Research Article

Micellar LC Separation of Sesquiterpenic Acids and Their Determination in Valeriana officinalis L. Root and Extracts

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A simple micellar liquid chromatography (MLC) method was developed and validated according to ICH Guidelines for the determination of sesquiterpenic acids (valerenic, hydroxyvalerenic, and acetoxyvalerenic acids) in root and rhizome extract from Valeriana officinalis L. and valerian dry hydroalcoholic extract. Samples were analyzed on Nucleosil C18 column (150 mm × 4.6 mm, 5 μm) using an isocratic mobile phase which consisted of Brij 35 (5% (w/v) aqueous solution; pH 2.3 ± 0.1 by phosphoric acid) and 1-butanol (6% (v/v)); UV detection was at 220 nm. Micellar mobile phase using allows to fully separate valerenic acids within 25 minutes. Linearity for hydroxyvalerenic, acetoxyvalerenic, and valerenic acids was 1.9–27.9, 4.2–63.0, and 6.1–91·3 μg·mL⁻¹, and limit of detection was 0.14, 0.037, and 0.09 μg·mL⁻¹, respectively. Intraday and interday precisions were not less than 2% for all investigated compounds. The proposed method was found to be reproducible and convenient for quantitative analysis of sesquiterpenic acids in valerian root and related preparations.

1. Introduction

Valeriana officinalis L. is the most common species of the genus Valeriana that is used for its medicinal properties [1]. The root and rhizome of the valerian plant is used medicinally for its sedative properties with indications including nervous tension, insomnia, anxiety, and stress. Valerian is also considered to have antispasmodic, anticonvulsant, and antidepressant effects.

The roots and rhizomes of Valeriana officinalis contain two main groups of constituents: sesquiterpenes of the volatile oil (valerenic acid and its derivatives, valeranone, valeranal, and kessyl esters) and valepotriates (valtrate, didrovaltrate, acevaltrate, and isovaleroyxyhydroxyvaltrate), in addition to other constituents such as flavonoids, triterpenes, lignans, and alkaloids [2, 3].

Valerian is available in a variety of formulations, including tablets, capsules, liquid, teas, and tinctures. Products may contain whole herb and/or a proprietary blend or may also be combined with other herbal supplements (e.g., lemon balm, hops, kava, St. John’s worth, etc.). Valerian root and some commercial products (drugs) are standardized according to the content of valerenic acid, but concentrations vary among products.

Valerenic acids are sesquiterpenes based on the dual ring valerane structure with the main representatives being valeric acid ((2E)-3-[(4S,7R,7aR)-3,7-dimethyl-2,4,5,6,7,7a-hexahydro-1H-inden-4-yl]-2-methylacrylic acid; C₁₅H₂₀O₂), acetoxyvalerenic acid, and hydroxyvalerenic acid. Chemical structures of these acids are presented in Figure 1.

Several methods, adopting different analytical techniques, have been reported to analyze sesquiterpenic acids: TLC [4–6], SFC [7], and HPLC-UV, which is the most commonly used method.

Bos et al. [8, 9] simultaneously analyzed valepotriates (valtrate, isovaltrate, acevaltrate, and didrovaltrate), valeric acids (valenic, hydroxyvalenic, and acetoxyvaleric), baldrinal and homobaldrinal by HPLC-DAD using LiChrospher 100 RP-18 column, and gradient elution of acetonitrile-water mobile phases. Total analysis time was 30 min.

Gobbeto and Lolla [10] proposed the HPLC method for the determination of valeric acids in valerian extracts using Hypersil ODS column and gradient elution with acetonitrile
containing 0.1% phosphoric acid-acetonitrile mobile phase; UV detection at 220 nm; analysis time was 40 min.

Bicchi et al. [7] developed supercritical fluid chromatography method with UV detection for the determination of valerenic acids and valepotriates in *valerian* extracts (analysis time is 20 min) and compared results, obtained during *valerian* extracts analysis that were determined by SFC and HPLC methods.

European Pharmacopoeia [11] for the analysis of valerenic acids in *valerian* root end extracts proposed HPLC-UV method with C18 column using an acetonitrile-phosphoric acid aqueous solution gradient elution. Detection at 220 nm and total analysis time is about 30 min (Figure 2).

