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Original article

Antihypertensive and antiarrhythmic properties of a *para*-hydroxy[bis(*ortho*-morpholinylmethyl)]phenyl-1,4-DHP compound: Comparison with other compounds of the same kind and relationship with log*P* values

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ABSTRACT

A new para-hydroxy[bis(ortho-morpholinylmethyl)]phenyl-1,4-DHP substituted compound, (4-(4-hydroxy-3,5-bis(morpholin-4-ylmethyl)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid diethyl ester, LQM300), with antihypertensive and antiarrhythmic properties, has been synthesized. Four pKa values of this compound have been determined with the aid of the program SQUAD, at pseudo-physiological conditions ($T = 37$ °C and $I = 0.15$ M) by UV spectrophotometry and at $T = 25$ °C and $I = 0.05$ M by Capillary Zone Electrophoresis (CZE). The log*P* = 2.7 ± 0.2 between *n*-octanol and water, has been estimated by UV spectrophotometry. The antihypertensive and antiarrhythmic efficacies as well as the log*P* values have been compared with other compounds of the same kind and related with their structure.

1. Introduction

Today there are many compounds used as antihypertensives [1–9]. Despite the great number of antiarrhythmic drugs known today, a satisfactory treatment does not exist and it has been necessary to develop long-lasting, oral route agents with minor indirect effects [6]. In Mexico arterial hypertension is a serious public health issue, so it is important to develop new compounds with antihypertensive activity, but less adverse effects and at more accessible costs. Recently the synthesis of some morpholinyl-methylphenols, as well as methyltiomorpholinylphenols, (LQM compounds, based on the Region 2 of changrolin molecule [10]) has been reported [11] and the antihypertensive and antiarrhythmic properties of some methyltiomorpholinylphenols have been demonstrated [12].

Due to the great potential observed for the LQM compounds, **1**–**3**, shown in Fig. 1, having antihypertensive and antiarrhythmic properties [12], it was decided to synthesize LQM300 (compound **4**, Fig. 2) that merges in the same molecule two fragments with antihypertensive properties: a 1,4-DHP moiety and a changrolin-Region 2-like moiety [10] (Figs. 1 and 2). The second fragment has also antiarrhythmic properties. With these structural changes it was expected that the new molecule had an ambivalent antihypertensive and antiarrhythmic action: blockage of Ca^{2+} channels (associated to 1,4-DHP moiety) and binding to Na^{+} channels (suggested for the changrolin-Region 2 moiety [13]).

The aim of the present work is to determine the antihypertensive and antiarrhythmic properties of the compound **4** (LQM300) and to determine its log*P* value and of the compounds **1**–**3**, between water and *n*-octanol, in order to compare it with those of similar molecules and trying to relate these values with the

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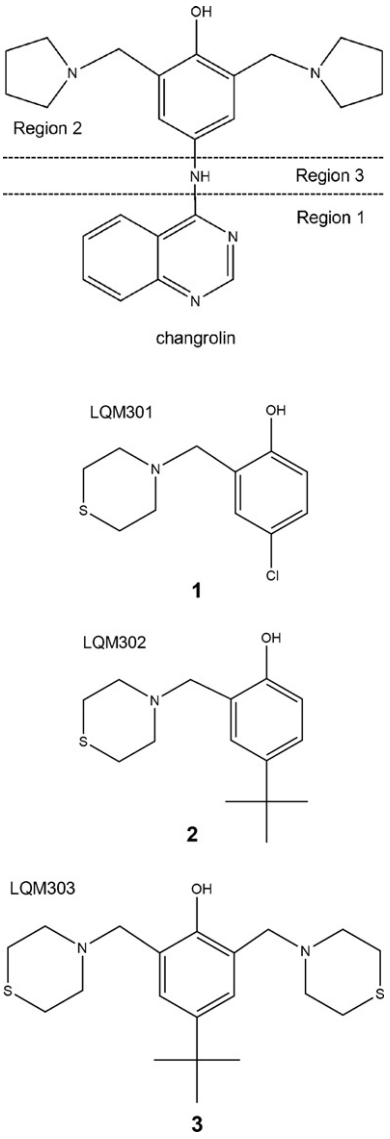

Fig. 1. Changrolin and some LQMs previously studied [12].

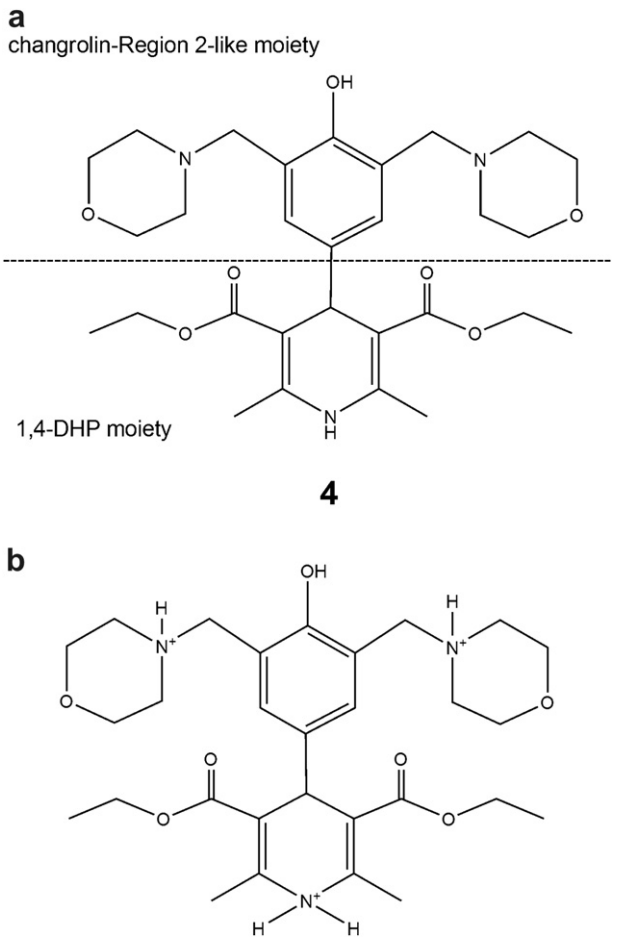

Fig. 2. Chemical structures of compound **4** (LQM300). a) Neutral species H(LQM300) = HL compound showing the 1,4-DHP and changrolin-like moieties. b) Fully protonated species $H_4(LQM300)^{3+} = H_4L^{3+}$.

antihypertensive efficacy of LQM compounds. It was decided to obtain the log*P* of compound **4** by determining its extracted fraction as a function of pH and its pKa values using Spectrophotometric (UV) and Capillary Zone Electrophoresis (CZE) studies through data processing by the program SQUAD [14–17].

