Therapeutic Drug Monitoring of Anticonvulsant Drugs by Micellar HPLC with Direct Injection of Serum Samples

Adrián Martinavarro-Domínguez,1,2 María-Elisa Capella-Peiró,1 Mayte Gil-Agustí,1 José V. Marcos-Tomás,2 and Josep Esteve-Romero1*

Background: We developed a micellar liquid chromatographic (MLC) procedure for the determination of three extensively monitored antiepileptics in serum samples: carbamazepine, phenobarbital, and phenytoin.

Methods: We determined the composition of the mobile phase after modeling the elution behavior of the antiepileptics in hybrid micellar mobile phases of sodium dodecyl sulfate (SDS) with different organic modifiers (propanol, butanol, or pentanol) in an experimental design that used five mobile phases, a C18 column, and ultraviolet detection. In the micellar chromatographic system, the serum samples can be injected directly.

Results: The optimum mobile phase was 70 mL/L butanol in 0.05 mol/L SDS, pH 7, in which the three antiepileptics were resolved in <10 min. Intra- and interday precision was evaluated at four different drug concentrations within the therapeutic range (n = 10); CVs were <2.1%. The method was applied to the analysis of 120 serum samples, and results were similar to those obtained by the TDx® method.

Conclusions: The MLC method allows chromatographic determination of three antiepileptics, using an interpretative strategy of optimization, without pretreatment of the serum samples and with direct injection in a hybrid micellar mobile phase of SDS–butanol. The method provides complete resolution and quantification of mixtures of two and three antiepileptics.

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Therapeutic drug monitoring is desirable mainly for adjusting dosage, avoiding side effects, and assessing patient compliance. One of the most important groups of drugs for monitoring are antiepileptics. Epilepsy is a common disease, with a current prevalence of ~0.7–0.8% and an incidence rate at which new cases occur of ~0.5%. Carbamazepine, phenobarbital, and phenytoin are regarded as the drugs of first choice to reduce the number and/or severity of partial and generalized tonic-clonic (grand mal) seizures in patients with epilepsy (1, 2).

Carbamazepine has been reported to be effective in the treatment of bipolar disorder (3). Table 1 shows the pK₈ and octanol/water partitioning coefficients (log Pₒ/w)³ of the three substances.

The most widely used methods for monitoring these drugs are chromatography and immunoassay (4). Reversed-phase HPLC (RP-HPLC) has been used to monitor antiepileptics with C₁₈ or C₈ columns and mobile phases with one or more organic solvents, acetonitrile (5–8), methanol (9), dichloromethane–n-hexane–methanol–acetonicloritrile (10), or n-hexane–ethanol–propan-2-ol (11). An important limitation of RP-HPLC methods with respect to immunoassays (12–15) is the need to treat the biological sample before injection. Thus, RP-HPLC methods include steps such as protein precipitation, liquid–liquid or solid-phase extraction on disposable cartridges, reextraction, and evaporation before injection into the chromatographic system. These procedures may be time-consuming and expensive and may introduce additional sources of error.

Micellar liquid chromatography (MLC) is a variant of RP-HPLC in which the mobile phase is composed of a surfactant in a concentration above the critical micellar concentration. In some cases a modifier, such 1-propanol, 1-butanol, or 1-pentanol, is added to decrease the reten-

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3 Nonstandard abbreviations: log Pₒ/w, octanol/water partitioning coefficient; RP-HPLC, reversed-phase HPLC; MLC, micellar liquid chromatography; SDS, sodium dodecyl sulfate; and LOD, limit(s) of detection.
tion factors and increase the efficiencies. The true starting point of MLC was the report by Armstrong and Nome (16), in which the authors used a three-phase partitioning model to explain the retention of solutes in a RP column with micellar mobile phases. Much of the work done since then has focused on better understanding of the interactions of the solutes inside the chromatographic column. Several experimental variables (type and concentration of surfactant and organic modifier, pH, ionic strength, and temperatures) can be used to better control the retention of solutes and increase the efficiency of the chromatographic peaks. Recently, the partitioning theory in MLC has been extended to include the effect of organic modifiers and acid–base equilibria (17–21) on retention. The stable and reproducible behavior of micellar mobile phases allows accurate prediction of the retention of solutes with a model that can also be used to optimize the separation of mixtures of solutes (22, 23). Over the last few years, MLC has been useful in the analysis of diverse groups of substances in pharmaceutical preparations, such as benzodiazepines, diuretics, steroids, or phenethylamines (24–27).

