 3-hydroxyquinolin-4(1H)-ones

for both phases exhibit very good correlation and therefore it is reasonable to consider the amino-P-C18 material useful for the determination of drug interaction with the cell membrane in the similar way as the IAM stationary phase is used [25].

In this work, the evaluation of HPLC as a tool for distribution coefficient estimation of 3HQs that differ by substitution in position 2 is described (Fig. 1) [26–28]. 3HQs are compounds with the notable importance in medicinal chemistry and it is necessary to have available reliable and fast methods for the determination of log $D_{7.4}$ that is one of fundamental parameters in description of ADME properties of drugs or their candidates. This is reason for the study of the HPLC potential for distribution coefficient determination of the 3HQs. Different reversed-phase stationary sorbents (columns packed with C18 silica gel as well as silica gel modified by hexyl-phenyl groups) including recent technological trends such as higher silanophilicity (XSelect HSS T3,

Table 1 Applied columns and their parameters

Column	Manufacturer	Length (mm)	ID (mm)	Particle size (μm)	Flow rates (mL min^{-1})	Carbon loading (%)
XBridge C18	Waters	50	3	3.5	1.0	18
Triart C18	YMC	50	3	5	1.0	20
Kinetex C18	Phenomenex	50	3	2.6	0.2	12
Cortecs C18+	Waters	50	3	2.7	0.5	5.7
XSelect HSS T3	Waters	50	3	2.5	0.4	11
XSelect phenyl-hexyl	Waters	50	2.1	2.5	0.2	14
Triart C18	YMC	20	3	5	3.0	20

Waters), rigorous endcapping (Triart C18, YMC), core shell (Kinetex, Phenomenex; Cortecs C18+, Waters), hybrid particle (Triart C18, YMC; XSelect C18, Waters) and/or charged surface hybrid particle (Cortecs C18+, XSelect CSH Phenyl-Hexyl, Waters) for 3-hydroxyquinolin-4(1H)-one derivatives were tested. To our knowledge, the application of tested sorbents especially hexyl-phenyl silica gel for distribution coefficient estimation have not been described so far. In the presented procedure commercially available and cheap standards such as anisole, acetophenone, benzyl alcohol, brombenzene, ethylbenzoate and trichlorethylene were applied. In an effort to increase the throughput of the HPLC method for log $D_{7.4}$ determination, we also evaluated almost aqueous mobile phase what would enable very fast log $D_{7.4}$ for individual analyte in one chromatographic run.

Experimental

Chemicals

The synthesis of 3HQ derivatives **1–10** have been already described in detail elsewhere [26–28]. The purity of the used 3HQ derivatives was >98 % according to HPLC. The structures of tested 3HQs are depicted in Fig. 1. Water was obtained using Milli-Q RG apparatus by Millipore (Millipore Intertech, Bedford, MA, USA) in our laboratory (18.2 MΩ). Methanol (HPLC gradient purity) was purchased from Sigma-Aldrich (Steinheim, Germany). For buffer preparation, 3-(*N*-morpholino)propanesulfonic acid (MOPS) (>99.5 %) from Sigma-Aldrich (Steinheim, Germany) and formic acid (p.a.) from Lach-ner (Neratovice, Czech Republic) were used. Octanol (for HPLC) and anisole, acetophenone, benzyl alcohol, brombenzene, ethylbenzoate and trichlorethylene (p.a.) were purchased from Sigma-Aldrich (Steinheim, Germany). Standard buffers (Hamilton Duracal buffers, pH 4.01 ± 0.01 and 7.00 ± 0.01) for pH meter calibration were purchased from Hamilton (Switzerland). pH measurements were performed with combined SENTEK P11 electrode (SENTEK, Braintree, United Kingdom) and pH meter pH50 XS Instrument. The pH meter was calibrated with standard buffers for pH 4.01 and 7.0.

Chromatographic Conditions

The HPLC system Alliance (Waters, MA, USA) consisted of quaternary pump, autosampler and photodiode array detector. Data acquisition was performed using Empower software (version 3). Applied chromatographic columns and their parameters are summarized in Table 1. The mobile phase consisted of different mixtures of methanol and aqueous buffer (20 mM MOPS, pH 7.4 or 0.1 % v/v formic acid, pH 3.0) in the range from 15 to 85 % of methanol depending on compound retention (Table 2). 0.25 % octanol (in respect to the volume of methanol) was used as masking agent. These conditions were adopted from [29]. In the case of almost aqueous mobile phase experiments 0.01 mol L^{-1} MOPS, pH 7.4 was applied. Octanol saturated water was used for preparation of buffers. 3HQs and standards were dissolved in methanol and diluted at conc. of 0.4 mmol L^{-1}. For almost aqueous mobile experiments 3HQs and standards were diluted to the concentration of 40 μmol L^{-1} in 10 % methanol in water. The injection volume was 5 μL. The mobile phase flow rates are listed in Table 1. Retention times t_r were measured at least from three separate injections and retention data were expressed as the logarithm of the retention factor log *k*.

