Qualitative and quantitative determination of ferulic acid by HPTLC and HPLC methods in root resin of Ferula Tadshikorum

Dildora Barakaeva, Nuritdin Mukarramov, Anarbay Babekov, Lola Zhamolova, Maksatbek Turatbekov

Abstract. Fast, simple, accurate, specific and reliable methods of HPTLC and HPLC have been developed and proposed, which can be used for the analysis of ferulic acid in plant extracts. The HPTLC method is economical because it uses a very small number of mobile phases that effectively dissolve ferulic acid, and the sample purification procedure associated with it is minimal. The developed methods can be widely used to determine the quality of Ferula tadshikorum resin and formulations containing it.

1 Introduction

Ferula tadshikorum Pimenov is a perennial, monocarpic, strongly and unpleasantly smelling herbaceous plant of the celery family (Umbelliferae) – Apiaceae (Umbelliferae), a large life cycle is carried out in 23-27 (30) years. It grows in the middle belt of mountains in the southern regions of the Republic – in Kashkadarya and Surkhandarya regions [1-3].

Medicinal raw materials are both underground (inspissated in the air milky juice of the roots) and the aboveground parts of the plant. The chemical composition of the inspissated milky juice of the roots is represented by resin (9.35-65.15%), gum (12-48%) and essential oil (5.8-20%). Ferulic acid, asarezinotanol, assarezinol and their ferulic derivatives: farnesiferol C and umbelliferon are isolated from the resin [4-5].

It is known from the literature that in the East, fetid gum (stinking gum), obtained from cuts made in the roots of fresh ferula, is used as a spice. Currently, industrial preparations of this resin, which is exported by tons as a spice to India, Iran, Pakistan, and Afghanistan, lead to depletion of stocks of these plants.

In Central Asia, gum resin is used as an anthelmintic, insecticidal, anticonvulsant, as well as for some nervous diseases and viral diseases of the reproductive system [6]. Of all the variety of known types of ferule activity, a promising area of use in medicine is their anti-inflammatory, antiparasitic, namely giardicidal activity. Giardiasis is known to be a widespread disease, especially in children. Research in this regard seems promising specifically for the Central Asian region. The most promising in this regard are alcohol extracts and resin of Ferula tadshikorum.
In recent years, the President of the Republic of Uzbekistan has adopted a number of resolutions and decrees on the need to create scientific and technical projects for the deep processing of ferula plants and other medicinal herbs, including those cultivated in the republic, for the development and creation of new medicines based on them, their raw materials and exports in order to ensure the production of necessary for medicine and agriculture production of medicines by processing raw ferule.

To this end, pharmacotoxicological studies on the basis of Ferula tadshikorum root resin, the antiparasitic activity of an alcoholic resin extract was revealed, which served as the subject of the invention [7] and the development of drugs with giardicidal activity.

The purpose of this study is to develop a method for standardizing the drug being created using the HPLC method [8-9]. Ferulic acid contained in the roots and resin of the plant was used as a standard.

2 Experimental Part

Chromatography conditions: chromatograph with automatic or manual dispenser, high pressure pump, UV detector, Supelco C18 column 5 µm, 150x4.6 mm.

Chromatographic run is performed at room temperature. UV detection is performed at a wavelength of 254 nm.

3 Result Discussion

Determination of ferulic acid by the HPTLC method. For the study, 10 ml of the test methanol resin solution was prepared at a concentration of 1 mg/ml and applied to the plate by spraying. The chromatographic plate was sprayed with the test solution using the CAMAG AUTOMATIC TLC SAMPLER 4. The distance between the tracks is 14.4 mm, the width of the tracks is 8 mm. For the analysis of substances, HPTLC Silicagel 60 F 254, Germany, 50 Glass plates were used, as an eluting mixture. Elution was performed using the CAMAG ADC 2 Automatic Developing Chamber. The plates were scanned at a wavelength of 254 nm in the CAMAG TLC SCANNER device, using the vision CATS by CAMAG program.