One of the main appeals of MLC is the possibility of determining drugs in physiologic fluids without the need for previous separation of the proteins present in the serum samples. The sodium dodecyl sulfate (SDS) micelles tend to bind proteins competitively, thereby releasing protein-bound drugs. Therefore, the drugs are free to partition into the stationary phase, whereas the proteins, rather than precipitating on the column, are solubilized and eluted with or shortly after the solvent front. The use of surfactants in direct injection is also much less complex than column-switching procedures, which require additional instrumentation (precolumns, switching valves, and HPLC pumps), and allows accurate and precise timing of valve switching for a successful separation (28). Compared with other eluents, the micellar mobile phases are less flammable, inexpensive, nontoxic, biodegradable, and can solubilize hydrophobic and hydrophilic analytes in complex matrices such as serum.

To our knowledge, there are no other published reports describing the RP-HPLC determination of antiepileptics in serum with direct injection. The aim of this work was to determine the chromatographic behavior of the three antiepileptics in serum samples to propose a quick and simple MLC procedure for the determination of these compounds without deproteinization.

**Materials and Methods**

**CALIBRATORS AND REAGENTS**

The drugs used were carbamazepine, phenytoin, and phenobarbital (Sigma). In the chromatographic optimization studies, stock solutions containing each drug at 20 mg/L were prepared by dissolving the substance in methanol and then suitably diluting them for analysis with 0.05 mol/L SDS–70 mL/L butanol, pH 7. In the TDx® system, we used the commercial reactants, calibrators, and controls from Abbott Laboratories. The reagents used to prepare the micellar mobile phases were SDS (99% purity), 1-propanol, 1-butanol, 1-pentanol, disodium hydrogen phosphate, and HCl (Merck). The drug solutions and the mobile phases were filtered through 0.45 μm nylon membranes (12 and 45 mm in diameter, respectively; Micron Separations). Distilled deionized water (Barnstead) was used throughout.

**CHROMATOGRAPHIC SYSTEM AND OTHER APPARATUS**

The chromatograph (Model HP 1100; Agilent) was equipped with a quaternary pump, an autosampler fitted with a Rheodyne valve, and an ultraviolet–visible detector (190–700 nm range). The flow rate, injection volume, and detection wavelength were 1.0 mL/min, 20 μL, and 220 nm, respectively. A conventional analytical column (250 × 4 mm i.d.) was packed with RP silica gel (Kromasil C18; particle size, 5 μm; Scharlab). The signal was recorded by a personal computer connected to the chromatograph through a HP Chemstation. The software Michrom (22, 23) was used for data handling. For absorbance and pH measurements, we used a Lambda 19 spectrophotometer (Perkin-Elmer) and a GLP 22 potentiometer (Crisron) equipped with a combined Ag/AgCl/glass electrode, respectively. Whole-blood samples, without anticoagulants, were centrifuged in a Sorvall RC-5B centrifuge (DuPont Instruments).