2. Chemistry

2.1. Synthesis and properties of the LQM compounds

2.1.1. Synthesis of LQM300 (compound **4**)

4-Hydroxyphenyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid diethyl ester was prepared from 4-hydroxybenzaldehyde with ethyl acetoacetate in ammonia gave the 1,4-dihydropyridine resulting from the normal Hantzsch reaction, Yield, 80%. Mp 230–232 °C. FT-IR (KBr): 3331, 1689, 1656, 1490, 1244, 1126, 720 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.25$ (t, $J = 7.1$ Hz, 6H), 2.34 (s, 6H), 4.12 (q, $J = 7.1$ Hz, 4H), 5.32 (s, 1H), 5.77 (s, 1H), 7.24–7.41 (m, 4H). ^{13}C NMR ($CDCl_3$): δ = 167.8, 148.5, 142.3, 130.4, 130.9, 127.9, 103.9, 59.8, 39.4, 19.3, 14.1.

4-(4-Hydroxy-3,5-bis(morpholin-4-ylmethyl)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid diethyl ester (compound **4**, Fig. 2) was prepared as described elsewhere [11] from 4-hydroxyphenyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid diethyl ester and thiomorpholine and formaldehyde (37%) were mixed in a round flask fitted with a condenser. The mixture was irradiated with infrared light for 10 min using a medicinal infrared lamp (250 Watts) and the reaction was monitored by TLC. Yield: 85%. The mixture was passed through a silica gel chromatography column using gradient hexane/ethyl acetate, than afforded one crystalline product.

4-(4-Hydroxy-3,5-bis(morpholin-4-ylmethyl)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid diethyl ester. Physico chemical properties. Mp 145–147 °C. IR (cm^{-1}; $CHCl_3$ film) 3450, 3540, 3056, 2932, 1691. ^1H NMR (300 MHz; $CDCl_3$; Me_4Si, δ_H): 6.93 (2H, s), 5.6 (1H, s, OH), 4.87 (1H, s, NH), 4.1 (4H,q, $J = 7.0$ Hz), 3.71 (8H, m), 3.51 (2H, s), 2.49, (8H, m), 2.35 (6H, s) 1.22 (6H, t, $J = 14.0$ Hz). ^{13}C NMR (δ $CDCl_3$): 167.7; 154.2; 143.3; 138.2; 128.7; 121.1; 104.4; 66.9; 59.5; 53.2; 38.7; 19.6; 14.3. FAB-MS (M + 1) 544 (20%), 456 (96%), 252 (100%). Calculated for $C_{29}H_{41}N_3O_7$, C 64.07%; H 7.60%; N 7.73%; O 20.60%.

2.1.2. Synthesis of LQM301, LQM302 and LQM303 (compounds **1**–**3**)

These compounds (Fig. 1) have been synthesized as described elsewhere [11,12].

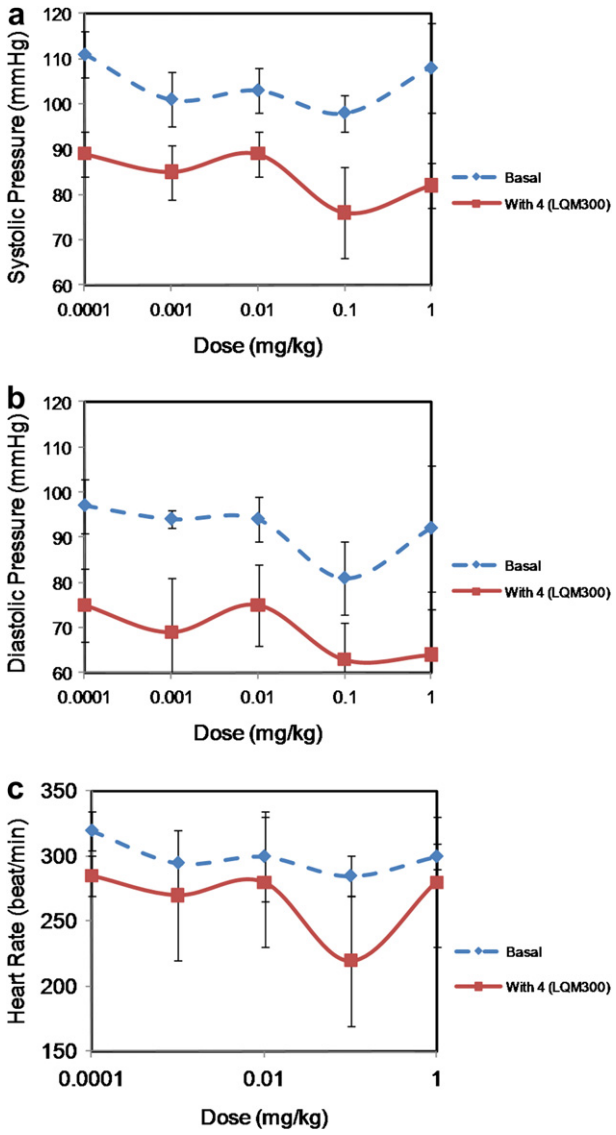

Fig. 3. Doses–effect curve (mean ± SD, $n = 5$) of the LQM300 compound for a) systolic pressure (SP), b) diastolic pressure (DP) and c) heart rate (HR), using IAP model in male Wistar rats anesthetized. The hypotensor effect of the LQM300 compound on the SP and DP such as HR diminution was found for every dose used.