As we can see, all LC methods required gradient elution techniques for separation and assay of sesquiterpenic acids in raw plant materials and drugs.

It was already described that micellar liquid chromatography (MLC) is an alternative for RP HPLC method [12, 13]. MLC is successfully applied to the analysis of drugs [14, 15] and biological fluids [16], and nowadays this method is widely used for the analysis of raw plant materials and plant containing drugs [17, 18]. One of MLC advantages that made this method preferable to HPLC is the simultaneous separation of both ionic and nonionic compounds, substances with different hydrophobicity without needing gradient elution.

The aim of this work is an investigation of possibility to separate valerenic acids using MLC method, to validate method according ICH validation requirements (specificity, accuracy, precision, and linearity), and an application of the developed method for the rapid sesquiterpenic acid analysis in raw materials (roots and rhizomes of *Valeriana officinalis*) and *valerian* dry extracts.

2. Experimental

2.1. Reagents and Materials. Acetoxyvalerenic acid (AcVal), hydroxyvalerenic acid (HVal), and valerenic acid (Val) were isolated from *Valeriana officinalis* L. in Plant Material Department of the Scientific and Expert Pharmacopoeial Centre, Ukraine, as described in [19]. Chemical structure was confirmed using IR, ¹H, and ¹³C NMR spectra (in the State Scientific Institution “Institute for Single Crystals” of the National Academy of Sciences of Ukraine). Chromatographic purity was determined by RP HPLC method [11]; those were more than 98.5% for all cases.
Valerian standardized dry extract EP CRS (0.38% C15H23O2 (valerenic acid) was from European Pharmacopoeia (Strasbourg, France).

1-butanol (1-BuOH), concentrated phosphoric acid, polyethylene glycol dodecyl ether (Brij 35), methanol (HPLC grade), and other chemicals were purchased from Fluka Chemie (Buchs, Switzerland). Double-distilled water was used in all experiments.

Fragmented underground parts of Valeriana officinalis L. were from “Sumyfitofarmacia Ltd,” Ukraine; valerian dry extracts were from “AIM Ltd,” Ukraine.

2.2. Apparatus. The chromatographic measurements were carried out with Hewlett Packard equipment (Agilent Technologies, Waldbronn, Germany) consisting of a Series 1050 pump, a Series 1050 spectrophotometric detector with the variable wavelength, and a Series 3395 integrator. The analytical column was the reversed-phase Nucleosil C18 (150 mm × 4.6 mm, 5 μm, Macherey-Nagel, Germany). The pH values were determined with a Beckman Φ-200 pH meter (Beckman Instruments, Fullerton, CA, USA) and Paratrode electrode (Metrohm AG, Herisau, Switzerland).

For the determination of peak purity, the Waters 2695 Separation Module (Waters, Milford, MA, USA) with Waters 996 Photodiode Array Detector (Waters) was used.

2.3. Standard Preparation. A stock standard solution was prepared by weighting an amount of hydroxyvalerenic acid, acetoxyvalerenic acid, and valerenic acid in a 100 mL volumetric flask, dissolving it in methanol to obtain concentrations of investigated compounds 0.0465 mg·mL−1 for hydroxyvalerenic acid, 0.105 mg·mL−1 for acetoxyvalerenic acid, and 0.152 mg·mL−1 for valerenic acid. Wang et al. [20] showed that its retention in approximately 0.06 M Brij 35 (6% w/v) as a micellar mobile phase. Preliminary experiment for valerenic acids separation showed that its retention time, peak symmetry, and ranges from 3 for hydroxyvalerenic acid to 5 for valerenic acid.

2.4. Sample Preparation

Valerian Root. Extract 1.50 g of powdered underground parts of Valeriana officinalis (1 mm) with 20 mL of methanol and heat on a water bath under a reflux condenser for 30 min. Allow to cool and filter through a paper filter into a 50 mL volumetric flask. Add another 20 mL of methanol, heat on a water bath under a reflux condenser for 15 min, cool, filter into the same 50 mL volumetric flask, and dilute to 50.0 mL with methanol [11].