**BLOOD SAMPLING AND ANALYSIS**

For the studies on patients, we obtained Review Board approval from the Ethics Committee of the Hospital Verge dels Lliria. Blood samples were collected from epilepsy patients who were treated with carbamazepine, phenobarbital, phenytoin, or mixtures containing two or three of these antiepileptics. The blood for monitoring was obtained at a suitable time and added to tubes containing separator gel (SST®, Becton Dickinson) and centrifuged 10 min at 1400 g, quickly separating the serum to avoid phenytoin absorption (29). After separation, serum samples were injected directly in 0.05 mol/L SDS–7 mL/L butanol (pH 7), the optimum mobile phase selected, and chromatographed on the C18 column at 25 °C; samples were processed simultaneously in the TDx automatic system for comparison purposes.

**Results and Discussion**

**OPTIMIZATION OF MOBILE PHASE COMPOSITION**

The three antiepileptics studied have different structures and, thus, different spectra. The maximum wavelengths were 280 nm for carbamazepine and 240 nm for phenobarbital and phenytoin. All optimization studies were performed at these wavelengths, but analysis of the mixtures was carried out at 220 nm to improve the signal, after we checked whether any other compound in the serum interfered at this wavelength. At 220 nm, the relative sensitivity with respect to the main maximum for each substance increased by a factor of 1.5–2.
The dissociation constants ($pK_a$) were 7.0, 7.4, and 8.3 (Table 1) for carbamazepine, phenobarbital, and phenytoin, respectively (30). Furthermore, in the SDS micellar medium, these values increased by 1 or 2 units; thus, one suspects that at pH 7 or lower, these substances must be positively charged and interacting with the micelles of SDS.

Also shown in Table 1 are the log $P_a/w$ values for the antiepileptics: 2.47, 2.45, and 1.47 for phenytoin, carbamazepine and phenobarbital, respectively (30). This means that these antiepileptics are moderately apolar substances and that one can expect an elution order in MLC of phenobarbital followed by carbamazepine or phenytoin.

We studied the possibility of using the same mobile phase to analyze the three antiepileptics in the serum samples. The retention times of the substances in pure micellar mobile phases without alcohol (0.05–0.15 mol/L SDS) was excessive, 72 and 47 min for phenytoin in 0.05 and 0.15 mol/L SDS, respectively, because of the strong association of these compounds with the nonmodified alkyl chains of the stationary phase. Thus, it was necessary to use hybrid micellar mobile phases with an added modifier to reduce the retention time. In 0.05 mol/L SDS plus 25 mL/L 1-propanol, the maximum retention time was 50 min; it was 20 min in 0.15 mol/L SDS plus 125 mL/L 1-propanol. On the other hand, the use of pentanol caused overlapping of the three substances. For these reasons, 1-butanol was selected for optimization studies.

The optimization procedure, which maximized the separation of the mixtures of compounds, was used to select the most suitable mobile phase. The mobile phase that permitted complete separation of the three drugs in an acceptable analysis time would be useful for the analysis of serum samples containing one, two, or all three antiepileptics.

To develop appropriate chromatographic conditions, we considered two different strategies. We first investigated the use of a single mobile phase to analyze mixtures of the three antiepileptics. The use of a set of experimental conditions to determine several drugs can be advantageous because it allows analysis of samples from individuals who have taken more than one drug without the need to change mobile phase composition. The second strategy involved the use of an optimal mobile phase for each combination. This may be necessary in some cases to achieve shorter retention times and accelerate the analyses. The development of both strategies was greatly facilitated by the capability of MLC to predict the retention of compounds by use of simple equations. The model (Eq. 1) used for these predictions was (23):

$$\log k = c_0 + c_1[M] + c_2[A] + c_{13}[M] + c_{22}[A]^2$$  (1)