Lipophilicity Parameters

Because the distribution coefficients of studied 3HQs have not been published yet the shake-flask method were applied for their determination using a standard procedure as described in [29]. Briefly, the protocol is as follows. The octanol and the aqueous phases were mutually saturated before the experiment. The volume ratio of the two phases was chosen so that adequate amount of the solute remained in the aqueous phase after equilibration. Equilibration time

Table 2 Content of methanol in mobile phase for individual compounds and columns (mobile phase composition was changed gradually with difference of 5 %)

Column	XBridge C18	Triart C18	Kinetex C18	Cortecs C18+	HSS T3	XSelect CSH phenyl-hexyl
Compound	% of MeOH	% of MeOH	% of MeOH	% of MeOH	% of MeOH	% of MeOH
Anisole	35–60	35–60	40–65	45–70	55–75	40–60
Acetophenone	40–65	35–60	40–65	45–70	55–75	40–60
Benzyl alcohol	35–60	35–60	40–65	45–70	55–75	40–60
Brombenzene	40–65	40–65	40–65	45–70	55–75	40–60
Ethylbenzoate	40–65	35–60	40–65	45–70	55–75	40–60
Trichloroethylene	40–65	40–65	40–65	45–70	55–75	40–60
1	35–60	35–60	40–65	45–70	50–75	40–60
2	35–60	35–60	40–65	45–70	50–75	40–60
3	35–60	35–60	40–65	45–70	50–75	40–60
4	35–60	35–60	40–65	45–70	50–75	40–60
5	15–35	15–35	25–50	45–70	50–75	40–60
6	35–60	35–60	40–65	45–70	50–75	40–60
7	35–60	45–65	50–80	45–70	50–75	40–60
8	35–60	35–60	40–65	45–70	50–75	40–60
9	35–60	35–60	40–65	45–70	50–75	40–60
10	60–80	60–80	60–85	55–80	55–80	40–60

was 1–4 h, depending on the drug. Centrifugation followed for 15 min at 2500 rpm. The aqueous phase, before and after equilibration, was analyzed by UV–VIS spectrophotometry. Distribution coefficients D were calculated according to Eq. (1):

$$D = \frac{A_0 - A_1}{A_1}\frac{V_{aq}}{V_{oct}}, \quad (1)$$

A_0 and A_1 being the absorbance before and after equilibration and V_{aq}, V_{oct} the volumes of aqueous and octanolic phase, respectively. Each determination was performed at least in triplicate and RSDs of determined log D values were <5 %.

The chromatographically derived distribution coefficients log D_{HPLC} were determined as follows: from sets of isocratic measurements at different methanol contents in mobile phase the values of log k_w for each standard were found. log k_w represents the intercept of the log k-% MeOH dependences which corresponds to 100 % aqueous phase. Similarly log k_w values for individual 3HQs were determined. Values of log D_{HPLC} were found out from the correlation between log k_w and log P for standards. In the case of the application of almost aqueous mobile phase log k (that can be considered as k_w) for standards and studied 3HQs were measured. From the log k–log P dependence for standards log D_{HPLC} values for 3HQs were derived. Data were fitted by linear regression using the method of least squares in MS Excel (version 14).

Results and Discussion

The relationship between retention factors and the fraction of the organic modifier φ follows the Schoenmaker's solubility parameter model expressed by Eq. (2) [29]:

$$\log k = A\varphi + B\varphi^2 + E\sqrt{\varphi} + \log k_w, \quad (2)$$

A, B, E are the fitting coefficients and log k_w is the intercept which corresponds to 100 % aqueous phase. If organic modifier fraction >0.2 and in the presence of masking agent the linear part of Eq. (2) is sufficiently large and can be used to derive extrapolated log k_w values according to Snyder's linear solvent strength model [30], expressed by Eq. (3):

$$\log k = -S\varphi + \log k_w, \quad (3)$$

The slope of the equation S is considered to be related to the specific hydrophobic surface area of the solutes and depends on the solute and the chromatographic system. Linear extrapolation is usually preferred and certain ranges of methanol concentration are suggested according to the lipophilicity magnitude of the solutes [19, 31].

Correlation Between log k_w and log P for Standards

Ordinarily available and cheap compounds such as anisole, acetophenone, benzyl alcohol, brombenzene, ethylbenzoate and trichlorethylene were chosen as standards. Values of log P of standards for correlation between log k_w and log P

Table 3 Snyder's solvent strength model data for standards and coefficients of determination R^2