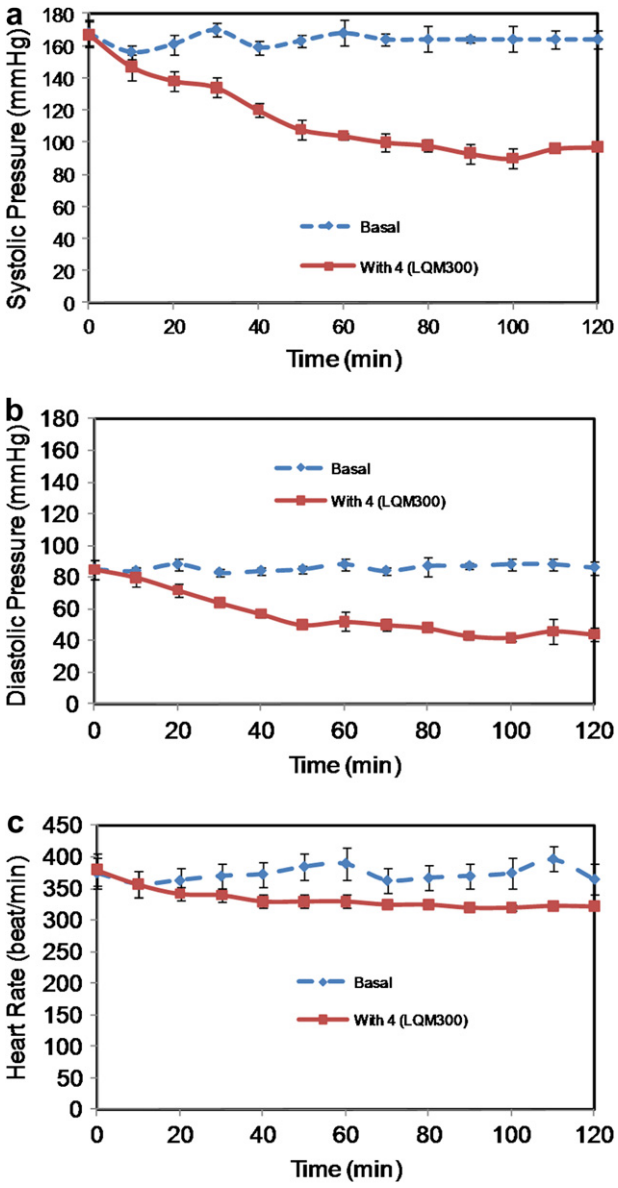

Fig. 4. Effect on a) SP, b) DP and c) HR of LQM300 after oral administration, using SHR model. In these cases a dose of 0.1 mg/kg was used. Results are given on mean ± SD, $n = 5$.

2.2. Chemicals

All chemicals were analytical grade including H_3PO_4, Na_2HPO_4, $Na_3PO_4 \cdot 12H_2O$ (Química Meyer, Mexico), $NaH_2PO_4 \cdot H_2O$ (Técnica Química, Mexico), sodium acetate (Merck), NaCl and boric acid (Productos Químicos Monterrey, Mexico).

3. Antihypertensive and antiarrhythmic properties of the LQM300 compound

3.1. Biological material

9-month old Male Wistar rats and male spontaneously hypertensive rats (SHR) were obtained from the Centro de Investigación y de Estudios Avanzados (CINVESTAV-IPN). All animal procedures were conducted according to the Federal Regulations for Animals Experimentation and Care (SAGARPA, NOM-062-ZOO-1999, Mexico).

Compound **4** was administrated using an intravenous physiological saline solution for the invasive model and an oral glucose solution (5%) in the spontaneous hypertenses rat model.

The studies of noninvasive arterial pressure model were carried out in a SPAM apparatus. The software used to collect data was SIEVART 1. This equipment is at the Instituto Nacional de Cardiología "Ignacio Chávez" from Mexico City.

3.2. Biological activity

3.2.1. Invasive arterial pressure model (IAP)

Rats were anesthetized by intra-peritoneal injection of sodium pentobarbital (40 mg/kg). Once the rat had reached a sufficient depth of anesthesia, carotid arteria was cannulated using a catheter model PE50, which was connected to a pressure transducer. Then, femoral vein was dissected using this route to

introduce in the rat different doses of compound **4** using a catheter PE10.

Basal heart rate, systolic and diastolic pressure were monitored, the tested doses of compound **4** in this work were 0.0001, 0.001, 0.01, 0.1 and 1.0 mg/kg. Data were collected using a Digi-Med pressure analyzer equipped with DMSI-200_1 software.

3.2.2. Non invasive arterial pressure model or spontaneous hypertenses rats (SHR)

Five spontaneously hypertensive rats were used. Heart rate, systolic and diastolic pressure were collected at the beginning and every 10 min for 2 h. After that the compound **4** was orally administrated (0.1 mg/kg) and heart rate, systolic and diastolic pressures were monitored in the same way.

3.3. Antihypertensive and antiarrhythmic properties

In order to obtain fast and reliable data about the activity of the compound **4**, the IAP Model was used, and it provided us a fast manner to know if this compound really decreases the arterial tension. Fig. 3 represents the curves dose-response for systolic and diastolic pressure (a and b, respectively) and heart rate (c) for the IAP model. The dose which showed more hypotensive activity was 0.1 mg/kg even on heart rate.

The SHR model was applied to evaluate the pharmacologic effect on the diastolic and systolic pressure and for heart rate too. The systolic and diastolic pressure reduction practically was initiated after oral administration, and reached its maximum effect after 50 min of the beginning of the experiment, and it remained until the end of the experiment (120 min) Figs. 4a and b. The effect on heart rate was also observed at the beginning until the end of the experiment, and it reached a maximum level after 40 min Fig. 4c.

4. Results and discussion

4.1. CZE experiments

The molecular structure of compound **4** (Fig. 2) permits to expect until four acid functions for the totally protonated species (H_4L^{3+}): the phenol and the three nitrogen atoms that might be protonated in an acid aqueous medium.

The effective mobility of compound **4** as a function of pH is shown in Fig. 5. The experimental values were fitted to the equation (1) using the program SQUAD, as explained before [16,17], with a chemical model involving four global formation equilibria.

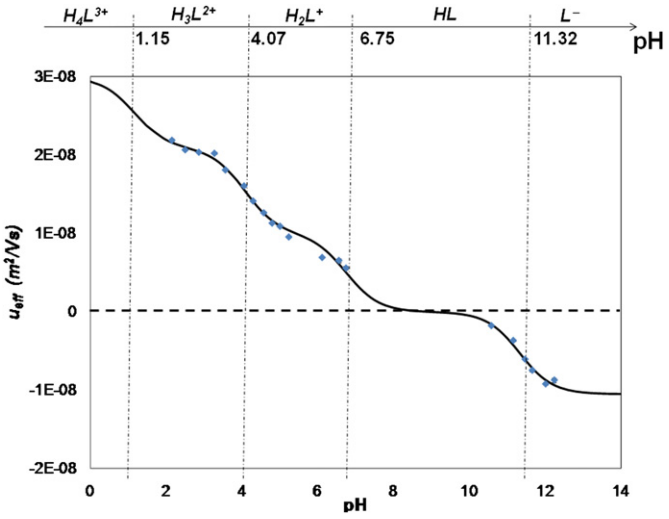

Fig. 5. Effective mobility of compound **4** (LQM300) as a function of the aqueous solution pH. Markers represent experimental values (the average of three replicates) while solid line represents fitting achieved with a four acid–base equilibria model and data of Table 1. Vertical lines signal the position of the pKa values determined at $T = 25$ °C and $I = 0.05$ M and in the top of the figure the predominance-zone diagram [18] of the species of compound **4** has been drawn.

