Aqueous preparations obtained from the herbal drugs (in what follows abbreviated as APHD) by the process of extraction belong to active substances and drugs. They are known from the time of Galenos, who is supposed to be the first who prepared the drugs from the herbal and animal material. Though his famous successor Paracelsus tried to isolate so called “effective principles” from natural material, his extracts having a heterogeneous composition, were finally assigned to those prepared by Galenos.

Many isolated and identified substances presented in the herbal drugs became models for synthesis of new drugs. Especially, the extracts and tinctures are rich in those substances and for this reason they have come to the fore of the research interests. The herbal drugs are justified still today and their use in the therapeutic praxis is well liked (1).

From aspects of the drug technology, the extraction is a manufacturing procedure applied to the system drug – leachant with the aim to obtain an extract with a complex of the contained substances of varied physical, chemical and technological properties (2).

The subjects of this study, presented in this paper, are tinctures and mixtures of extracts. Their physical properties, stability and influence of the light are evaluated and the tinctures prepared by various methods and manufacturers are compared.

Within the framework of this study are evaluated four types of the tinctures: Belladonnae tinctura, Gentianae tinctura, Cinchonae tinctura and Valerianae tinctura, and three mass-manufactured drugs: Bucofit, Cynarofit and ºaliafit.

The study was aimed at determinations of: 1. density, dry matter, index of refraction, pH value, content of ethanol, influence of the light on these parameters as well as the global appearance of samples. In parallel to that, the stability of samples Tinctura valerianae prepared by two different manufacturers and the samples of magistraliter preparations were compared. It was found that storing samples delivered by Calendula j.s.c. does not significantly influenced their stability neither in the light nor in the dark, kept at the temperature of 20-25°C over the time interval of 6 months. All samples were in agreement with the norms of companies as well as with both Czechoslovak (ČSL 4) and Slovak (SL 1) pharmacopoeias. Besides, the results obtained show that a kind of extraction methods (percolation, maceration) does not influence neither quality nor stability of the samples Tinctura valerianae.

Key words: herbal extracts, tinctures, evaluation, physico-chemical properties
membrane of the mouth cavity. It exhibits a mild antimicrobial, anti-inflammatory, anti-ache and deodorant effect.

**CYNAROFIT**
Cynarofit is a supporting nourishing preparation for protection of the liver, releasing spasms, lowering the fat levels in blood. It also facilitates the digestibility of the meal and the process of digestion.

**ºALIAFIT**
ºaliafit is a supporting disinfectant exhibiting a favorable effect on burns, wounds, bites of insects, muscles rigor etc.

**BELLADONNAE TINCTURA**
For preparation of the tincture, a dried leaf of the genus of *Atropa belladonna* (Solanaceae) Belladonnae folium is used. The APHD contains tropan alkaloids (hyoscyamine, atropine, and scopo-lamine) coumarin derivatives, flavonic glycosides and tannins. It is used as a neurotropic parasympathetic, spasmyloytic, mydriatic, anti-emetic and antiparkinsonian drug. The tincture is not used solely, but as a component of mass production. The efficacy must be standardized.

**GENTIANAE TINCTURA**
The tincture is prepared from the dried non-fermented radix with subterranean part of the genus of *Gentiana lutea* (Gentianaceae). The APHD contains secoriodic embitterments. It is used as an amarum-stomachicum, a component of cholagoges and roborances.

**CINCHONAE TINCTURA**
For preparation of the tincture a dried bark of the trunk, branches or radices of the grown genus of Cinchona (Rubiaceae) is used. The APHD contains chinin alkaloids (chinin, chinidine, cinchonine, cinchonidine). The tincture is used as a component of stomachics-digestives).

**VALERIANAE TINCTURA**
For preparation of the tincture a dried rhizome with radices of the genus of Valeriana officinalis (Valerianaceae) – Valerianae radix is used. The APHD contains essential oil, seskwiterpenes and valepotriates. It is used as a mild ataractics, anxiolyticum, sedative and spasmyloyticum.