	Anisole	Acetophenone	Benzyl alcohol	Brombenzene	Ethylbenzoate	Trichloroethylene
XBridge C18 pH 3.0						
S	−0.0233	0.0209	−0.0141	−0.0342	−0.0300	−0.0299
log k_w	1.6804	1.1243	0.5127	2.7423	2.2409	2.3495
R^2	0.9950	0.9922	0.9537	0.9989	0.9975	0.9981
XBridge C18 pH 7.4						
S	−0.0238	−0.0209	−0.0167	−0.0302	−0.0301	−0.0299
log k_w	1.6965	1.1074	0.6545	2.8185	2.2368	2.3400
R^2	0.9972	0.9916	0.9844	0.9944	0.9972	0.9981
Triart C18 pH 3.0						
S	−0.0262	−0.0227	−0.0162	−0.0360	−0.0326	−0.0321
log k_w	2.0505	1.4338	0.7733	3.0339	2.5927	2.6551
R^2	0.9983	0.9985	0.9863	0.9992	0.9990	0.9993
Triart C18						
S	−0.0261	−0.0210	−0.0162	−0.0339	−0.0315	−0.0324
log k_w	2.0525	1.3440	0.7803	3.4680	2.5434	2.6701
R^2	0.9982	0.9962	0.9885	0.9999	0.9984	0.9991
Kinetex C18 pH 3.0						
S	−0.0270	−0.0220	−0.0196	−0.0355	−0.0316	−0.0310
log k_w	1.8198	1.1595	0.7466	2.7429	2.2885	2.3296
R^2	0.9998	0.9994	0.9982	0.9999	0.9996	0.9998
Kinetex C18 pH 7.4						
S	−0.0270	−0.0226	−0.0216	−0.0364	−0.0331	−0.0316
log k_w	1.8100	1.1821	0.8401	2.7867	2.3576	2.3629
R^2	0.9981	0.9978	0.9881	0.9997	0.9970	0.9989
Cortecs C18+ pH 3.0						
S	−0.0281	−0.0253	−0.0246	−0.0364	−0.0333	−0.0326
log k_w	1.8522	1.2932	0.8428	2.7921	2.3778	2.3977
R^2	0.9977	0.9925	0.9736	0.9974	0.9983	0.9985
Cortecs C18+ pH 7.4						
S	−0.0263	−0.0225	−0.0183	−0.0348	−0.0319	−0.0314
log k_w	1.7873	1.1740	0.5574	2.7277	2.3339	2.3571
R^2	0.9951	0.9918	0.9646	0.9983	0.9977	0.9982
HSS T3 pH 3.0						
S	−0.0267	−0.0221	−0.0320	−0.0179	−0.0317	−0.0351
log k_w	1.8933	1.2536	2.4267	0.7407	2.4707	2.8167
R^2	0.9958	0.9880	0.9978	0.9996	0.9982	0.9985
HSS T3 pH 7.4						
S	−0.0270	−0.0234	−0.0325	−0.0213	−0.0319	−0.0353
log k_w	1.8957	1.3083	2,4370	0.9504	2.4565	2.7953
R^2	0.9924	0.9761	0.9838	0.9767	0.9970	0.9973
XSelect phenyl-hexyl pH 3.0						
S	−0.0304	−0.0242	−0.0178	−0.0374	−0.0337	−0.0289
log k_w	1.7921	1.1506	0.4972	2.5806	2.1921	1.9737
R^2	0.9965	0.9920	0.9782	0.9920	0.9963	0.9881
XSelect phenyl-hexyl pH 7.4						
S	−0.0277	−0.0227	−0.0184	−0.0380	−0.0337	−0.0351
log k_w	1.6228	1.0596	0.5057	2.5931	2.1783	2.2625
R^2	0.9994	0.9956	0.9877	0.9999	0.9993	0.9978

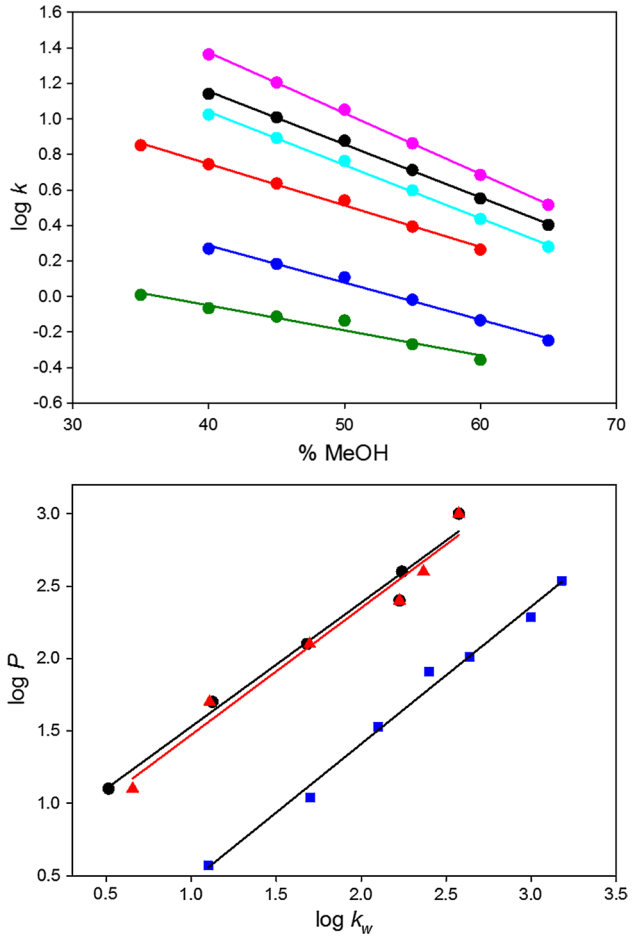

Fig. 2 Illustration of Snyder's solvent strength model equations for standards at pH 7.4 and log k_w–log P correlations at pH 3.0 (*black circles*), pH 7.4 (*red triangles* both for column XBridge C18) and for almost aqueous mobile phase (*blue squares* YMC Triart C18 short)

were taken from the literature [32] (log P values for anisole 2.1, acetophenone 1.7, benzyl alcohol 1.1, brombenzene 3.0, ethylbenzoate 2.6 and trichloroethylene 2.4). Standards were chosen in order to a range of log P values that are suitable for biological treatment (from 1 to 3) was covered. For simplicity and high method throughput, only six standards were measured.