$$u_{\text{eff}} = u_{\text{LQM300}} = u_L f_L + u_{HL} f_{HL} + \ldots + u_{H_jL} f_{H_jL} + \ldots + u_{H_nL} f_{H_nL}$$
$$= \frac{z_L \lambda_L}{F} f_L + \frac{z_{H_2L^+} \lambda_{H_2L^+}}{F} f_{H_2L^+} + \frac{z_{H_3L^{2+}} \lambda_{H_3L^{2+}}}{F} f_{H_3L^{2+}} + \frac{z_{H_4L^{3+}} \lambda_{H_4L^{3+}}}{F} f_{H_4L^{3+}}$$
$$\approx \left(z_L f_L + z_{H_2L^+} f_{H_2L^+} + z_{H_3L^{2+}} f_{H_3L^{2+}} + z_{H_4L^{3+}} f_{H_4L^{3+}} \right) \frac{\lambda_{\text{LQM300}}}{F} = \bar{z}_{\text{LQM}} \frac{\lambda_{\text{LQM300}}}{F} \quad (1)$$

where f_{HjL} ($j \in \{0, 1, 2, 3, 4\}$) are the fractions of the compound **4** (LQM300) species (that contain L in the system). [19], Table 1.

Analyzing the information obtained by CZE for compound **4** it seems that $pKa_1 = 1.15$ may be related to the nitrogen atom of the 1,4-DHP moiety [1] and, by analogy with the behavior of some pyperidinylmethylphenols with bulky non-polar substituents in *para* position, previously studied [17], the $pKa_2 = 4.07$ and $pKa_3 = 6.75$ values could be associated to the nitrogen atoms of morpholines. Then, the $pKa_4 = 11.28$ should be linked then to the phenol group. In this way, the neutral species HL could be molecular and *non-zwitterionic*.

4.2. UV spectrophotometry

The absorption spectra obtained in this work for $[HL] = 8.83 \times 10^{-5}$ M aqueous solutions (compound **4**) at different pH values for pseudo-physiological conditions ($T = 37$ °C and 0.15 M ionic strength ($\boldsymbol{I}$)) are shown in Fig. 6.

It is remarkable that at this concentration the compound forms a solid in the $6.7 < \text{pH} < 10.4$ range. Then, absorbance values of 23 absorption spectra –corresponding to aqueous solutions of 8.83×10^{-5} M of the compound **4** in the $1.5 \leq \text{pH} \leq 6.8$ and $10.4 \leq \text{pH} \leq 13.1$ pH ranges and in the $230 \text{ nm} \leq \lambda \leq 425$ nm range (with increments of $\Delta\lambda = 5$ nm) were introduced to program SQUAD, in order to refine global formation constants and molar absorptivity coefficients for several models. The best refinement is presented in Table 1. The molar absorptivities obtained in the same refinement are shown in Fig. 7.

4.3. logP determinations

*4.3.1. Distribution ratio, logP′ = f(pH), between n-octanol and aqueous solutions for compound **4** (LQM300)*

The absorption spectra obtained to quantify compound **4** (LQM300) concentration in *n*-octanol ($[\text{LQM300}]_o$) is shown in Fig. 8a, for the concentrations of the calibration curve. A comparison of the molar absorptivities of the neutral $HL = H_{\text{LQM300}}$ species (Fig. 8b), in aqueous and *n*-octanol phases, indicates that the

Table 1
pKa values (pKa$_i$) of compound **4** (LQM300) obtained with the aid of program SQUAD, from CZE and spectrophotometric data, shown in Figs. 5 and 6.

Acidity equilibria	pKa$_i \pm \sigma$[a]	
	CZE	Spectrophotometry
	$T = 25$ °C and [b]$I = 0.05$ M	$T = 37$ °C and [b]$I = 0.15$ M
$H_4L^{3+} \rightleftarrows H_3L^{2+} + H^+$	1.153 ± 0.24	1.5[c]
$H_3L^{2+} \rightleftarrows H_2L^{+} + H^+$	4.076 ± 0.084	4.118 ± 0.021
$H_2L^{+} \rightleftarrows HL + H^+$	6.748 ± 0.039	7.172 ± 0.023
$HL \rightleftarrows L^- + H^+$	11.323 ± 0.050	11.2798 ± 0.0087
Electrical and optical properties of the five species involved obtained with the aid of program SQUAD	Ionic effective mobilities of species $(u^z_{HjL} \pm \sigma) \times 10^8$/m^2 V^{-1} s^{-1} $\sigma_{reg} = 0.05825$[d]	Absorptivities at $\lambda = 360$ nm $(\epsilon^z_{HjL} \pm \sigma)$/L mol^{-1} cm^{-1} $\sigma_{reg} = 0.0063$[d]
H_4L^{3+}	3.000[c]	5788 ± 170
H_3L^{2+}	2.091 ± 0.094	5413 ± 40
H_2L^{+}	1.000[c]	5548 ± 27
HL	0.000[c]	7900 ± 64
L^-	−1.048 ± 0.054	9030 ± 34

[a] σ represents the standard deviation of the statistical estimator.
[b] I represents the ionic strength of the solution.
[c] Fixed during refining.
[d] σ_{reg} represents the standard deviation of the regression, fitting mobilities or absorbances.

predominant species at pseudo-physiological conditions is practically the same (due to the equality of both molar absorptivities). This gives evidence to associate to the neutral species the molecular non-zwitterionic character, as explained before by the electrophoretic mobility curves. The wavelength selected to work for analysis of samples of extraction systems was 360 nm and linear regression of the calibration curve gives: $A^{(360)} = (7370 \pm 340)[LQM300]_o + (0.0037 \pm 0.0034)$, when concentrations are given in molarity.

Absorbance values of the *n*-octanol phase have been recorded and interpolated in the calibration curve shown in Fig. 8c, after phase separation for the liquid–liquid extraction experiments, in order to obtain $[LQM300]_o$; the concentration of compound **4** on aqueous phase $[LQM300]_{aq}$ has been obtained by difference of total LQM300 and LQM300 in *n*-octanol and, then the fraction of compound **4** in the octanol phase (E_{LQM300_o}) in the extraction may be calculated by definition (equation (2)).

$$E_{LQM300_o} = 100\% \frac{v_o [LQM300]_o}{n_{LQM300_T}} \quad (2)$$

where v_o represents the volume of *n*-octanol phase while n_{LQM300_T} represents the total amount of substance of compound **4** in the extraction system.