**MATERIALS AND METHODS**

<table>
<thead>
<tr>
<th>APHDs</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>SL1 (atest)</th>
<th>In light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bukofit</td>
<td>0.9192</td>
<td>0.9165</td>
<td>0.9182</td>
<td>0.9183</td>
<td>0.9181</td>
<td>0.9184</td>
<td>0.900 – 0.925</td>
<td>0.916</td>
</tr>
<tr>
<td>Cynarofit</td>
<td>0.9776</td>
<td>0.9780</td>
<td>0.9785</td>
<td>0.9802</td>
<td>0.9782</td>
<td>0.9784</td>
<td>0.970 – 1.010</td>
<td>0.979</td>
</tr>
<tr>
<td>ºaliafit</td>
<td>0.8803</td>
<td>0.8791</td>
<td>0.8817</td>
<td>0.8808</td>
<td>0.8829</td>
<td>0.8830</td>
<td>0.860 – 0.910</td>
<td>0.883</td>
</tr>
<tr>
<td>Belladonnae</td>
<td>0.8928</td>
<td>0.8937</td>
<td>0.8936</td>
<td>0.8950</td>
<td>0.8954</td>
<td>0.8957</td>
<td>0.889 – 0.908</td>
<td>0.896</td>
</tr>
<tr>
<td>tinct.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentianae</td>
<td>0.9167</td>
<td>0.9173</td>
<td>0.9166</td>
<td>0.9173</td>
<td>0.9194</td>
<td>0.9190</td>
<td>0.890 – 0.920</td>
<td>0.92</td>
</tr>
<tr>
<td>tinct.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cinchonae</td>
<td>0.9116</td>
<td>0.9129</td>
<td>0.9145</td>
<td>0.9117</td>
<td>0.9153</td>
<td>0.9146</td>
<td>0.910 – 0.920</td>
<td>0.914</td>
</tr>
<tr>
<td>tinct.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valerianae</td>
<td>0.9065</td>
<td>0.9051</td>
<td>0.9072</td>
<td>0.9059</td>
<td>0.9077</td>
<td>0.9076</td>
<td>0.898 – 0.916</td>
<td>0.907</td>
</tr>
<tr>
<td>tinct.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 2. pH values of the APHDs

<table>
<thead>
<tr>
<th>APHDs</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>SL1 (attests)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In dark</td>
<td>In light</td>
<td></td>
</tr>
<tr>
<td>Bukofit</td>
<td>4.69</td>
<td>4.63</td>
<td>24.5</td>
</tr>
<tr>
<td>Cynarofit</td>
<td>5.79</td>
<td>5.77</td>
<td>24.6</td>
</tr>
<tr>
<td>Laliatit</td>
<td>6.38</td>
<td>5.89</td>
<td>24.7</td>
</tr>
<tr>
<td>Belladonnae tinct.</td>
<td>5.82</td>
<td>5.70</td>
<td>24.8</td>
</tr>
<tr>
<td>Gentianae tinct.</td>
<td>5.07</td>
<td>4.94</td>
<td>24.8</td>
</tr>
<tr>
<td>Cinchonae tinct.</td>
<td>5.08</td>
<td>5.07</td>
<td>24.7</td>
</tr>
<tr>
<td>Valerianae tinct.</td>
<td>6.13</td>
<td>6.11</td>
<td>24.8</td>
</tr>
<tr>
<td>Valerianae tinct. IVAX</td>
<td>5.99</td>
<td>-</td>
<td>24.2</td>
</tr>
<tr>
<td>Valerianae tinct. magistraliter</td>
<td>5.99</td>
<td>-</td>
<td>24.6</td>
</tr>
</tbody>
</table>

### Table 3. Values of the refraction index over the time span 09/2005 – 02/2006

<table>
<thead>
<tr>
<th>APHDs</th>
<th>Refraction index</th>
<th>Requirements of SL1 (attests)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In dark</td>
<td>In light</td>
</tr>
<tr>
<td>Bukofit</td>
<td>1.3675</td>
<td>1.3670</td>
</tr>
<tr>
<td>Cynarofit</td>
<td>1.3610</td>
<td>1.3605</td>
</tr>
<tr>
<td>Laliatit</td>
<td>1.3690</td>
<td>1.3690</td>
</tr>
<tr>
<td>Belladonnae tinct.</td>
<td>1.3645</td>
<td>1.3640</td>
</tr>
<tr>
<td>Gentianae tinct.</td>
<td>1.3690</td>
<td>1.3680</td>
</tr>
<tr>
<td>Cinchonae tinct.</td>
<td>1.3700</td>
<td>1.3685</td>
</tr>
<tr>
<td>Valerianae tinct.</td>
<td>1.3690</td>
<td>1.3680</td>
</tr>
<tr>
<td>Valerianae tinct. IVAX</td>
<td>1.3670</td>
<td>1.3670</td>
</tr>
<tr>
<td>Valerianae tinct. magistraliter</td>
<td>1.3670</td>
<td>1.3675</td>
</tr>
</tbody>
</table>

chamomilla L. Asteraceae, extract of the herbage of Mentha piperita L. Lamiaceae (4).

LALIAFIT SPRAY – the extract of the stem of Lilium candidum L. Liliaceae, extract of the herbage of Salvia officinalis L. Lamiaceae (5).

BELLADONNAE TINCTURA (radix), Atropa belladonna L., Solanaceae.

CINCHONAE TINCTURA (bark), Cinchona succirubra Pavon, Rubiaceae.

GENTIANAE TINCTURA (radix), Gentiana lutea L., Gentianaceae.

VALERIANAE TINCTURA (radix) Valeriana officinalis L., Valerianaceae.