It was reported [6, 7] and it is in agreement with our experience as well that low pH of mobile phase is suitable for 3HQs separation on reversed phase sorbents otherwise broad tailing peaks were observed. That is why besides of mobile phase with MOPS buffer (pH 7.4), formic acid mobile phase (pH 3.0) was applied. From Table 3 it is evident that Snyder's dependences between solvent strength and retention factor were linear and coefficients of determination R^2 were >0.99 for almost all applied standards and columns. Moreover, the linearity of retention factor–solvent strength dependences as well as log k_w values of standards were not significantly affected by pH of mobile phase. This fact can be easily explained if poor ionizability of applied standards at pH 3.0 and 7.4 is considered and therefore marginal effect of pH on retention of standards can be expected. In Fig. 2 an illustration of Snyder's solvent strength model equations for standards is depicted. The correlation between log k_w and log P for standards were nearly linear (Table 4). The high determination coefficients R^2 were achieved for XSelect Hexyl-Phenyl (0.9895) and XBridge C18 (0.9776) columns at pH 3.0. The negligible pH effect on the relationship between log k_w and log P for standards is appreciable from log k_w–log P correlations at pH 3.0 and pH 7.4 for column XBridge C18 depicted in Fig. 2.

Log *k*-solvent Strength Dependences for 3HQs

The equations of linear regression of log k-solvent strength dependences as well as R^2 for compounds **1**–**10** and applied columns are summarized in Table 5. In Fig. 3 illustrative chromatograms of derivative **1** at different methanol content in mobile phase are depicted. Generally, for the most compounds determination coefficients for log k-solvent strength dependences as a measure of linearity were >0.99. The lower R^2 values were observed for compounds **1** (Kinetex pH 7.4), **3** (XBridge pH 3.0 and 7.4), **5** (Xbridge pH 3.0; Kinetex pH 3.0 and 7.4) and **8** (Kinetex pH 7.4). From mentioned it seems that if linearity of log k-solvent strength dependences was considered the most suitable column was Triart C18 column. In Fig. 4 an illustrative comparison of

Table 4 Correlation between log P and log k_w for standards at pH 3.0, 7.4 and almost aqueous mobile phase pH 7.4

Column	pH 3.0	R^2	pH 7.4	R^2
XBridge C18	$\log P = 0.8546 \log k_w + 0.6745$	0.9776	$\log P = 0.8756 \log k_w + 0.5993$	0.9690
Triart C18	$\log P = 0.9259 \log k_w + 0.3521$	0.9490	$\log P = 0.9232 \log k_w + 0.3772$	0.9506
Kinetex C18	$\log P = 0.9360 \log k_w + 0.4628$	0.9701	$\log P = 0.9563 \log k_w + 0.3935$	0.9622
Cortecs C18+	$\log P = 0.9010 \log k_w + 0.4148$	0.9749	$\log P = 0.8139 \log k_w + 0.6670$	0.9756
HSS T3	$\log P = 0.9339 \log k_w + 0.5375$	0.9692	$\log P = 0.9185 \log k_w + 0.3370$	0.9627
XSelect phenyl-hexyl	$\log P = 0.8910 \log k_w + 0.6381$	0.9895	$\log P = 0.8355 \log k_w + 0.7266$	0.9687
Triart C18 (2 cm)			$\log P = 0.9479 \log k_w - 0.4854$	0.9905

Table 5 Snyder's solvent strength model data for 3HQs **1–10** and coefficients of determination R^2