Valerian Dry Extract. Place about 0.5 g of valerian dry extract in a 25 mL volumetric flask, add 15 mL methanol, mix, and sonicate during 10 min. Then the solution is diluted to 25.0 mL with methanol and mix [11].

All samples must be filtered through a 45 μm HPLC filter (DynaCard HPLC filter, Microgon, USA) before analysis.

2.5. Chromatographic Separation. A 5% (w/v) aqueous solution of Brij 35 with 6% (v/v) of 1-butanol, adjusted to pH 2.3 ± 0.1 by phosphoric acid, was prepared and used as mobile phase. The flow rate was set at 1.0 mL·min−1, and the injection volume was 10 μL. The chromatographic runs were carried out at 40.0 ± 0.1°C. Detection wavelength was chosen at 220 nm.


3. Results and Discussion

Nonionic surfactants, unfortunately, are not widely used for micellar liquid chromatographic separation of compounds with different hydrophobicity. It was not found any recommendation in the literature about an approximate nonionic surfactant concentration, which is required for different hydrophobicity substances separation, as described for sodium dodecyl sulfate as micellar-forming agent [12]. In [20–22] it was found that concentration of nonionic surfactants to be micellar eluents should be sufficiently large—5–10% (w/v).

Figure 1 provides the chemical structures and some physical-chemical characteristics of investigated compounds: hydrophobicity as lgPow (logarithms of partition constant in 1-octanol-water system) and pKa values. Hydrophobicity of compounds is not quite different and ranges from 3 for hydroxyvalerenic acid to 5 for valerenic acid. Wang et al. [20] separated quinazoline derivatives with hydrophobicity 2–4 using 0.06 M Brij 35 (6% w/v) as a micellar mobile phase.

Preliminary experiment for valerenic acids separation showed that its retention in approximately 0.06 M Brij 35 mobile phase was about 75 min, and peaks asymmetries of investigated compounds were high.

According to pKa values, at pH value below 4, the predominant form of compounds in solution is molecular forms—all ionizable groups are fully protonated, and at pH above 6 compounds are negatively charged. A mobile phase for acid separation should be acidified. Preliminary experiments have shown that when acidified 0.06 M Brij 35 containing micellar mobile phase used for separation of the investigated compounds, peak symmetry is lower than 2; it is appropriate to the assay determination.

It was investigated retention time, peak symmetry, and resolution for different micellar mobile phases to choose an optimal micellar mobile phase composition. Brij 35 concentrations in mobile phases were varying from 2% to 8% (w/v) with a step of 1%, and micellar mobile phase organic modifier (1-bithanol) was varying from 1% to 10% (v/v) with the same step. The mobile phase pH in all cases was about 2.3 ± 0.1.

The optimization procedure [23, 24] that was used for choosing the optimal mobile phase composition showed that
for all micellar mobile phases were obtained good chromatographic characteristics: sesquiterpenic acid resolution was more than 1.5, and peak symmetry was 1.5–2. Lowest retention time (12 min) was obtained when 8% (w/v) of Brij 35 and 10% (v/v) of 1-BuOH as micellar mobile phase components were used. Unfortunately, this micellar mobile phase has high viscosity, and column input pressure was unacceptable: 350–370 bar whereas chromatograph pump upper pressure limit is 400 bar. When a mobile phase with low viscosity (2% (w/v) of Brij 35 and 1% (v/v) of 1-BuOH) was used for sesquiterpenic acids separation, total analysis time was about 90 min.

Optimum between micellar mobile phase viscosity (relatively low viscosity) and analysis time (till 25 min) was found, when Brij 35 concentration of 5% (w/v) and 1-BuOH volume fraction of 6% (v/v) were chosen.

In addition, the efficiency of the separation and peak asymmetry can be improved by using high temperatures due to faster mass transfer of solutes between mobile and stationary phases [15], and for reducing of mobile phase viscosity. A column temperature 40 °C was chosen, because higher temperature (50 and 60 °C) had not exerted significant influence on the chromatographic behavior of investigated compounds.

3.1. Validation of the MLC Method. The procedures and parameters used for the validation of the chromatographic method developed in this study are those described in [25, 26].