On the other hand, the fraction of compound **4** in the *n*-octanol phase may be obtained through equation (3), for a system with buffering in pH and phosphates [20]:

$$E_{LQM300_o} = \frac{rP\prime}{1 + rP\prime} \quad (3)$$

where r is the volume ratio of *n*-octanol to aqueous phase while P' is the conditional partition coefficient (or distribution ratio, DR) of compound **4**, given by the equation (4).

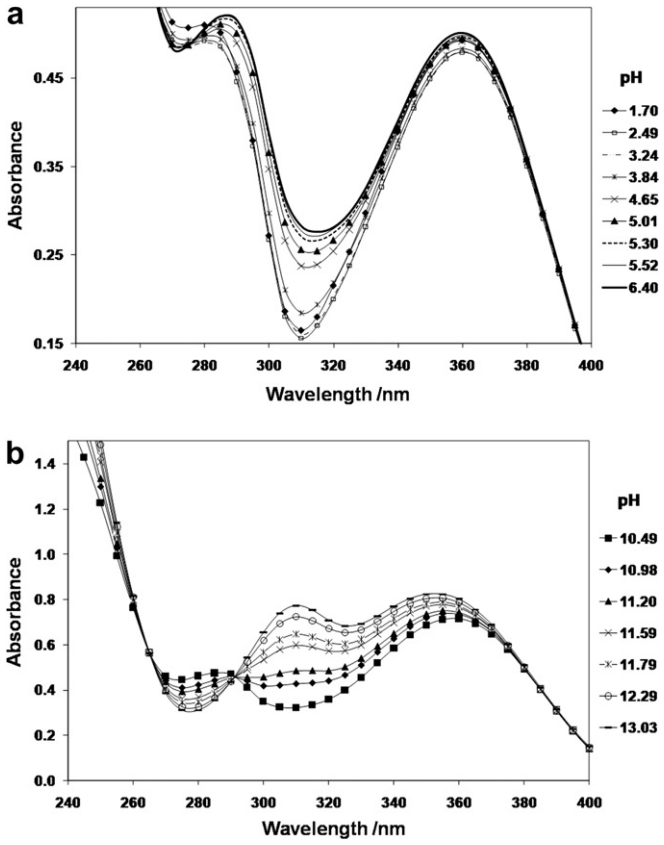

Fig. 6. Representative absorption spectra of compound **4**. $[LQM300] = 8.83 \times 10^{-5}$ M solutions. a) Experimental behavior in acidic media. b) experimental behavior in basic media.

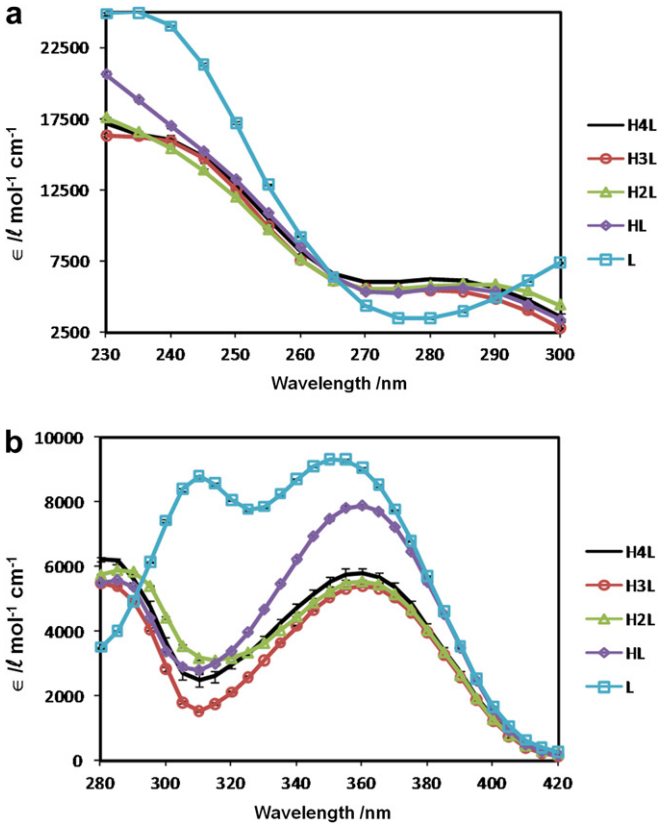

Fig. 7. Molar absorptivity coefficients (ε) of the compound **4** (LQM300) species obtained by absorbance data refined with program SQUAD (Table 1). Error bars are only appreciated for the (more uncertain) coefficient of H_4L^{3+} species. a) Short UV wavelengths. b) Long UV wavelengths.

$$P\prime = \frac{[HL]_o}{[L^-]_{aq}+[HL]_{aq}+[HL^+]_{aq}+[H_2L^{2+}]_{aq}+[H_3L^{3+}]_{aq}}$$

$$= \frac{P\beta_1[H^+]}{1+\beta_1[H^+]+\beta_2[H^+]^2+\beta_3[H^+]^3+\beta_4[H^+]^3} = Pf_{HL} \quad (4)$$

By combining equations (3) and (4) and remembering that for this compound $r = 1$, equation (5) was obtained.