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
XBridge pH 3.0										
S	−0.0208	0.0259	−0.0290	−0.0296	−0.0190	−0.0275	−0.0352	−0.0352	−0.0243	−0.0490
$\log k_w$	1.6582	1.4687	1.7523	1.7851	0.4930	1.3883	2.5357	2.1647	1.3338	3.9205
R^2	0.9989	0.9996	0.9798	0.9982	0.9702	0.9999	0.9992	0.9993	0.9994	0.9999
XBridge pH 7.4										
S	−0.0243	−0.0254	−0.0279	−0.0255	−0.0229	−0.0272	−0.0352	−0.0352	−0.0242	−0.0471
$\log k_w$	1.4682	1.4541	1.7158	1.4518	0,5886	1.3788	2.5327	2.1123	1.3309	3.7929
R^2	0.9904	0.9956	0.9795	0.9998	0.9931	0.9956	0.9993	0.9989	0.9975	0.9997
Triart pH 3.0										
S	−0.0285	−0.0275	−0.0269	−0.0294	−0.0188	−0.0271	−0.0382	−0.0370	−0.0254	−0.0516
$\log k_w$	1.9383	1.8195	1.8969	1.9157	0.6228	1.6509	2.9821	2.5000	1.6431	4.3062
R^2	0.9985	0.9950	0.9987	0.9993	0.9961	0.9987	0.9992	0.9986	0.9977	0.9997
Triart pH 7.4										
S	−0.0286	−0.0274	−0.0273	−0.0281	−0.0257	−0.0277	−0.0394	−0.0365	−0.0261	−0.0506
$\log k_w$	1.9333	1.8193	1.9242	1.8446	1.1424	1.6849	3.0501	2.4822	1.6786	4.2312
R^2	0.9976	0.9981	0.9987	0.9910	0.9981	0.9989	0.9994	0.9992	0.9976	0.9994
Kinetex pH 3.0										
S	−0.0299	−0.0288	−0.0292	−0.0296	−0.0278	−0.0290	−0.0396	−0.0415	−0.0284	−0.0495
$\log k_w$	1.8260	1.7151	1.8631	1.7581	0.9320	1.5938	2.8926	2.5695	1.6344	3.9760
R^2	0.9920	0.9982	0.9999	0.9982	0.9621	0.9983	0.9995	0.9997	0.9995	0.9993
Kinetex pH 7.4										
S	−0.0358	−0.0290	−0.0281	−0.0290	−0.0208	−0.0292	−0.0394	−0.0467	−0.0272	−0.0497
$\log k_w$	2.1701	1.7222	1.7912	1.7278	0.5839	1.6077	2.6595	2.8596	1.5684	3.9871
R^2	0.9741	0.9981	0.9916	0.9999	0.9678	0.9998	0.9997	0.9872	0.9997	0.9994
Cortecs C18+ pH 3.0										
S	−0.0317	−0.0315	−0.0293	−0.0305	−0.0267	−0.0320	−0.0388	−0.0450	−0.0301	−0.0529
$\log k_w$	1.8158	1.7141	1.7760	1.6578	0.5436	1.5515	2.7580	2.5674	1.5657	4.1554
R^2	0.9765	0.9902	0.9955	0.9942	0.9191	0.9888	0.9956	0.9923	0.9921	0.9994
Cortecs C18+ pH 7.4										
S	−0.0282	−0.0273	−0.0256	−0.0261	−0.0144	−0.0257	−0.0380	−0.0397	−0.0259	−0.0531
$\log k_w$	1.6563	1.5410	1.6377	1.4520	0.1024	1.1566	2.7467	2.2583	1.4033	4.2149
R^2	0.9959	0.9940	0.9893	0.9971	0.9029	0.9960	0.9988	0.9964	0.9931	0.9978
HSS T3 pH 3.0										
S	−0.0320	−0.0314	−0.0301	−0.0270	−0.0202	−0.0271	−0.0363	−0.0379	−0.0282	−0.0491
$\log k_w$	2.0668	1.9641	2.0457	1.6661	0.6240	1.5444	2.7904	2.4264	1.7223	4.1110
R^2	0.9761	0.9935	0.9959	0.9937	0.9168	0.9824	0.9980	0.9972	0.9945	0.9994
HSS T3 pH 7.4										
S	−0.0291	−0.0282	−0.0273	−0.0273	−0.0166	−0.0277	−0.0372	−0.0390	−0.0270	−0.0526
$\log k_w$	1.8472	1.7289	1.8353	1.6655	0.4731	1.5457	2.8009	2.4307	1.6111	4.2818
R^2	0.9938	0.9946	0.9951	0.9918	0.9779	0.9964	0.9958	0.9937	0.9960	0.9963
XSelect CSH pH 3.0										
S	−0.0330	−0.0341	−0.0337	−0.0365	−0.0242	−0.0346	–	−0.0494	−0.0314	−0.0520
$\log k_w$	2.1002	2.0104	2.1624	2.1295	0.7496	2.0090	–	3.4228	1.7748	2.5307
R^2	0.9927	0.9944	0.9773	0.9968	0.9647	0.9990	–	0.9998	0.9982	0.9999
XSelect CSH 7.4										
S	−0.0302	–	−0.0281	−0.0302	−0.0160	−0.0281	–	−0.0399	−0.0275	–
$\log k_w$	1.9391	–	1.8472	1.7765	0.2870	1.6292	–	2.6218	1.5546	–
R^2	0.9959	–	0.9939	0.9973	0.9955	0.9856	–	0.9947	0.9923	–

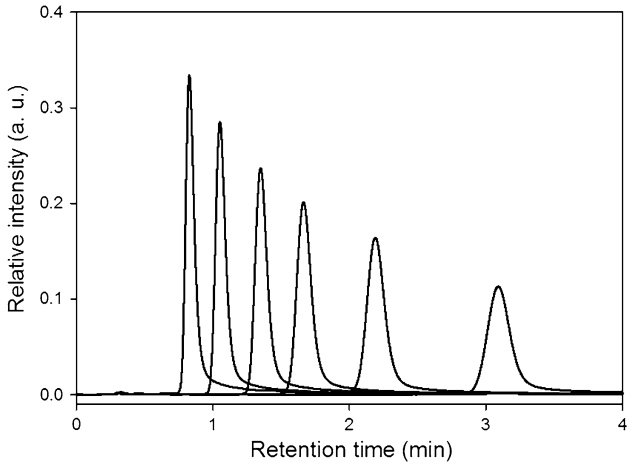

Fig. 3 Illustrative chromatograms for derivative **1** at different methanol content in mobile phase (YMC Triart C18; with increasing retention time 60, 55, 50, 45, 40 and 35 % MeOH)