where $k$ is the retention factor, [M] and [A] are the surfactant and alcohol concentrations, respectively, and $c_0$, $c_1$, $c_2$, $c_{13}$, and $c_{22}$ are the adjustment coefficients. This equation was nonlinearly fitted according to the Powell method (31), using the retention data obtained from injections of the antiepileptic solutions in five mobile phases containing the following combinations of SDS and 1-butanol: 0.05 mol/L SDS plus 10 or 70 mL/L 1-butanol; 0.10 mol/L SDS plus 40 mL/L 1-butanol; or 0.15 mol/L SDS plus 10 or 70 mL/L 1-butanol. All buffers contained phosphate buffer at pH 7. The retention factors ($k$) and efficiencies ($N$) were measured according to Foley and Dorsey (32). Asymmetry factors ($B/A$), with $B$ defined as the distance between the center and the tailing edge and $A$ the distance between the center and the leading edge of the chromatographic peak, were measured at 10% of peak height. Table 2 shows the coefficients in Eq. 1 for each drug when butanol was used. These allowed the prediction of mobile phase composition containing butanol for any desired retention time and provided a simple way of optimizing the separation of mixtures.

Optimizing the resolution of the mixtures of the three drugs by use of a procedure based on the sequential variation of the composition of the mobile phase was difficult because of the changes in the elution order of the antiepileptics. However, accurate prediction of the retention according to Eq. 1 allowed the application of an interpretive procedure to predict the optimal resolution,

Table 1. $pK_a$ and log $P_a/w$ data for the antiepileptics.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$pK_a$</th>
<th>log $P_a/w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>7.0</td>
<td>2.45</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>7.4</td>
<td>1.47</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>8.3</td>
<td>2.47</td>
</tr>
</tbody>
</table>

Table 2. Coefficients from Eq. 1 used to predict the chromatographic behavior of the antiepileptics.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$c_0$</th>
<th>$c_1$</th>
<th>$c_2$</th>
<th>$c_{13}$</th>
<th>$c_{22}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>3.44</td>
<td>-13.15</td>
<td>-20.98</td>
<td>106.7</td>
<td>11.89</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>4.19</td>
<td>-12.24</td>
<td>-22.68</td>
<td>98.39</td>
<td>8.40</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>2.83</td>
<td>-8.25</td>
<td>-17.56</td>
<td>90.79</td>
<td>-32.33</td>
</tr>
</tbody>
</table>

Fig. 1. Global resolution diagram drawn according to the peak-to-valley criterion for the three antiepileptics, eluted in mobile phases containing 0.05–0.15 mol/L SDS and 10–70 mL/L 1-butanol at pH 7. Values for butanol are shown as volume fractions ($v/v$).
following a criterion that uses the valley-to-peak ratios (Eq. 2):

\[ r = \prod_{i=1}^{n-1} \frac{X_{i,i+1}}{\sum_{i=1}^{n-1} X_{i,i+1}} \]  (2)

where \( X_{i,i+1} = 1 - (h_1/h_2) \), \( h_1 \) is the height of the valley between two adjacent peaks, and \( h_2 \) is the interpolated height between the maxima of both peaks measured at the abscissa of the valley. The global function of resolution, \( r \), may vary from 0 to 1, and the proximity to 1 indicates the performance of the separation. The function was maximized to obtain the optimal mobile phase.

Incorporation of the shape of the chromatographic peaks in the optimization procedure improved the results. The reliable simulation of peak shape for any mobile phase of the variable space was carried out with an asymmetrical gaussian function where the standard deviation is a first-degree polynomial function (Eq. 3):

\[ h(t) = H \exp \left[ \frac{1}{2} \left( \frac{t - t_R}{s_0 + s_1(t - t_R)} \right)^2 \right] \]  (3)

where \( H \) and \( t_R \) are the height and time at the peak maximum, respectively, \( s_0 \) is the standard deviation of a symmetrical gaussian peak describing the central region of the experimental peak; and \( s_1 \) is a coefficient that quantifies its skewness. The coefficients \( s_0 \) and \( s_1 \) are related to the efficiency and asymmetry factor. These parameters were interpolated from the data obtained in the three experimental mobile phases closest to the simulated mobile phase. When we used Eq. 1 and the mathematical treatment described here, the relative global error in the prediction of capacity factors for the three antiepileptics was 1.9%.