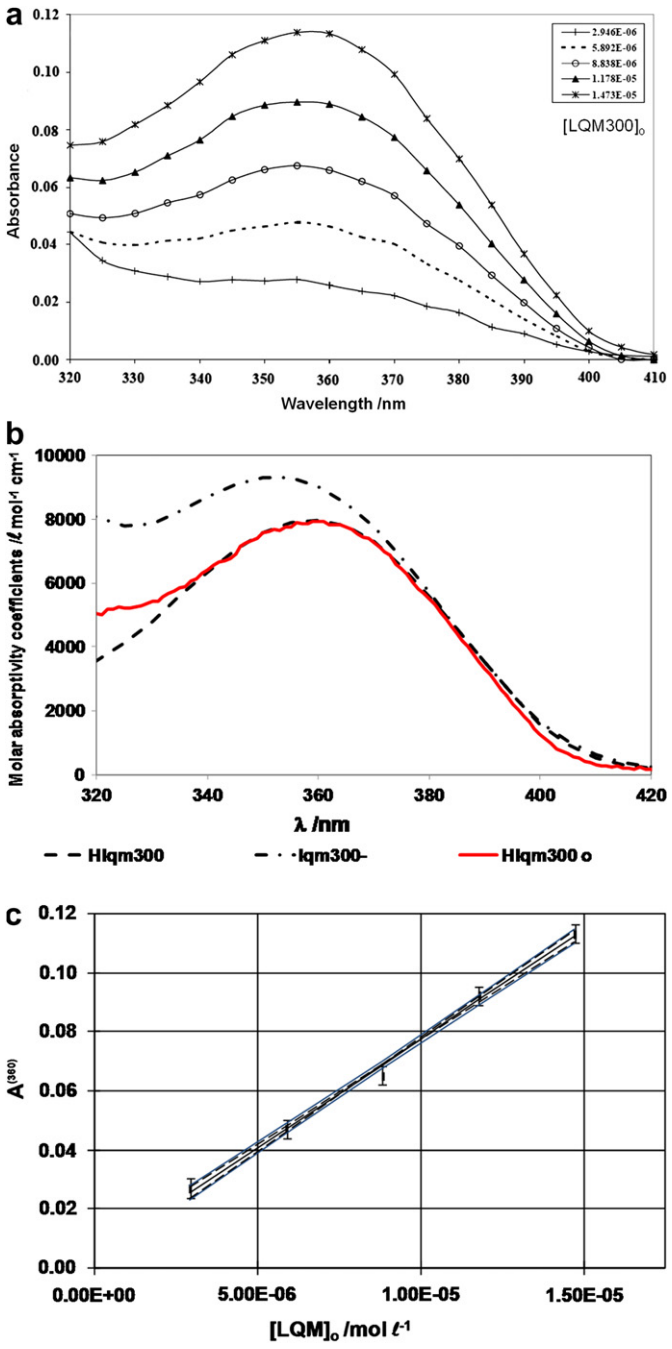

Fig. 8. Results for the calibration curve of the quantification of $[LQM300]_o$. a) Absorption spectra of HL in *n*-octanol. b) Molar absorptivity coefficients of aqueous neutral ($HLQM300$) and anionic ($HLQM300^-$) species, and octanolic neutral species ($HLQM300^\circ$) of compound **4**. It is relevant the similarity of the absorptivity coefficients of the neutral species in both phases. c) Graphic representation of regression curve. The error bars are represented over the experimental points in the absorbance direction; the solid line represents the maximum verisimilitude line, the dashed lines represent the slope confidence limits at 95% significance level and the curve lines (hyperboles) represent the confidence limits of the absorbances at the same significance level.

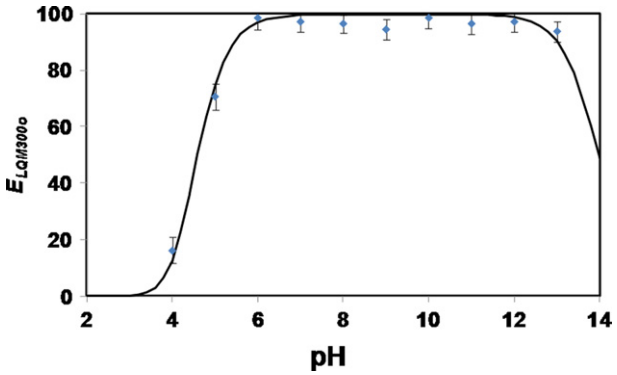

Fig. 9. Fraction of compound **4** (LQM300) in the *n*-octanol phase. Markers represent experimental points and solid line is the fitting obtained with equation (5) for a $\log P = 2.7 \pm 0.2$.

$$E_{LQM300_o} = 100\%\left(\frac{1}{1+1/(Pf_{HL})}\right) \quad (5)$$

The fitting of the curve shown in Fig. 9 has been obtained by substituting the global formation constants given for pseudo-physiological conditions (Table 1) and a value of $P = 10^{2.7}$ in equation (5). Furthermore, from Fig. 9 it can be seen that at pseudo-physiological conditions $P' = P = 10^{2.7}$.

4.3.2. *logP between n-octanol and water for LQM301, LQM302 and LQM303 (compounds **1**–**3**)*

The experimentation followed for compound **4** (LQM300) has demonstrated that the complete set of pKa values might explain its extraction behavior. But, as this experimentation is very long, it was decided to determine only the log*P* value of compounds **1**–**3** (LQM301, LQM302 and LQM303) between *n*-octanol and water, in order to compare it with that obtained for compound **4**. The volume of the aqueous solution was raised to obtain better precision for these three estimators. The linear regression equations of the calibration curves obtained were $A^{(286)} = (10.49 \pm 0.08) \times 10^{-3}[LQM301]_o + (-0.0306 \pm 0.0050)$, $A^{(286)} = (12.12 \pm 0.07) \times 10^{-3}[LQM302]_o + (0.1383 \pm 0.0029)$, and $A^{(280)} = (10.04 \pm 0.11) \times 10^{-3}[LQM303]_o + (0.1280 \pm 0.0058)$; when concentrations are given in parts per million (ppm). The extracted fractions of LQM301, LQM302 and LQM303 were obtained from equation (6) and then its *P* values from equation (7).

$$E_{LQM30X_o} = \frac{[LQM_{30X}]_o}{[LQM_{30X}]_{o,\ initial}} \quad (6)$$

$$P_{LQM30X} = \frac{1}{r}\left(\frac{E_{LQM30X_o}}{1-E_{LQM30X_o}}\right) \quad (7)$$

Table 2 shows the comparison of log*P* values of compound **4** (LQM300) with other LQMs and other antihypertensive substances.

Table 2
log*P* values between *n*-octanol and water for several antihypertensive substances.

Compound	log*P*	Reference
1 (LQM301)	>3.048	This work
2 (LQM302)	1.96 ± 0.03	This work
3 (LQM303)	2.03 ± 0.08	This work
4 (LQM300)	2.7 ± 0.2	This work
Nifedipine	2.40	[1]
Felodipine	3.86	[21]
Captopril	1.02	[1]
Losartan	4.01	[21]