3.1.1. Specificity (Selectivity). The specificity of the method was determined by analyzing valerian-standardized dry extract EP CRS and methanol extract of valerian root. Methanolic extract was analyzed without and with addition of investigated compounds; increasing peak areas indicate the presence of valerenic acids in the chromatogram. Selectivity of the MLC method was assessed by the comparison of the spectrum extracted from the chromatogram of valerian-standardized dry extract and methanolic extract obtained using photodiode array detector; peak purity was also determined.

Figure 3 shows a chromatogram and UV spectra obtained for valerian-standardized dry extract, which contains (see Figure 2) hydroxyvalerenic, acetoxyvalerenic, and valerenic acids. The same spectra were obtained for valerian methanolic extract. The peaks purity was more than 98.5% in all cases.

3.1.2. Linearity. A linear plot was obtained from six different concentrations of working standard solutions using four replicate injections [25, 26]. The regression line was calculated as $Y = a + bC$, where $C$ was the analyte concentration.
predicted responses were not observed. The systematic deviations between experimental and linear relationship between amount of analyte and detector obtained by linear least-squares regression. A general acceptance criterion to the linearity performance of the regression. This parameter should be comparable to the relative standard deviation obtained in precision studies within the given concentrations range. Relative standard error of slopes was obtained to be less than RSD of precision. The results obtained from these analyses are listed in Table 4 as mean recovery. The table shows that there is no significant difference between assay results either within days or between days, implying that the reproducibility of MLC method was good. In all instances, the % R.S.D. values were less than 2%—criterion for an intra-assay precision [28].

3.1.4. Accuracy. The accuracy of the proposed method was tested in some ways. First, samples of known concentration (working standard solutions) were analyzed, and measured values were compared with the true value. The results obtained from determination of accuracy, expressed as percentage recovery, are summarized in Table 2. Accuracy criterion for the assay method is that the mean recovery will be ± 2% for each concentration over the investigation range [28]. The recovery of the proposed method was good.

Second, this study was performed by addition of known amounts of studied compounds to a solution of valerian dry extract (standard addition method). The resulting mixtures were analyzed by proposed MLC method, and the obtained results were compared with the expected results. The excellent recoveries of standard addition method (Table 2) suggested good accuracy of the proposed method.

Third, accuracy of MLC method was determined by comparing results obtained by proposed method with results from the validated method using reversed-phase HPLC [11]. MLC and RP-HPLC results for sesquiterpenic acids assay were compared. Statistically analysis of the results obtained for both methods using variance ratio F-test shows that there is no significant difference between these results. The calculated F values were less than those of the theoretical values at 95% confidence level (see data from Table 3).

3.1.5. Precision. The precision as intraday and interday reproducibility, expressed as RSD %, was characterized by the spread of data from replicate determinations.

For the intraday reproducibility, that is, repeatability, we performed nine determinations covering the specified range of the method. Working standard solutions were analyzed (four replicates each). Interday precision of the method was checked on three different days by preparing and analyzing working standard solutions (four replicate injections) under the same conditions.

The results obtained from these analyses are listed in Table 4 as mean recovery. The table shows that there is no significant difference between assay results either within days or between days, implying that the reproducibility of MLC method was good. In all instances, the % R.S.D. values were less than 2%—criterion for an intra-assay precision [28].

3.2. Analysis of Plant Material and Drug. Sesquiterpenic acids (valerenic, acetoxyvalerenic, and hydroxyvalerenic acids) were quantified using developed MLC method in the valerian root and rhizomes and valerian dry extract (chromatograms of investigated compounds are presented in Figure 4). Fragmented underground parts of Valeriana officinalis L. cultivated in 2008–2011 years were from “Sumyfitofarmacia Ltd,” Ukraine; valerian dry hydroalcoholic extracts obtained from above-mentioned valerian root were from “AIM Ltd,” Ukraine.

