Post marketing surveillance of two St. John's wort and four liquorice products in Iran's market

S. Esmaeili*, F. Naghibi and M. Mosaddegh

Traditional Medicine and Materia Medica Research Center (TMRC), School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, I.R.Iran.

Abstract

With the tremendous expansion in the use of herbal medicine worldwide, safety and efficacy as well as quality control of herbal medicine have become important concerns for both health authority and public. In this paper, the analysis of two St. John's wort (*Hypericum perforatum*) products and four liquorice (*Glycyrrhiza glabra*) products by spectrophotometric and HPLC methods, is explained. Moreover chromatographic fingerprinting was done by TLC scanner. Hypericin was chosen as the marker of St. John's wort products and 18 β-glycyrrhetic acid was chosen as the marker for post marketing survey of four liquorice commercial formulations. The content of total hypericins that can be expressed as hypericin exist in the sample was 0.008% (mg/100mg) in product A/1 and 0.01% in product B/2. According to the analyses, the content of 18 β-glycyrrhetic acid in the samples were between 0.002-0.05% (mg/100mg). It is suggested that manufacturers should commit to proper quality control procedures and ensure that label claims for content and dosage are accurate and realistic.

Keywords: *Hypericum perforatum; Glycyrrhiza glabra;* Post marketing surveillance

INTRODUCTION

The past decades has seen a significant increase in the use of herbal medicines, among them St John's wort and liquorice products. The evaluation of active components of herbal drugs as well as the quality control of chemical drugs began to play an important role in the pharmaceutical and cosmetic industries. Standardization of herbal products has become a means for assuring or attempting to assure the quality of herbal preparations. There are inherent difficulties in trying to apply pharmaceutical current good manufacturing practices to botanical products. The problems are due to variability within the same plant materials, from grower to grower and crop to crop. Besides, variability may occur in harvesting and post harvest handling.

*Corresponding author: Dr. S. Esmaeili
Tel. 0098 21 88776027, Fax. 0098 21 88776027
Email: sesmaeili@tmrc.org

Moreover, herbals have multiple constituents including active, inactive unknown compounds and elements which are dietary rather than therapeutic, so that standardization become more difficult. Moreover, monitoring of herbal medicines stability providing reference standards are more difficult than that of chemically synthesized medicines. Spectrophotometric and chromatographic techniques have been used for a long time to evaluate single chemical entity of drug substances. The use of chromatographic techniques for herbal drugs tends to focus on identification and assessment of the stability of the constituents observed by chromatography. Chromatographic techniques such as high performance liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography (GC) and capillary
electrophoresis (CE) have been used for identity tests. Spectroscopic methods such as infrared (IR), nuclear magnetic resonance (NMR) and ultra violet-visible (UV-Vis) may also be used for fingerprinting (1). The British Herbal Pharmacopoeia has had an emphasis on using TLC profiles to characterize herbal materials, relying on the use of different spraying reagents and TLC profiles to identify characteristic and active principles of herbal materials (2). Chromatographic fingerprinting of poly herbal formulations has also been reported. HPLC have also been used to analyze herbal formulations (3). A method for identification and, where possible, assay of the plant preparation should be developed. If identification of an active principle is not possible, it should be sufficient to identify a characteristic substance to ensure consistent quality of the preparation (4). However, it should only serve for internal batch control. Single or multiple markers can be used to ensure that the concentration and ratio of components in an herbal mixture are present in reproducible levels in raw materials, manufacturing intermediates, and in the final dosage forms. In this manner, multiple markers or chromatographic methods give information assisting quality control and assuring batch to batch consistency.

In Iran more than 110 herbal medicinal products have been licensed for production by the Ministry of Health and Medical Education. Post marketing survey is necessary to assure the quality and safety of herbal products. Traditional Medicine and Materia Medica Research Center has been appointed by Ministry of Health, department of food and drug for doing this study on most consumed herbal drugs in the market. In this paper, post marketing survey of six products of two medicinal plants (St. John's wort and liquorice), widely used medicinally in Iran, is reported.

MATERIALS AND METHODS

St. John's Wort

The following procedure was used for hypericin measurement. In a 100 ml round-bottomed flask, 0.8 g of powdered drug, 60 ml of a mixture of 20 volumes of water and 80 volumes of tetrahydrofuran were introduced. The mixture was boiled in a paraffin-bath at 70 °C under a reflux condenser for 30 min. The supernatant was centrifuged (2 min at 700 g) and decanted into a 250 ml flask. The residue was taken up with 60 ml of above mentioned solvent and was heated again under a reflux condenser for 30 min. The supernatant was decanted after centrifugation and evaporated to dryness. The dried extract was dissolved in 15 ml of methanol with the aid of ultrasound and and diluted to 25 ml using a measuring flask. 10 ml of the extract was centrifuged and filtered through a syringe filter (0.2 µm). The first two milliliters of the filtrate was discarded. 5 ml of the filtrate was introduced into a measuring flask and was diluted to 25 ml with methanol. The absorbance of the test solution was measured for hypericin which is related to total hypericins at 590 nm (4). For qualitative study, 10 µl test solution and 5 µl of the reference solution were applied on silica plate as 10 mm bands and developed in a mixture of 100 volumes of ethyl acetate, 11 volumes of formic acid, 11 volumes of acetic acid and 26 volumes of water. The plate was allowed to dry at 100 to 105 °C for 10 min. The plate was sprayed with a 10 g/l solution of natural product reagent: polyethylene glycol reagent. The fingerprint of samples was obtained by TLC scanner at 365 nm (5,6).