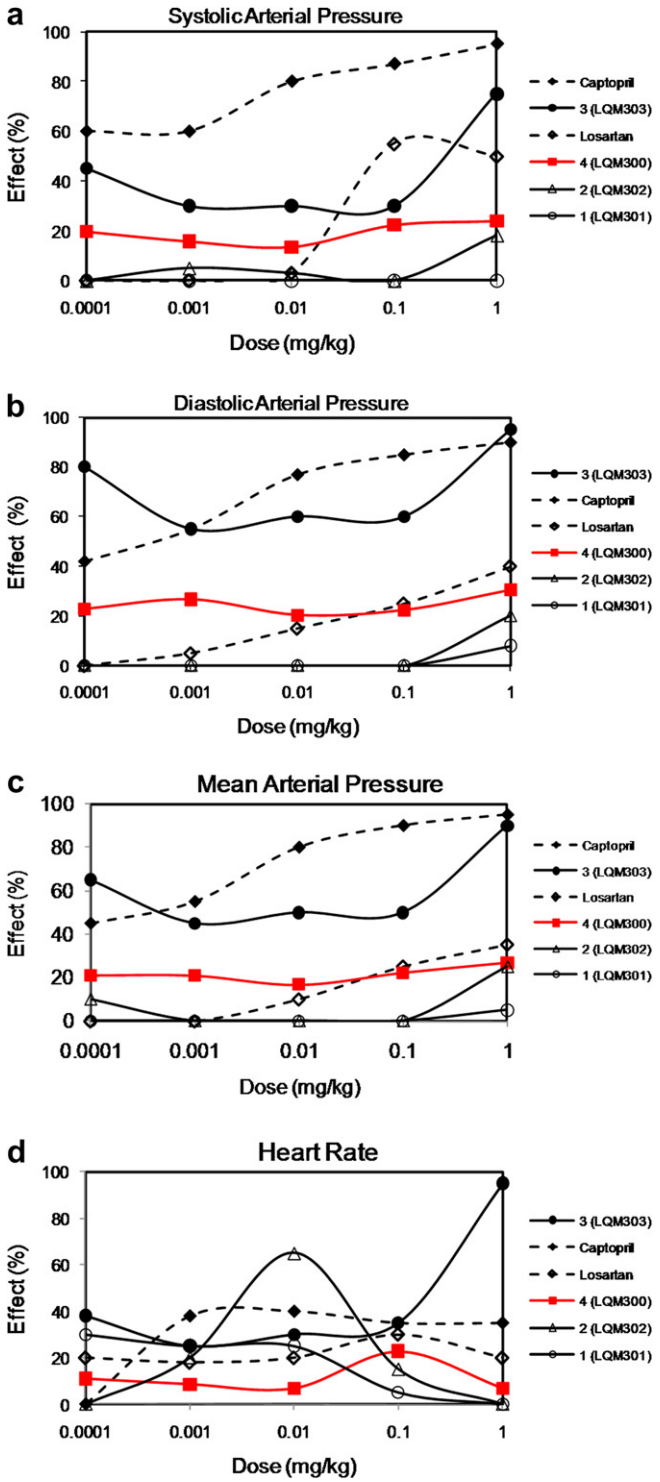

Fig. 10. Comparison of the dose–effect percentage curves of the compound **4** (LQM300) and other compounds using IAP model in male Wistar rats anesthetized. a) Effect on the systolic pressure. b) Effect on the diastolic pressure. c) Effect on the mean pressure. d) Effect on heart rate. The effect of compound **4** is intermediate to other LQM compounds and similar to the effect of losartan. Data taken from Figs. 3 and 4 for compound **4**, and from Ref. [12] for all the other compounds.

The values obtained in the present work show good compatibility with those reported for other antihypertensives. In fact, it seems that a chloride substituent in the LQM compounds originate higher solubility and affinity to the *n*-octanol phase, as it occurs with the other antihypertensive compounds (losartan and felodipine), given higher log*P* values than for the other substituents. On the other hand, the order observed for log*P* (**2** ≤ **3** < **4**) may be explained by the size of the substituent in the *para* position of the changrolin-Region 2-like moiety of the molecules, as expected: higher the size of the substituent, higher the log*P* value.

*4.3.3. Comparison of antihypertensive and antiarrhythmic effects of compound **4** (LQM300) with captopril, losartan, and **1**–**3** compounds (LQM301, LQM302 and LQM303) and relationship with log P values*

The antihypertensive and antiarrhythmic effects of compound **4** (LQM300) and other substances are presented in Fig. 10. The curves of the effect percentage as a function of the dose, for the Invasive Arterial Pressure (IAP) model in male Wistar rats anesthetized, are given for antihypertensive (Figs. 10a–c) and antiarrhythmic (Fig. 10d) properties. In general and for a dose of 1 mg/kg the antihypertensive effect of the compound **4** is placed between compounds **3** and **2** or **1**. This may be due to the bulky substituent 1,4-DHP in the *para* position of the bis(morpolinylmethyl)phenol of compound **4** with respect to the *tert*-butyl substituent in the *para* position of the bis(tiomorpholinylmethyl)phenol of compound **3**. Then, it seems that it is important to maintain the double nitrogen-ring substitution (morpholines or tiomorpholines *ortho* to the hydroxyl group of the phenol) in the structure, to have good antihypertensive properties. The antiarrhythmic effect of compound **4** is poorer than for the others LQMs at a dose lower or equal to 0.1 mg/kg. In general the antihypertensive and antiarrhythmic effects of compound **4** are similar to those of losartan.

5. Conclusions

The four pKa values of a compound **4** (LQM300) have been determined by UV spectrophotometry (at pseudo-physiological conditions) and CZE (at 25 °C and 0.05 M of ionic strength). These values and extraction of the compound **4** (LQM300), between *n*-octanol and water, have been used to determine the log*P* value. The order observed of log*P* values of LQM compounds determined in this work follows the order: **2** ≤ **3** < **4** < **1**.

A comparison of log*P* values of compound **4** with respect to other LQM compounds, as well as the percentage of the effect as a function of dose for antihypertensive properties, suggests that there is a maximum effect for intermediate values of log*P* – around 2.0 – between *n*-octanol and water when there is a double *ortho* substitution in the phenol ring (of LQM compounds) and may be a molecular non-zwitterionic neutral species. The antiarrhythmic properties seem not to be so dependent of these remarks, because compound **4** has good antiarrhythmic effect for low doses.

6. Experimental protocols

6.1. UV spectrophotometry

6.1.1. Equipment conditions

UV absorption spectra were recorded with a Perkin–Elmer Lambda 18 spectrophotometer, using quartz cells of 1 cm optic path length, during titration of basic solution of compound **4** (LQM300) with acid solution of **4**, and *vice versa*.

6.1.2. Samples preparation

A stock aqueous solution of **4** was prepared by solving 5 mg at the minimum amount of HCl 0.1 M required in a 50 ml volumetric flask and filling to the mark with deionized water. 12 ml of the stock aqueous solution of **4** were mixed with necessary amounts of NaOH and NaCl in a 50 ml volumetric flask and filling to the mark with deionized water, in order to obtain the basic solution of **4** (pH ≈ 13

and 0.15 M of ionic strength). In a similar way the acid solution of **4** was prepared (pH ≈ 1 and 0.15 M of ionic strength), but changing NaOH by HCl.