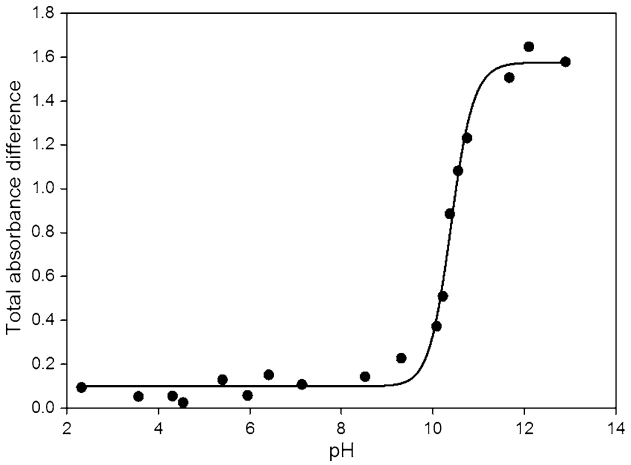

Fig. 5 Dependence of total absorbance difference on pH for **1** and nonlinear regression of measured data

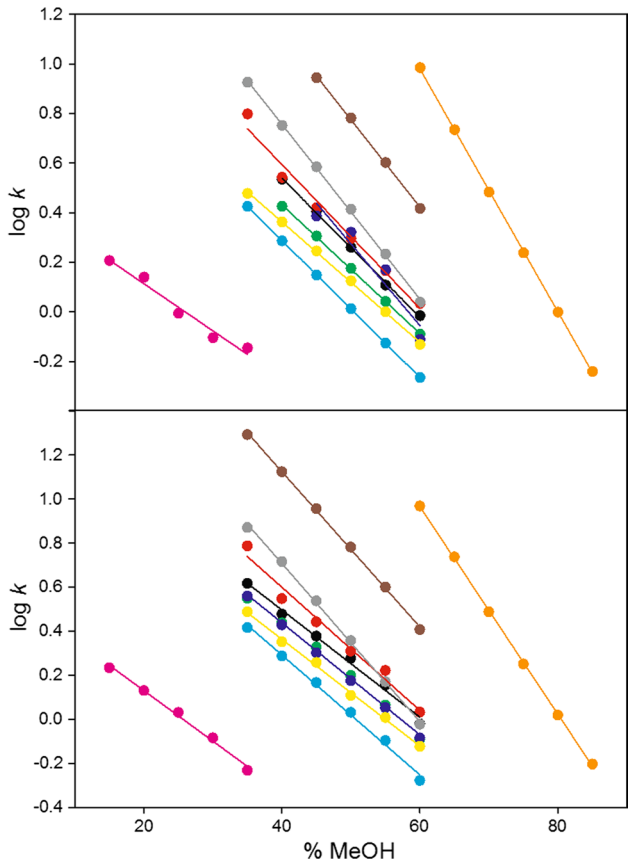

Fig. 4 Illustrative comparison of dependences of log k on methanol percentage for pH 3.0 (*top*) and pH 7.4 (*below*) for Triart C18 column (**1**, *black*; **2**, *green*;, **3**, *red*; **4**, *blue*; **5**, *pink*; **6**, *cyan*; **7**, *brown*; **8**, *gray*; **9**, *yellow*; **10**, *orange*)

dependences of log k on methanol percentage for pH 3.0 (top) and pH 7.4 (below) for Triart C18 column is shown. The pH effect on Snyder's solvent strength model data was more apparent than in the case of standards. Especially, values of the intercept which corresponds to 100 % aqueous phase log k_w differed for some 3HQs measured at different pH (e.g., log k_w for **5** and column Kinetex C18 was 0.9320 and 0.5839 for pH 3.0 and 7.4, respectively). The difference between values measured at pH 3.0 and 7.4 was least for column Triart C18 that is evident from Fig. 4, where log k-methanol percentage dependences measured for pH 3.0 (top) and 7.4 (below) are presented. But generally the pH effect on the distribution constant estimation of 3HQs was not substantial. The possible explanation can consist in acido-basic properties of studied 3HQs. It can be expected that 3HQs do not occur in any dissociated form in aqueous solution at pH 3.0 and 7.4 and therefore their retention properties do not change significantly. But, even so, the peak shape improved in acidic mobile phase. To prove this theory the acid dissociation constant (pK_a) was determined for **1** as a representative with the use of UV–VIS spectrophotometry [33]. The normalized dependences of total absorbance difference on pH are placed in Fig. 5 and pK_a for **1** was determined as 10.25. The pK_a value indicates the forming of deprotonated anionic form in strong alkaline solution and supports our assumption of nonionic form at acidic and neutral pH.