The global resolution diagrams obtained are depicted in Fig. 1. The best resolution value was obtained with 0.05 mol/L SDS/20 mL/L butanol (\( r = 0.999 \)) with an analysis time of 18 min. Fig. 1 also shows how resolution values near unity (maximum value) can be obtained by use of mobile phases that contain 0.05–0.11 mol/L SDS and 10–70 mL/L 1-butanol.

The mobile phase selected as optimum was 0.05 mol/L SDS plus 70 mL/L 1-butanol, which gave excellent resolution (\( r = 0.997 \)) and allowed the analysis time to be

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Table 3. Slope, intercept, correlation coefficient (\( r \)), and LOD in the calibration of the antiepileptics eluted with 0.05 mol/L–70 mL/L butanol (pH 7).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Slope</th>
<th>y-Intercept, milli-units of absorbance</th>
<th>( r )</th>
<th>LOD, ( \mu g/L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>63.27</td>
<td>8.12</td>
<td>0.99998</td>
<td>10</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>15.77</td>
<td>6.47</td>
<td>0.99994</td>
<td>50</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>9.02</td>
<td>0.35</td>
<td>0.99997</td>
<td>10</td>
</tr>
</tbody>
</table>

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Fig. 2. Simulated chromatogram (a) and real chromatograms of serum samples (diluted 1 mL to a final volume of 10 mL), collected from epilepsy patients, containing 20 mg/L phenobarbital (b), 8 mg/L carbamazepine (c), or 10 mg/L phenytoin (d). Peaks: 1, phenobarbital; 2, carbamazepine; 3, phenytoin. The range for the y axis is 0–40 milli-units of absorbance for panels b and c and 0–15 milli-units of absorbance for panel d.
reduced to 10 min. Fig. 2a shows the simulated chromatogram for the mixture of the three drugs in the optimum mobile phase. Panels b–d of Fig. 2 show the experimental chromatograms for serum samples containing the antiepileptics phenobarbital (Fig. 2b), carbamazepine (Fig. 2c), or phenytoin (Fig. 2d). The agreement between the simulated and experimental chromatograms was good. In the optimal mobile phase (0.05 mol/L SDS plus 70 mL/L 1-butanol), the capacity factors were 3.8, 5.7, and 10.8 for phenobarbital, carbamazepine, and phenytoin, respectively.

**SERUM SAMPLE BACKGROUND ABSORBANCE**

When serum is injected directly into the chromatographic system, the wide band at the beginning of the chromatogram and the peaks produced by endogenous compounds at diverse retention times can seriously affect the detection of the least retained drugs. When the concentration is appropriate, dilution of the serum sample before its injection is advisable to reduce the width of the protein band. Furthermore, the injection of a large number of serum samples can shorten the life of the column or may require frequent regeneration of the stationary phase to avoid changes in retention times because of the adsorbed matrix. This also makes the injection of diluted samples

<table>
<thead>
<tr>
<th>Compound</th>
<th>Intraassay CV, %</th>
<th>Interassay CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$c_1$</td>
<td>$c_2$</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>1.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>1.9</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*a* Drug concentrations for $c_1$, $c_2$, $c_3$, and $c_4$ were 4, 6, 8, and 10 mg/L for carbamazepine; 15, 20, 30, and 40 mg/L for phenobarbital; and 10, 12, 15, and 20 mg/L for phenytoin. Compounds were eluted with 0.05 mol/L SDS–70 mL/L butanol, pH 7.