Liquorice

Sample preparation

2 g of liquorice products was dissolved in 40 ml of methanol. The mixture was shook for 30 min. The supernatant was centrifuged (5 min at 700 g) and decanted.
The residue was taken up with methanol and decanted after 30 min, repeated twice. The supernatants were added together and evaporated to a concentrated solution (0.2 mg/ml). Then it was filtered through a syringe filter (0.2 μm) and analyzed with HPLC.

**Chromatographic analysis**

HPLC chromatograms were obtained using Shimadzu HPLC system with a 20 μl sample loop. The HPLC analysis was completed using a C-18 reversed phase column (VP-ODS, 250 × 4.6 mm). The column effluent was monitored with a variable wavelength photodiod-array detector (SPD-10A) which has the ability to scan from 200-800 nm. The detector was connected to a computer and the data were analyzed by class VP software. Determination of 18 β-glycyrrheticin acid was done by using acetonitril/phosphoric acid 85 % (3:1, pH=2.5) at flow rate of 0.6 ml/min (0-8 min), 0.4 ml/min (8-20 min). The detector wavelength was adjusted to 230 nm (7).

For TLC fingerprint 10 μl of the test solutions and 5 μl of the standard solution was applied. Development was carried out over a path of 15 cm by a mixture of petroleum ether/benzen/ethylacetate/acetic acid (20/40/14/1). The plate was allowed to dry for 10 min followed by spraying with anisaldehyde/sulphu-ric acid reagent. The fingerprinting of samples was obtained by TLC scanner at 258 nm (6).

**Standard curve preparation**

Standard solutions of 18 β-glycyrrheticin acid were prepared by weighing a known quantity and creating standards by serial dilution with methanol, resulting in final concentrations of 1, 0.25, 0.0625, 0.0156, 0.0039 mg/ml. A blank solution was also prepared with methanol. Each set of standards and a blank was analyzed chromatographically. The standard curves, each run in triplicate, were prepared. The peak area was recorded and standard curves were constructed by linear regression (0.9992) of peak area and concentration.

**RESULTS**

Hypericin was chosen as a marker for post marketing survey of St. John's wort products. The spectrophotometric and TLC scanner fingerprints showed that all commercial products contained hypericin. The amount of hypericin was determined in St. John's wort products (Table 1). Total content of hypericin products A/1 and B/2 were measured as 0.008% and 0.01% (mg/100mg), respectively. 18 β-glycyrrheticin acid was chosen as the marker for post marketing survey of four liquorice commercial formulations. Stacked plots of HPLC chromatograms are shown in Fig. 1. 18 β-glycyrrheticin acid is clearly observed at 10.4 min in the chromatogram of standard which matches

**Table 1.** Tabulated data for products purchased in Iran

<table>
<thead>
<tr>
<th>Company/Product</th>
<th>Description of label</th>
<th>Active compound</th>
<th>Measured active compound (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/1</td>
<td>St. Johns wort tablet, 0.2 % hypericin</td>
<td>Hypericin</td>
<td>0.008</td>
</tr>
<tr>
<td>B/2</td>
<td>St. Johns wort drop, 0.2 % hypericin</td>
<td>Hypericin</td>
<td>0.010</td>
</tr>
<tr>
<td>C/3</td>
<td>Liquorice powder (5% root)</td>
<td>18 β-glycyrrheticin acid</td>
<td>0.004</td>
</tr>
<tr>
<td>D/4</td>
<td>Liquorice tablet (5% extract)</td>
<td>18 β-glycyrrheticin acid</td>
<td>0.002</td>
</tr>
<tr>
<td>C/5</td>
<td>Liquorice tablet (400 mg extract)*</td>
<td>18 β-glycyrrheticin acid</td>
<td>0.050</td>
</tr>
<tr>
<td>C/6</td>
<td>Liquorice tablet (380 mg extract)*</td>
<td>18 β-glycyrrheticin acid</td>
<td>0.030</td>
</tr>
</tbody>
</table>

* with decreased glycyrrhizin
% mg/100mg in product
with commercial formulations of liquorice. The HPLC and TLC scanner fingerprints show that all commercial products contained 18 β-glycyrrhetinic acid. The amount of 18 β-glycyrrhetinic acid was determined in each liquorice product (Table 1). According to the analyses, the content of 18 β-glycyrrhetinic acid in the samples are between (0.002-0.05% mg/100mg). The results of the recoveries were 99.60%–102.81% with relative standard deviations between 0.01% and 1.58%, which were in acceptable ranges. The relative standard deviation of repeat-ability test was also acceptable (2.96%).

![HPLC Chromatograms](image)

**Fig. 1.** Stacked plots of HPLC chromatograms of the 18 β-glycyrrhetinic acid (St) and a commercial liquorice product

**DISCUSSION**

The amount of hypericin measured in the products does not match with the claimed amount of hypericin in the brochure. It is not easy to figure out if the low amount of the active principle is due to the raw plant material used for drug manufacturing or incorrect determination of the product expiration date. It is noteworthy that the amount of the measured hypericin in the products is in effective dose for hypericin as mentioned in commission E monograph (8).

Unfortunately no measurable data of active component exist on the label of four liquorice products available in the market. The reason may be the absence of therapeutic dose in the existing references. Therefore, there is no possibility to match the obtained result with the brochure.

From these experiments it can be concluded that:

1) The therapeutic range of medicinal plants for specific diseases should be determined either by local authority or international herbal pharmacopoeia.

2) Product labels and package inserts should be understandable to the consumer or patient. The package information should include all necessary information on the proper use of the product such as quantitative list of active ingredient(s), dosage form and indication as is suggested in the WHO guidelines (4).

3) If it is believed that the quality, safety and efficacy of herbal medicines is the consumer’s right, therefore monitoring and evaluation of herbal medicine after marketing is necessary to be carried out, continuously.

**ACKNOWLEDGEMENT**

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