DETERMINATION OF QUERCETIN IN LOTUS LEAVES EXTRACT AND GLYCYPHRHIZIN IN LIQUORICE ROOTS EXTRACT BY SPECTROFLUORIMETRIC METHODS

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ARTICLE INFO

Article history
Received 15/10/2014
Available online
30/11/2014

Keywords
Quercetin,
Glycyrrhizin,
Spectrofluorimetry,
Extracts.

ABSTRACT
A simple, accurate, sensitive and reproducible spectrofluorimetric method has been developed for the analysis of quercetin in lotus (Nelumbo nucifera) leaves extract and glycyrrhizin in liquorice (Glycyrrhiza glabra) root extract. Quercetin and glycyrrhizin show strong fluorescence in methanol having excitation wavelength at 242nm and 272nm respectively. The emission wavelength of quercetin and glycyrrhizin were obtained 515nm and 545nm respectively. The calibration curves for quercetin and glycyrrhizin were linear in the range from 10-70ng/ml and 100-600pg/ml respectively. The proposed methods were statistically validated and successfully applied in extracts. The limit of detection for glycyrrhizin and quercetin were found to be 0.28ng/ml and 4.65ng/ml respectively. The limit of quantification for quercetin and glycyrrhizin were found to be 0.83ng/ml and 14.11pg/ml respectively. The percentage recovery was found to be in the range of 98% and 101%.

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INRODUCTION

Glycyrrhiza glabra (Fabaceae), also known as liquorice and sweet wood, is native of mediteeanean and certain areas of Asia. \(^{[1]}\) In modern medicine, liquorice extracts are often used as a flavouring agent to mask bitter taste in preparation, and as an expectorant in cough and cold preparations. Glycyrrhizin, active constituent of liquorice has anti-carcinogenic, anti-inflammatory activity and significant anti-oxidant and hepatoprotective properties. \(^{[2,3]}\) Different analytical methods have been reported for the determination of Glycyrrhizin in different verities of Glycyrrhiza roots, which involved methods using, spectrophotometry, RP-HPLC with UV detection \(^{[4]}\), HPLC with DAD \(^{[5]}\), HPTLC \(^{[6]}\), ion pair HPLC \(^{[7]}\), etc. Some groups have described HPLC methods for the analysis of glycyrrhizin in biological fluids. \(^{[8]}\)

Nelumbo nucifera Gaertn. (Nymphaceae) is a large aquatic herb with stout creeping and yellowish white colored rhizome. Leaves, flowers, roots, embryos, rhizomes, and seeds of N. nucifera has been claimed to possess various medicinal values and have pharmacologic properties, including hepatoprotection, anti-oxidant activity, antipyretic effects, and prevention of atherosclerosis and fatty liver. \(^{[9]}\) The leaves of N. nucifera are bitter, sweet and neutral. It is known for cleaning heat, resolving summer heat and stop bleeding. \(^{[10]}\) Quercetin is an important bioflavonoid constituent in lotus leaves has antidepressant activity, hepatoprotective activity, anti tumor activity, DNA protection, anti-inflammatory action, and cardioprotective activity, etc. \(^{[11,12,13]}\) Different analytical methods have been also reported for the determination of quercetin which involved methods using, Spectrophotometry \(^{[14]}\), RP-HPLC with UV detection \(^{[15]}\), HPTLC \(^{[16]}\), GC-MS \(^{[17]}\) and also in biological samples \(^{[18]}\).

The aim of this study was to develop spectrofluorimetric methods which are less tedious, more rapid and more sensitive than the previously reported methods for estimating quercetin and glycyrrhizin. This selective fluorimetric assays are simple and sensitive enough to be useful for determining these active constituents in herbal extracts as well as in herbal preparations without any pre sample treatment.

MATERIALS AND METHODS

Instrumentation:

All fluorescence measurements were done on a spectrofluorimeter RF-5301 PC (Shimadzu, Japan), with non fluorescent quartz cell of 1 cm path length. Data acquisition was performed using RFPC software version 2.04. Quercetin and glycyrrhizin show strong fluorescence in methanol having excitation wavelength at 242nm and 272nm respectively. The emission wavelength of quercetin and glycyrrhizin obtained 515nm and 545nm respectively. The scan speed is super and slit width is 5nm.

Materials:

Glycyrrhizin (95%) and quercetin (90%) were purchased from Sigma Aldrich. AR grade methanol (Spectrochem) was used as a solvent. Dried roots of Glycyrrhiza glabra were purchased from the local stores. The fresh leaves of Nelumbo nucifera were collected from the local garden of Vadodara. Both the plant materials were identified by Dr. P.S. Nagar, Asst. Prof., Botany Department, The M. S. University of Baroda. The voucher specimens (Bot/20214/aut) of the herbs have been deposited in the Pharmacy department, The M.S. University of Baroda.

Extract preparation:

Lotus leaf powder was defatted with petroleum ether (60-80º) then extracted with alcohol water mixture (80:20) using successive solvent extraction procedure. Then marc was filtered and concentrates the filtrate. The semi solid material was dried using lyophilisation. Liquorice root powder was defatted with petroleum ether (60-80º) then extracted with water using successive solvent extraction procedure. Then marc was filtered and by concentrating the filtrate we get dry extract.

Standard solutions and calibration:

Standard stock solutions of quercetin and glycyrrhizin (1000µg/ml) were prepared by dissolving 10 mg of pure drug in 10 ml methanol. Appropriate and accurate aliquots of the stock solutions were transferred to 10 ml calibrated flasks and diluted up to the volume with methanol in the range of 10-70ng/ml for quercetin and 100-600pg/ml for glycyrrhizin.

Sample preparation:

10 mg of each extract was dissolved in 10 ml methanol individually. The solutions were sonicated for 15 min and were filtered through whatman filter paper No. 40. Further dilutions were made in methanol.