**Table 5. Recoveries of the antiepileptics and CVs (n = 10) obtained for the compounds eluted with 0.05 mol/L SDS–70 mL/L pentanol, pH 7.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Added, mg/L</th>
<th>Recovered, mg/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>4</td>
<td>4.02</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5.98</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7.93</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.86</td>
<td>0.8</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>15</td>
<td>14.84</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.89</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.91</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>40.12</td>
<td>0.6</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>10</td>
<td>9.9</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>11.87</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15.09</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.85</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Fig. 3. Chromatograms of serum samples (diluted 1 mL to 10 mL) collected from epilepsy patients treated with binary or ternary mixtures of the antiepileptics. (a), 6 mg/L carbamazepine and 18 mg/L phenobarbital; (b), 9 mg/L carbamazepine and 10 mg/L phenytoin; (c), 33 mg/L phenobarbital and 15 mg/L phenytoin; (d), 7 mg/L carbamazepine, 20 mg/L phenobarbital, and 12 mg/L phenytoin. The range for the $y$ axis is 0–40 milli-units of absorbance. Peaks: 1, phenobarbital; 2, carbamazepine; 3, phenytoin.
advisable. Therefore, it was decided that the analysis should be carried out after dilution of the samples with a solution containing 9 g/L NaCl. For the antiepileptic drugs, the sensitivity achieved after a 1:10 dilution (1 mL of sample + 9 mL of saline) was adequate for their detection in serum. No changes in retention times were observed after at least 250 consecutive injections of serum onto the C18 column.

**FIGURES OF MERIT**

Calibration curves were constructed for each antiepileptic drug based on the measured areas of the chromatographic peaks after triplicate injection of six solutions of the drugs at increasing concentrations within the therapeutic ranges: 8–12, 15–40, and 10–20 mg/L for carbamazepine, phenobarbital, and phenytoin, respectively. The curves were obtained for aqueous solutions of the analytes and for serum samples that were not diluted and were diluted 1:10. The slopes of the calibration curves in the absence and presence of serum were similar, the intercepts were statistically zero, and the linear regression coefficients for plots of peak areas vs concentration were always >0.999 (Table 3). Table 3 also shows the limits of detection (LOD; in μg/L, based on the 3 SD criterion) that were appropriate for monitoring the antiepileptics; these LOD were similar to those obtained with the TDx method.

Shown in Table 4 are the intraassay precision (average of 10 determinations covering the specified range for the procedure made the same day) and the interassay precision (average of intraassay values taken over 10 days throughout a 2-month period) at four different drug concentrations within the therapeutic ranges of each drug according to the ICH Harmonised Tripartite Guideline (34). The CVs were always <2.1%.

**ANALYSIS OF SERUM SAMPLES**

To demonstrate the usefulness of this procedure, we added known amounts of the antiepileptics at four different concentrations within the therapeutic range to each drug blank plasma sample, which were provided by the Analytical Service of the Verge dels Llíris Hospital in Alcoi (Alacant, Spain). As shown in Table 5, recoveries were satisfactory.

**METHOD COMPARISON**

Results obtained by the MLC method were compared with results obtained with TDx method. Both methods were applied to the serum samples provided by the Hospital Verge dels Lliris. Shown in Fig. 3 are some of the chromatograms obtained by the MLC method for serum samples containing binary or ternary mixtures of the antiepileptics. As shown in Table 6, serum concentrations of the three antiepileptics obtained by the two methods (n = 40) showed good correlation. The MLC method gave slightly lower results for the three drugs compared with TDx. However, there was no substantial difference between the two methods in the practical sense of clinical drug monitoring.

In conclusion, our results indicate that the MLC procedure can be used for the analysis of mixtures of the three frequently prescribed antiepileptics in serum samples, with analysis times <10 min. The method is sensitive enough for routine analysis of the drugs at therapeutic serum concentrations, with LOD similar to those for TDx method and those usually reported in the literature, taking into account that the serum sample was injected without any previous treatment to separate or concentrate the analytes.

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**Table 6. Linear regression of results obtained with the MLC method vs the TDx.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linear regression (n = 40)</th>
<th>ρ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>MLC = 0.958 (TDx) –0.916</td>
<td>0.9230</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>MLC = 1.063 (TDx) –5.176</td>
<td>0.9747</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>MLC = 0.892 (TDx) –1.105</td>
<td>0.9682</td>
</tr>
</tbody>
</table>