Correlation Between log k_w and log P for 3HQs

The distribution coefficients of 3HQs were determined from the correlations between log P and log k_w for standards and corresponding pH and column. In Table 6 distribution coefficients determined by means of "shake-flask" method and HPLC method are listed and can be compared. Distribution coefficient data provided by HPLC method were in relatively good agreement with those obtained by

Table 6 Comparison of partition/distribution coefficients determined by means of "shake flask" method and HPLC method

Compounds	Shake-flask	xBridge pH 3.0	xBridge pH 7.4	Triart pH 3.0	Triart pH 7.4	Kinetex pH = 3.0	Kinetex pH 7.4	Cortecs pH 3.0	Cortecs pH 7.4	HSS T3 pH 3.0	HSS T3 pH 7.4	XSelect pH 3.0	XSelect pH 7.4	Triart short
1	2.30	2.06	1.86	2.03	2.01	2.13	2.40	2.05	2.02	2.26	2.03	2.51	2.35	2.15
2	2.00	1.91	1.85	1.94	1.93	2.03	2.00	1.96	1.92	2,18	1.92	2.43	–	2.57
3	2.00	2.13	2.06	2.00	2.00	2.16	2.06	2.01	2.00	2.24	2.02	2.56	2.27	2.88
4	2.02	2.14	1.84	2.01	1.94	2.07	2.01	1.91	1.85	1.93	1.87	2.54	2.21	2.70
5	1.02	2.13	1.15	1.00	1.46	1.35	1.00	0.90	0.75	1.06	0.77	1.31	0.97	1.34
6	1.51	1.84	1.79	1.81	1.83	1.93	1.90	1.81	1.69	1.83	2.02	2.43	2.09	1.47
7	2.69	2.76	2.72	2.85	2.77	3.06	2.83	2.90	2.90	2.86	2.91	–	–	3.33
8	2.36	2.46	2.38	2.47	2.37	2.78	3.00	2.73	2.51	2.56	2.57	3.69	2.92	3.21
9	2.13	1.80	1.75	1.80	1.83	1.96	1.87	1.83	1.81	1.97	1.82	2.22	2.02	2.22
10	3.67	3.86	3.73	3.89	3.59	4.01	4.00	4.16	4.10	3.97	4.27	2.89	–	3.57

shake-flask method. The highest R^2 for log P and log k_w correlations for 3HQs (Table 7) were achieved for Triart C18 and Cortecs C18+ columns and both pH 7.4 (0.9436 and 0.9756, respectively). In Fig. 6 log P–log k_w dependences for Cortecs C18+ column are shown and it is evident that the regression curves are similar for both pH. Triart C18 and especially Cortecs C18+ columns (pH 7.4) provided good estimation of log $D_{7.4}$ in the comparison with log $D_{7.4}$ values determined by means of shake-flask method as a standard method (Table 6). These values of regression coefficients are comparable with those published previously for different sets of compounds (R^2 0.9490 [19]; R^2 0.9370 or R^2 0.9430 only for basic compounds [19]). When XSelect phenyl-hexyl column was applied for 3HQs separations a significant peak broadening and tailing was observed, especially at pH 7.4. For some 3HQs due to this peak broadening the chromatograms could not be evaluated and therefore corresponding data are missing in Tables 5 and 6. In spite the fact that this sorbent provided the best linearity for the correlation between log P and log k_w for standards XSelect phenyl-hexyl column seemed inappropriate for 3HQ separation at applied conditions. The Triart C18 sorbent is described by the manufacturer as a hybrid silica material with little metal impurities and rigorously endcapped. Interactions of an analyte with residual silanol groups and/or surface impurities can significantly affect the equilibrium between mobile phase and the stationary phase thus resulting distribution coefficient. And a restriction on these interactions can be considered as possible explanation why the Triart C18 sorbent provided satisfactory results. Similarly, the introducing of positive charge on silica surface is used for blocking the interaction with the silanols and that could be possible explanation of relatively good results that were achieved for Cortecs C18+ column.

Application of Almost Aqueous Mobile Phase

In an effort to increase the throughput of the HPLC method for log $D_{7.4}$ determination we evaluated mobile phase (0.01 mol L^{-1} MOPS, pH 7.4) that contained 3 % of acetonitrile. The reason for the application of the almost aqueous mobile phase was an assumption that the relatively lengthy measurement of log k-solvent strength dependences can then be omitted. The measured retention factors can be considered as the intercept k_w in Eq. (3). A short C18 column (length 2 cm, YMC Triart C18) was applied for these experiments and flow rate of 3 mL min^{-1} so that the analytes were eluted from the column in acceptable retention times (the most lipophilic compound that was tested, **10**, was eluted in 21 min). The fully aqueous mobile phase could not be applied because of very broad peaks of tested 3HQs and long retention times. p-xylene (log P 3.18) was chosen to prove that the method is suitable for lipophilic compounds

Table 7 Correlation between log $D_{7.4}$ (shake-flask method) and log D determined by HPLC for 3HQs at pH 3.0 and 7.4