The percentage of valerenic acid, acetoxyvalerenic acid, and hydroxyvalerenic acid, in root and rhizomes was, according to [11] not less than 0.17% of sesquiterpenic acids expressed as valerenic acid, calculated with the reference to

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hydroxyvalerenic acid</th>
<th>Acetoxyvalerenic acid</th>
<th>Valerenic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration range (μg·mL⁻¹)</td>
<td>1.9–27.9</td>
<td>4.2–63.0</td>
<td>6.1–91.2</td>
</tr>
<tr>
<td>Detection limit (μg·mL⁻¹)</td>
<td>0.037</td>
<td>0.09</td>
<td>0.14</td>
</tr>
<tr>
<td>Quantitation limit (μg·mL⁻¹)</td>
<td>0.11</td>
<td>0.27</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Parameters of regression equation \( Y = a + b \cdot C \), where \( C \) is the concentration of compound in μg·mL⁻¹, \( Y \) is the peak area

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hydroxyvalerenic acid</th>
<th>Acetoxyvalerenic acid</th>
<th>Valerenic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept ( a )</td>
<td>( -5.5 \cdot 10^3 )</td>
<td>( -7.8 \cdot 10^4 )</td>
<td>( -1.6 \cdot 10^4 )</td>
</tr>
<tr>
<td>SD of the intercept ( S_a )</td>
<td>( 0.3 \cdot 10^3 )</td>
<td>( 0.6 \cdot 10^4 )</td>
<td>0.1 \cdot 10^4</td>
</tr>
<tr>
<td>Slope ( b )</td>
<td>( 27.1 \cdot 10^3 )</td>
<td>( 21.3 \cdot 10^4 )</td>
<td>23.8 \cdot 10^4</td>
</tr>
<tr>
<td>SD of the slope ( S_b )</td>
<td>( 0.2 \cdot 10^3 )</td>
<td>( 0.2 \cdot 10^4 )</td>
<td>0.2 \cdot 10^4</td>
</tr>
<tr>
<td>RSD of the slope, %</td>
<td>0.74</td>
<td>0.94</td>
<td>0.84</td>
</tr>
<tr>
<td>Correlation coefficient ( r )</td>
<td>0.9995</td>
<td>0.9994</td>
<td>0.9995</td>
</tr>
</tbody>
</table>

\( (μg\ mL^{-1}) \), and \( y \) was the peak area. The calibration plot was obtained by linear least-squares regression.

Calibration parameters, such as slope, intercept, and correlation coefficient are provided in Table 1. The correlation coefficient \( r \) was close to unity; hence, there was a linear relationship between amount of analyte and detector signal. The systematic deviations between experimental and predicted responses were not observed.

Since the coefficient of correlation is not suitable as a general acceptance criterion to the linearity performance of an analytical procedure [27], the relative standard error of slope was used as a parameter with respect to precision of the regression. This parameter should be comparable to the relative standard deviation obtained in precision studies within the given concentrations range. Relative standard error of slopes was obtained to be less than RSD of precision.

3.1.3. Limit of Detection and Quantitation. The limits of detection (LOD) and quantitation (LOQ) were calculated in accordance with the 3.3 \( s/m \) and 10 \( s/m \) criteria, respectively, where \( s \) is the standard deviation of the peak area for the sample, and \( m \) is the slope of the calibration curve, determined from linearity [25]. Both values were calculated and presented in Table 1.

3.1.4. Accuracy. The accuracy of the proposed method was tested in some ways. First, samples of known concentration (working standard solutions) were analyzed, and measured values were compared with the true value. The results obtained from determination of accuracy, expressed as percentage recovery, are summarized in Table 2. Accuracy criterion for the assay method is that the mean recovery will be 100 ± 2% for each concentration over the investigation range [28]. The recovery of the proposed method was good.