Fig. 1. Chromatograms at 254 nm: a) the methanol fraction of the resin; b) ferulic acid - standard

Ferulic acid was determined by high performance liquid chromatography (HPLC) using an external standard. Ferulic acid was selected as the standard sample, and a comparative determination relative to the standard was carried out using the device. The device was previously calibrated according to the standard.

Preparation of a standard sample solution. 1 mg (precisely weighed amount) of the standard ferulic acid sample is placed in a 50 ml graduated flask, 1 ml of methyl alcohol is added and stirred in an ultrasonic apparatus for 10 minutes.

Preparation of a test sample solution. 10 mg (precisely weighed amount) of the test drug is placed in a 50 ml graduated flask, 10 ml of methyl alcohol is added and stirred in an ultrasonic apparatus for 10 minutes, stirred and filtered through a 0.45 µm membrane filter.
Chromatography conditions: For the analysis, 10 ml of the test methanol resin solution at a concentration of 1 mg/ml and standard ferulic acid 1 mg/ml were prepared, which were subjected to HPLC analysis on a Shimadzu chromatograph with a UV detector at a wavelength of 320 nm. A column with silica gel (5 µm), with octadecylsilane, Supelco C18, 150x4.6 mm, 5 µm was used as the stationary phase. Elution was carried out with a mixture (acetonitrile:water:formic acid 20 ml/1000 ml) - 20:80 in a gradient system at room temperature with an eluent flow rate of 0.5 ml/min. As a result, ferulic acid was identified on the chromatogram of the samples, the retention time of which coincided with the retention time of the standard ferulic acid sample and was 4.6 minutes.

Table 1. Gradient conditions for the mobile phase

<table>
<thead>
<tr>
<th>Time, min.</th>
<th>Solvent A</th>
<th>Solvent B</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.00</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>20.00</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>24.00</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2. Diagram: gradient conditions for the mobile phase

Solvent A – methanol
Solvent B – acetonitrile: (water : formic acid 20 ml/1000 ml) – 20:80
The analysis time is 30 minutes, room temperature, flow is 0.5 ml/min, sample volume is 20 µl.

Other peaks are allowed.

To do this, the eluent is passed through the column for 30 minutes at a rate of 0.5 ml/min. Three samples of Ferula tadshikorum were collected from the roots of a vegetating plant with an interval of 1 month.

4 Result Discussion

Sample 1 – F. tadshikorum resin of the first harvest (July); sample 2 – resin of the second harvest (August); sample 3 – resin of the third harvest (September).

Table 2. The results of the analysis of ferulic acid and resin samples of F. tadshikorum

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Retention time</th>
<th>Square Высота</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard</td>
<td>4.58</td>
<td>937404</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Standard and test solutions are chromatographed. The calculation of the mass fraction (X%) of ferulic acid in the drug preparation is performed in accordance with the formula presented below:

\[ X = \frac{S \times m \times P}{S_0 \times m} \]

- \( S \) – the average value of the peak area of ferulic acid in a standard solution;
- \( S_0 \) – the average value of the peak area of ferulic acid in the solution of the drug sample;
- \( m \) – ferulic acid standard weighed amount, g;
- \( m_0 \) – drug sample weighed amount, g;
- \( P \) – the content of ferulic acid in the standard, in %.

According to the results of HPLC analysis, it was found that in the Ferula tadshikorum resin of the first harvest, the content of ferulic acid reaches 20%, in the resin of the second harvest – 10-15%, and in the resin of the third harvest the amount of ferulic acid – more than 5%.

5 Conclusion

It has been established that the proposed HPLC method is fast, simple, accurate, specific and reliable for the determination of ferulic acid. The mobile phase used to develop the method effectively dissolves ferulic acid, therefore, the developed method can be used to analyze ferulic acid in plant extracts. Statistical analysis proves that the developed method is reproducible. The method is economical because it uses a very small number of mobile phases, and the sample purification procedure associated with it is minimal. The developed HPLC method can be widely used to control the quality of Ferula tadshikorum resin and formulations containing it.
Thus, a modern HPLC qualitative and quantitative method for determining ferulic acid in the resin of the roots of the Ferula tadshikorum plant growing in Uzbekistan has been developed.

**References**

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