6.1.3. Spectrophotometric titrations

A volume of 25 ml of basic (acid) solution of **4** was placed on a glass cell and titrated with the acid (basic) solution of **4**, in order to decrease (increase) the pH by 0.25 pH units. The temperature of the cell was maintained at 37 ± 0.1 °C with a PolyScience 9105 water recirculator bath and the inert atmosphere was achieved passing a nitrogen flow over the solution. The titrated solution was stirred magnetically with a Corning PC-353 device. The pH of the solution was measured with a Mettler Toledo MA235 pH meter equipped with a combined electrode INLAB 413. The pH-meter was calibrated previously with commercial 4, 7 and 10 buffer solutions.

6.2. Capillary zone electrophoresis (CZE)

6.2.1. Equipment conditions

CZE experiments were carried out with a PACE-MDQ Capillary Electrophoresis System (Beckman-Coulter, Fullerton CA, US) equipped with a diode array detector and temperature control cartridges. Instrument control and data processing were made with 32 KARAT 5.0 Software. The wavelength detection was set at 254 nm. New and untreated fused silica capillary (from Polymicro Technologies, Phoenix USA) of 60 cm (10 cm to detector in the short side and 50 cm in the long side) and 75 μm of internal diameter was used to carry out the electropherograms. The voltage applied was set at 20 kV. The sample was injected hydrodynamically at 5 psi for 0.5 s.

6.2.2. Capillary conditioning

Before use the capillary was flushed with 1.0 M NaOH for 10 min and then with deionized water for 10 min. In order to achieve repeatable migration times, the capillary was rinsed with water 3 min, buffer running 5 min before each run.

6.2.3. Preparation of buffer solutions for CZE experiments

Since the studied compound is a complex molecule, it was studied in a wide range of pH (2–12.5). The electrolyte solutions were prepared using a worksheet designed in our Lab to adjust the ionic strength, considering the quantity of ions produced at each pH value; this worksheet shows the quantity of NaCl to add (if necessary) in order to obtain the required ionic strength, 0.05 M in this case. In order to obtain the pH requested we used the following ionic pairs.

pH range	Reagents used
2.0 – 3.5	$H_3PO_4/H_2PO_4^-$
3.5 – 5.7	CH_3COOH/CH_3COO^-
6.0 – 8.2	$H_2PO_4^-/HPO_4^{2-}$
8.2 – 10.7	$H_3BO_3/H_2BO_3^-$
10.9 – 12.5	HPO_4^{2-}/PO_4^{3-}

The pH of each buffer solution was measured by a Beckman Φ 310 pH-meter and a Futura Gel-Filled combined electrode.

6.2.4. Sample preparation

A stock methanol solution of 450 μg of compound **4** was prepared. An aliquot of 100 μL of this solution was added to 1.7 ml of buffer solution at the pH required in order to obtain an adequate concentration of the compound. Sample was injected hydrodynamically to the capillary from the last solution.

6.2.5. Effective electrophoretic mobility determination (u_{eff})

Migration times of **4** ($t_m = t_{LQM300}$) and acetone ($t_o = t_{eof}$, used as electroosmotic flow (eof) marker) were obtained for different pH values. Effective mobility (u_{eff}) of LQM300 was calculated as the difference of measured mobility (or apparent mobility, u_{app}) and electroosmotic mobility (u_{eof}), by applying the equation (8):

$$u_{eff} = u_{LQM} = u_{app} - u_{eof} = \frac{L_t L_d}{V}\left(\frac{1}{t_{LQM}} - \frac{1}{t_{eof}}\right) \quad (8)$$

where L_t represents the total length of the capillary, L_d accounts for length from injection to detector and V the applied voltage for electro-migration

6.3. logP determination

In order to determine the logarithmic value of the partition coefficient (log*P*) between *n*-octanol and water, experiments have been designed to determine the *n*-octanol fraction of compound **4** as a function of pH. For compounds **1**–**3** experiments have been designed to determine its corresponding fraction at neutral pH.

*6.3.1. Solutions for the calibration curves of compounds **1**–**4***

A convenient mass of compounds **1**–**4** was solved in *n*-octanol in order to obtain a stock solution of concentration 5.00×10^{-4} M, 4.00×10^{-4} M, 3.00×10^{-4} M, and 1.47×10^{-4} M, respectively. The solutions for each calibration curve were prepared by taking 5–25 ml with volumetric pipettes and diluting with *n*-octanol to 25.00 ml in a volumetric flask.

*6.3.2. Absorbance as a function of **1**–**4** compounds concentration*

The absorption UV spectrum of each LQM solution was recorded in a Perkin–Elmer Lambda 18 spectrophotometer, using quartz cells with an optical path length of 1 cm.

6.3.3. Preparation of pH phosphate buffers

Several buffer solutions of phosphates were prepared in a similar way that those used for CZE experiments, but a 0.1 M phosphates concentration, but without fixing the ionic strength. The pH of each solution was adjusted using a Mettler Toledo MA235 pH-meter equipped with a combined electrode INLAB 413. The pH-meter was calibrated previously with commercial 4, 7 and 10 buffer solutions.

*6.3.4. Extraction procedure of compound **4** and interpolation on the calibration curve*

5 ml of stock solution of compound **4** were mixed with 5 ml of the pH buffer desired in an extraction flask. Vigorous stirring was undertaken during 2 min and the phases were allowed to separate. As a blank for absorbance measurements was used a similar mixture in each case, but changing *n*-octanol in place of the stock solution of **4**. The UV absorption spectra for the *n*-octanol phase were recorded in the Perkin–Elmer Lambda 18 spectrophotometer. The concentration of compound **4** in the *n*-octanol phase was obtained by interpolation of the absorbance measured on the calibration curve.

*6.3.5. Extraction procedure of compounds **1**–**3** and interpolation on the calibration curve*

10 ml of **1**, **2** or **3** stock solution were mixed with 90 ml of deionized water Type I. Vigorous stirring was undertaken during 5 min and the phases were allowed to separate. As a blank for absorbance measurements was used a similar mixture in each case, but changing *n*-octanol in place of the LQM300 stock solution. The UV absorption spectra for the *n*-octanol phase were recorded in the Perkin–Elmer Lambda 18 spectrophotometer. The concentration of **1**, **2** or **3** compound in the *n*-octanol phase was obtained by

interpolation of the absorbance measured on the calibration curve and this determination was repeated three times.

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