Measurement of fluorescence:

Quercetin and Glycyrrhizin showed strong fluorescence in methanol. The excitation wavelength for quercetin and glycyrrhizin were 242nm and 272nm respectively. The excitation wavelengths were kept constant and the emission wavelength were scanning in the range of 450 to 600nm for quercetin and 220 to 770nm for glycyrrhizin.
RESULTS AND DISCUSSION
Validation of method:
The proposed method was validated as per ICH guidelines for linearity, precision, accuracy, sensitivity and robustness. [19]

Linearity and range:
The emission wavelength of quercetin and glycyrrhizin were obtained 515nm and 545nm respectively. Linearity was evaluated by analyzing a series of standard solutions of six different concentrations in the range of 10-70ng/ml (Y= 1.198x + 67.033; correlation coefficient R^2= 0.9997) for quercetin and 100-600pg/ml (Y= 0.0993x + 126.24; correlation coefficient R^2= 0.9979) for glycyrrhizin. The overlain spectra over the linearity range are shown in figure 1 and figure 2.

![Figure 1: Overlain spectra of Quercetin in methanol.](image1)

![Figure 2: Overlain spectra of Glycyrrhizin in methanol.](image2)
Accuracy:
Recovery studies were done at three different levels. The preanalyzed samples were spiked with 80%, 100% and 120% of the standard markers were reanalyzed by the proposed method. The estimation was made in triplicate. Percentage recovery was calculated from the amount of drug found in solution. The percentage recovery studies revealed that the recovery levels lie between 98% and 101%. Recovery results demonstrated that the proposed method was unaffected in the presence of other extracts and thus highly accurate as shown in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quercetin (λ&lt;sub&gt;em&lt;/sub&gt;=515nm)</th>
<th>Glycyrrhizin (λ&lt;sub&gt;em&lt;/sub&gt;=545nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration range (µg/ml)</td>
<td>10-70ng/ml</td>
<td>100-700pg/ml</td>
</tr>
<tr>
<td>Detection limit (µg/ml)</td>
<td>0.28ng/ml</td>
<td>4.65pg/ml</td>
</tr>
<tr>
<td>Quantitation limit (µg/ml)</td>
<td>0.83ng/ml</td>
<td>14.11pg/ml</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = y = 1.4474x + 169.93</td>
<td>y = 0.0993x + 126.24</td>
</tr>
<tr>
<td>Correlation coefficient (R²)</td>
<td>R² = 0.9989</td>
<td>R² = 0.9979</td>
</tr>
<tr>
<td>Accuracy (% recovery ± standard deviation)</td>
<td>80% 98.77 ± 0.21</td>
<td>100 % 99.14 ± 0.37</td>
</tr>
<tr>
<td>Precision (%RSD)*</td>
<td>Intraday 0.81</td>
<td>Interday 1.59</td>
</tr>
<tr>
<td></td>
<td>Interday 1.59</td>
<td>Interday 1.44</td>
</tr>
</tbody>
</table>

*Mean value of six determinations

Precision:
Precision was estimated by the determination of the repeatability of the method. Repeatability was assessed using three determinations at each of three different concentrations (covering the specified range of the method), in a day for intra-day precision and on three different days for inter-day precision. Both intra-day as well as inter-day precision were carried out, showed that %RSD (Relative Standard Deviation) is less than 2.0.

Detection and Quantitation limits:
Calibration curves were repeated three times and the SD (Standard Deviation) of the intercepts was calculated. According to ICH recommendation (1966), the approach based on the SD of the response and the slope was used for determining the limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were measured as follows.
LOD = 3.3*SD/slope of calibration curve
LOQ = 10* SD/slope of calibration curve
SD = standard deviation of intercepts
The theoretical values were assessed practically. The detection limits were found to be 0.28ng/ml and 0.83ng/ml for quercetin and 4.65pg/ml and 14.11pg/ml for glycyrrhizin.

Stability:
The standard and sample solutions of quercetin and glycyrrhizin in methanol were stored at room temperature for 72 hrs and 5°C in refrigerator for 5 days. The solutions were found to be stable and no changes in fluorescence were observed.

Specificity:
According to ICH document for specificity, the method is specific when the results are unaffected by presence of other constituents in the extracts, which can be observed from the results of recovery of extracts.

Analysis in extracts:
The proposed spectrofluorimetric was applied to the determination of quercetin in hydro alcoholic extract of *Nelumbo nucifera* leaves and glycyrrhizin in aqueous extract of *Glycyrrhiza glabra*. Satisfactory results were obtained which were in good agreement. The results are shown in Table 2.
Table 2: Percent yield of quercetin and glycyrrhizin (%w/w) in lotus leaves and liquorice roots extract respectively.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Marker compound measured</th>
<th>% Yield of marker*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lotus leaves extract</td>
<td>Quercetin</td>
<td>5.58</td>
</tr>
<tr>
<td>Liquorice root extract</td>
<td>Glycyrrhizin</td>
<td>9.17</td>
</tr>
</tbody>
</table>

*Found from regression equation stated in table 1

CONCLUSION

The spectrofluorimetric method developed for quercetin and glycyrrhizin are valid with respect to linearity, sensitivity, accuracy, reproducibility and precision. The developed method was found to be accurate, precise, reproducible and stable, which indicated that this method can be used for routine quality control of quercetin and glycyrrhizin in commercial herbal preparation. Owing to the detectability and sensitivity of the drug in nano and pico range, as an added benefit of spectrofluorimeter, the method can also be used for bioanalytical purpose.

Authors’ Statements

Competing Interests

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Authors are thankful to the University Grants Commission, New Delhi for providing Junior Research Fellowship.

REFERENCES