Column	pH 3.0	R^2	pH 7.4	R^2
XBridge C18	$\log D = 0.9514k_w + 0.2474$	0.8536	$\log D = 1.0528k_w - 0.0646$	0.8639
Triart C18	$\log D = 1.0787k_w - 0.0399$	0.9193	$\log D = 1.1262k_w - 0.1839$	0.9436
Kinetex C18	$\log D = 1.0863k_w + 0.0488$	0.9044	$\log D = 1.1720k_w - 0.1463$	0.9077
Cortecs C18+	$\log D = 1.1939k_w - 0.3647$	0.9271	$\log D = 1.2243k_w - 0.5017$	0.9499
HSS T3	$\log D = 1.1434k_w - 0.4133$	0.5810	$\log D = 1.2301k_w - 0.4492$	0.9033
XSelect CSH phenyl-hexyl	$\log D = 0.5443k_w + 1.3592$	0.3960	$\log D = 1.0810k_w + 0.0585$	0.7813
Triart C18 short			$\log D = 0.6139k_w + 0.9867$	0.6994

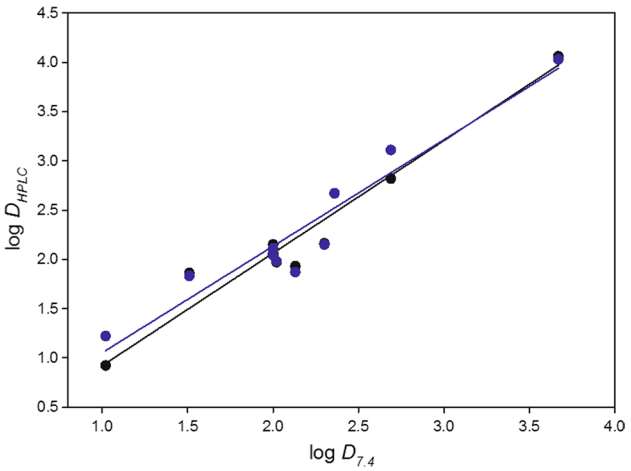

Fig. 6 Correlation between log $D_{7.4}$ (shake-flask method) and *log D* determined by HPLC for Cortecs C18+ column (*black*, pH 7.4; *blue*, pH 3.0)

as well. From Fig. 2 (blue squares) it is evident that log k_w–log P dependence for standards was linear and coefficient of determination R^2 was 0.9905 (Table 4). The distribution coefficients of 3HQs were determined from the correlations between log P and log k_w for standards. The determined log $D_{7.4}$ values of tested 3HQs are listed in Table 6. These results show that the correlation between log $D_{7.4}$ determined by shake-flask method and HPLC with almost aqueous mobile phase are worse (Table 7) than in the case of method based on measurement of log k-solvent strength dependences. But the described procedure offers very simple and high-throughput alternative for an estimation of log $D_{7.4}$ of 3HQs. The excellent log P–log k_w correlation for standards prompts that the procedure could be suitable for the determination of log $D_{7.4}$ for other compounds that would show better chromatographic behavior in neutral pH than investigated 3HQs.

Conclusions

The potential of reversed-phase HPLC for the estimation of distribution coefficient $D_{7.4}$ of selected 3-hydroxyquinolin-4(1H)-ones as compounds with significant biological activity was studied. Various stationary phases with C18 as well as hexyl-phenyl modification that reflect current trends in RP-HPLC development were tested. Due to poor chromatographic behavior of 3HQs at physiologic pH on reversed-phase sorbents (significant peak tailing) the separations at pH 3.0 were performed as well. Surprisingly, the pH change did not affect significantly the partition coefficients of 3HQs. Very affordable and common standards such as anisole, acetophenone, benzyl alcohol, brombenzene, ethylbenzoate and trichlorethylene were applied in the described methodology. The best linearity (R^2 0.9895) of the correlation between log P and log k_w for standards was obtained for hexyl-phenyl sorbent, but this stationary phase was shown to be unsuitable for HPLC separation of 3HQs. The highest linearity of the relationship between log $D_{7.4}$ (R^2 0.9499) determined by the classic shake-flask method and log D determined by means of HPLC for 3HQs was attained with Cortecs C18+ column at pH 7.4. The described methodology with Cortecs C18+ as stationary phase offers fast and accurate estimation of log $D_{7.4}$ of tested 3HQs. Especially, if the studied 3HQs are poorly soluble in water or water/dimethyl sulfoxide mixtures and a precipitation may occur during the shake-flask method experiments the HPLC offers the effective alternative. In an effort to increase the throughput of the HPLC method for log $D_{7.4}$ determination, we evaluated almost aqueous mobile phase that contained only 3 % of acetonitrile. Although the worse correlation between log $D_{7.4}$ determined by shake-flask method and HPLC with almost aqueous mobile phase was observed, the described procedure offers very simple and high-throughput alternative for the estimation of log $D_{7.4}$ and could be applied for extensive compound libraries.

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Compliance with Ethical Standards

Conflict of interest Author Tereza Volná declares that she has no conflict of interest. Author Kamil Motyka declares that he has no conflict of interest. Author Jan Hlaváč declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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