All delivered by Calendula – the joint stock company (JSC), Nová Lubovňa, Slovakia.

VALERIANAE TINCTURA magistraliter (prepared by maceration), delivered by: IVAX Pharmaceuticals, Ltd., Opava, Czech Republic.
**Measurement of the index of refraction**

The index of refraction was measured by a refractometer. The average of three measurements was evaluated.

**Measurement of pH**

The pH value was determined by a digital pH-meter.

**Determination of the content of ethanol**

The content of ethanol was calculated with the formula (6):

$$x = \frac{50 \times d_{20}^2 \times y}{q}$$

where $x$ is the content of ethanol in APHD expressed in the mass percentage, $y$ is the content of ethanol in mass percentage corresponding to the density, $d_{20}$ is a relative density of the obtained distillate (50 mL) at 20°C and $q$ is the amount of sample.

**Storage of extracts**

The extracts were divided into 2 halves. The first half was stored at the temperature of 20-25°C on the dark place in the tightly closed flasks. The second half was exposed to day light, because the
RESULTS AND DISCUSSION

Since besides the active substances, all extracts contain also other accompanying substances, an aim of the extraction was to prepare an APHD in which the active substances would be presented in the optimal amounts, respectively, in the amounts requested by the pharmacopoeias and norms, and which would have both the suitable properties and stable dosage form (7).

Evaluation of the quality of APHD is not satisfactorily resolved so far. A reason is that they are multi-component mixtures of the active, adjuvant and bulky materials and their mutual ratios and properties are variable and often unknown. Tinctures and extracts are evaluated by quality indices such as the consistency, appearance, color, taste, solubility, density, evaporate, percentage of alcohol, glycerol and content of the active agents.

Regarding the evaluation of the density of the samples [stored in the dark and the light in accordance with the Slovak pharmacopoeia (8) over month intervals, it can be concluded that for majority of the extracts no significant differences were observed.

The maximum difference between the lowest and the highest density varied in the range from 0.0026 to 0.0039 g/cm³. The densities were compared with the values from attests and with values in the Slovak pharmacopoeia. (Tab. 1). All determined values were appropriate.
The pH values ranged within 4.69-6.38 and satisfy requirements of the attests (Tab. 2).

The values of the index of refraction ranged within the interval 4.69-6.38. By comparison with the values stated in attests it can be ascertained that the samples meet the requirements (Tab. 3). The dry matter of the particular APHDs varied in the range 0.95-5.975 (Tab. 4) and met requirements of the attests and in case of tinctures also those of pharmacopoeia. Lower values were obtained with Laliofit (0.95-1.05 %), but the certificate does require such data, therefore, the stated value is only informative.

The amount of ethanol varied within the range 32.3-79.9% (Tab. 5), what is in agreement with the requirements of the attests and pharmacopoeia ČSL 4 (9). Higher content of ethanol (79.9% m/m) was found in Laliafit; independently whether it was stored in the dark or in the light.

The results of the evaluation of appearance, namely the color, dim and sheer over the time span from Sept. 2005 to Feb. 2006 are shown in Table 6. In case of the samples stored in the light it was observed change of the color deepness in four cases (Laliafit and Valerianae tinct., Gentiana tinct. and Valerianae tinct.) In two cases (Cynarofit and Valerianae tinct.), samples turbidity was observed after 6 months, independently whether they were stored in the dark or in the light.

Values of the density and index of refraction were no influenced by the way of storage. Change of pH was recorded in case of Laliafit only (in the dark 6.38 and 5.89 in the light). In spite of that it is conform to the attest.

The above stated parameters were also evaluated for samples of Valerianae tinct., which were prepared by different producers and different methods. In particular there were studied the Valerianae tinct. delivered by Calendula J.S.C. Nová Lubovňa and Valerianae tinct. from company IVAX Pharmaceuticals Ltd., Opava, which was prepared by percolation and Valerianae tinct. magistraliter prepared by maceration. It was found that they obey both the pharmacopoeia requirements and attestations. The results are summarized in Table 7.

CONCLUSIONS

The part of our research described in this paper was aimed at the study of the APHD’s stability, namely the evaluation of physical properties like density, index of refraction, dry matter, pH, content of ethanol, outer appearance and how the light can influence these properties.

In view of this aim, there were compared samples of Valerianae tinct. prepared by different manufacturers and methods. It was revealed that the APHDs delivered by Calendula J.S.C, Nová Lubovňa, which underwent the six months lasting tests at the temperature 20-25°C in the dark place in tightly closed flasks were stable and obeyed all requirements stated in both the attestations and pharmacopoeia (8, 9). The exposition of the extracts to the light lasting six months did not have any significant influence on their stability.

Besides, the results obtained imply that the type of extraction method used do not influence neither quality nor stability of the Valerianae tinct. samples.

REFERENCES

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