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Third, accuracy of MLC method was determined by comparing results obtained by proposed method with results from the validated method using reversed-phase HPLC [11]. MLC and RP-HPLC results for sesquiterpenic acids assay were compared. Statistically analysis of the results obtained for both methods using variance ratio F-test shows that there is no significant difference between these results. The calculated F values were less than those of the theoretical values at 95% confidence level (see data from Table 3).
Table 2: Accuracy results of MLC method: analysis of sesquiterpenic acid mixtures and standard addition technique.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Added (μg·mL⁻¹)</th>
<th>As found* (μg·mL⁻¹)</th>
<th>% Recovery</th>
<th>Δ</th>
<th>Added, μg·mL⁻¹</th>
<th>As found*, μg·mL⁻¹</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyvalerenic</td>
<td>1.90</td>
<td>1.88</td>
<td>99.0</td>
<td>−1.0</td>
<td>0</td>
<td>—</td>
<td>2.10</td>
</tr>
<tr>
<td></td>
<td>9.30</td>
<td>9.25</td>
<td>99.5</td>
<td>−0.5</td>
<td>1.90</td>
<td>4.20</td>
<td>2.20</td>
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<tr>
<td></td>
<td>27.9</td>
<td>27.8</td>
<td>99.6</td>
<td>−0.4</td>
<td>5.60</td>
<td>7.74</td>
<td>2.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.3</td>
<td>11.4</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean, %R.S.D.</td>
<td>99.4, 0.8</td>
<td>Mean, %</td>
</tr>
<tr>
<td></td>
<td>4.20</td>
<td>4.19</td>
<td>99.8</td>
<td>−0.2</td>
<td>0</td>
<td>—</td>
<td>33.4</td>
</tr>
<tr>
<td>Acetoxyvalerenic</td>
<td>21.0</td>
<td>20.85</td>
<td>99.3</td>
<td>−0.7</td>
<td>12.6</td>
<td>46.3</td>
<td>33.7</td>
</tr>
<tr>
<td></td>
<td>63.0</td>
<td>63.1</td>
<td>100.1</td>
<td>+0.1</td>
<td>21.0</td>
<td>54.2</td>
<td>3.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29.4</td>
<td>63.5</td>
<td>34.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean, %R.S.D.</td>
<td>99.7, 1.1</td>
<td></td>
<td>Mean, %</td>
<td>101.2</td>
<td></td>
</tr>
<tr>
<td>Valerenic acid</td>
<td>6.10</td>
<td>6.07</td>
<td>99.5</td>
<td>−0.5</td>
<td>0</td>
<td>—</td>
<td>50.7</td>
</tr>
<tr>
<td></td>
<td>30.4</td>
<td>30.5</td>
<td>100.3</td>
<td>+0.3</td>
<td>18.2</td>
<td>69.4</td>
<td>51.2</td>
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<tr>
<td></td>
<td>91.2</td>
<td>91.4</td>
<td>100.2</td>
<td>+0.2</td>
<td>30.4</td>
<td>80.7</td>
<td>50.3</td>
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<tr>
<td></td>
<td></td>
<td>Mean, %R.S.D.</td>
<td>100.0, 1.1</td>
<td></td>
<td>Mean, %</td>
<td>100.6</td>
<td></td>
</tr>
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</table>

*Mean value of the four determinations.

Figure 4: Chromatograms obtained during *Valeriana officinalis* L. root and extract analysis. HVal: hydroxyvalerenic acid, AcVal: acetoxyvalerenic acid, and Val: valerenic acid. (a) valerenic acids standard solution; (b) methanolic extract of *Valeriana officinalis* L. root and rhizome; (c) chromatogram of *valerian* hydroalcoholic dry extract.
the dried drugs. Ukrainian Pharmacopoeia [29] required plants that grown in Ukraine and Russia regions, sum of sesquiterpenic acids not less than 0.10% (as valeric acid, calculated with the reference to the dried drugs).

Valerian dry hydroalcoholic extract should contain minimum 0.25% of sesquiterpenic acids, expressed as valeric acid (dry extract) [11].

Table 3 provides the results of the determination of sesquiterpenic acids in various analytical subjects, which was obtained using micellar liquid chromatographic method. As can be seen from Table 3, not all raw materials satisfied the European Pharmacopoeia [11] requirements for valerian root, but all results fulfilled requirements of Ukrainian Pharmacopoeia. All results for valerian dry extract analysis comply with requirements of European Pharmacopoeia [11].

### 4. Conclusions

A simple and reliable MLC method for the simultaneous determination of sesquiterpenic acids (valeric, acetoxyvaleric, and hydroxyvaleric acids) in Valeriana root and rhizomes and Valeriana dry extract was developed. Chromatographic method with using micellar mobile phase does not require gradient elution which is widely used in reversed-phase HPLC methods. That is the main advantage of MLC that allows separating compounds with different hydrophobicity in a single run without the gradient elution.

The method was completely validated showing satisfactory data for all the parameters tested. This method is also ecofriendly for its low concentration of organic solvent, as compared to other analytical techniques.

### References


