Development and Validation of UV Spectrophotometric Method for the Estimation of Kaempferol in Kaempferol: Hydrogenated Soy PhosphatidylCholine (HSPC) Complex

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ABSTRACT

Introduction: Kaempferol (3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a natural flavonoid belongs to a subcategory of flavonol family. The kaempferol – hydrogenated soy phosphatidylcholine (HSPC) complex was obtained by refluxing and freeze drying method. Ultra violet (UV) – visible spectrophotometric method has been developed for the determination of kaempferol in kaempferol – HSPC complex. Objective: A validated UV – visible spectrophotometric method for determination of kaempferol in Kaempferol – HSPC complex. Materials and Methods: The Kaempferol – HSPC complex (Phytosomes) were prepared by dissolving both kaempferol and HSPC in 1, 4 – dioxane for refluxing up to 2 h and freeze dried. The spectrophotometric detection of kaempferol was done at absorption maximum ($\lambda_{\text{max}}$) of 365 nm and 265 nm using methanol as solvent. The developed method was validated as per ICH guidelines. Result: The kaempferol content in Kaempferol – HSPC complex was found to be 79.32% and 79.19% at 365 nm and 265 nm. Kaempferol demonstrated good linearity in concentration range of 2-12 $\mu$g/ml ($r^2 > 0.99$) at 365 nm and 2-14 $\mu$g/ml ($r^2 > 0.99$) at 265 nm. Precision and mean recoveries were found to be in the range of (%RSD 0.0957 and 0.0580) and (% RSD 0.1461 and 0.0959) and 99.70% and 91.85% at 365 nm and at 265 nm. Limit of detection and limit of quantification were found to be (0.015 $\mu$g/ml and 0.0191 $\mu$g/ml) and (0.0457 $\mu$g/ml and 0.0579 $\mu$g/ml) respectively. Conclusion: The developed method was found to be simple, specific, economic, reliable, accurate, precise, reproducible and used as a quality control tool for analysis of Kaempferol.

Keywords: Freeze drying, hydrogenated soy phosphatidylcholine, Kaempferol, method validation, UV – visible spectrophotometer

INTRODUCTION

Kaempferol (3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), a yellow compound with a low molecular weight (MW: 286.2 g/mol), is a common natural flavonoid which representative of the subcategory of flavonol. This flavonoid is abundant in many plant-derived foods and traditional medicine. It is widely distributed in the plant kingdom such as onion, kale, endive and tea along with formed as secondary metabolites through the phenylpropanoid biosynthetic pathway. Although it has broad spectrum importance, researcher have been isolated it from different plants like Asclepias cyriaca L., Crocus sativus L., Cassia alata L., Capsella bursa-pastoris L., Leptadenia pyrotechnica L., and broccoli and also reported its various pharmacotherapeutic effects like anticancer, antioxidant, anti-inflammatory and hepatoprotective etc. kaempferol revealed low to moderate absorption, which results poor bioavailability ~2%. It is hydrophobic in nature and freely soluble in methanol, 1, 4 – dioxane, Ethanol and dimethylformamide. In a wide range of HPLC and HPTLC techniques were reported for estimation of kaempferol in extracts, ginkgo biloba tablets, phytosome formulation (Kaempferol – phospholipids complex) at around 360 nm. Literature survey revealed that no simple UV – visible method has

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been reported for estimation of kaempferol in phytosome formulations.

**MATERIALS AND METHODS**

**Materials**

Phospholipion 90 H (Hydrogenated Soy Phosphatidyglycholine) was received as gift sample from lipoid GmbH, Germany. Kaempferol standard was purchased from imani international group (Pharmaceutical) Co., Ltd, China. All other chemical used were of either pharmaceutical or analytical grade.

**Preparation of kaempferol – HSPC complex**

The complex was prepared with Kaempferol and HSPC at a molar ratio of (1:1 w/w). Weighed amount of Kaempferol (mol. wt., 286.2) and HSPC (mol. wt., 790) were taken in 100 ml round bottom flask and 20 ml of 1, 4-dioxane was added. The mixture was refluxed at a temperature not exceeding 50°C for 2 h to get a clear solution. The obtained solution was freeze dried. The resultant Kaempferol complex (yield 92% w/w) was kept in an amber colored glass bottle flushed with nitrogen and stored at room temperature.

**Method development**

**Instrument**

Double beam UV – visible spectrophotometer (JASCO V-630).

**Preparation of standard stock solution**

To find out the wavelength maximum absorption ($\lambda_{\max}$) of kaempferol, the standard stock solution (1000 $\mu$g/ml) of kaempferol was prepared by weighing accurately 5 mg of pure drug into 5 ml volumetric flask and dissolved with a minimum quantity of methanol and dissolved with a minimum quantity of methanol and final volume was made up to mark with methanol.

**Preparation of working stock solution**

The working stock solution of kaempferol (100 $\mu$g/ml) was prepared by diluting 1 ml of standard stock solution to 10 ml with methanol.

**Selection of ($\lambda_{\max}$)**

The working stock solution was further diluted with methanol to get (10 $\mu$g/ml) concentration (1ml to 10 ml). This solution was scanned between the wavelength regions of 200-400 nm against methanol as blank. The UV spectra were shown in [Figure 1] and absorption curve showed two characteristics absorption maxima at 365 nm and 265 nm. Hence both ($\lambda_{\max}$) were selected for analysis of kaempferol.

**Validation of the analytical method**

The developed analytical method was validated as per ICH guidelines and prepared different series of diluted solutions (2-12 $\mu$g/ml and 2-14 $\mu$g/ml) were analyzed for linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ).

**Linearity**

The linearity for this method at various concentrations of the range between 2-12 $\mu$g/ml and 2-14 $\mu$g/ml were analyzed at 365 nm and 265 nm [Tables 2, 3]. The absorbance v/s concentration plot for kaempferol was found to be linear in [Figures 2, 3].

**Figure 1.** UV spectrum of kaempferol in methanol.
Precision
The precision studies were performed by repeatability (intra-day) and intermediate precision (inter-day). The intra-day precision was determined by analyzing 10 μg/ml of kaempferol at three different time points within a day. Inter-day precision was determined by analyzing same concentration of solutions at three different points for three days, and average % RSD was calculated.24

Accuracy
Accuracy is the closeness of the test results obtained by the method to the true value. Accuracy was evaluated by performing recovery studies by spiking pre – analyzed sample solutions of of kaempferol with three different concentrations of 8 μg/ml, 10 μg/ml and 12 μg/ml and % recovery was computed.24

LOD and LOQ
LOD is the lowest concentration of analyte in a sample that an analytical process can reliably differentiate from background levels, and LOQ is the lowest concentration of the calibration curve that can be measured with an acceptable accuracy and precision. In this method, LOD and LOQ were based on a standard deviation of the response and slope of the calibration curve using following equations.24

\[
\text{LOD} = 3.3 \times \frac{S}{M}; \quad \text{LOQ} = 10 \times \frac{S}{M}
\]

Where S is the standard deviation of the absorbance of the sample and M is the slope of the calibration curve.

RESULT AND DISCUSSION
The kaempferol was found to be soluble in methanol. The absorption maximum (λ_max) was found to be 365 nm and 265 nm as shown in [Figure 1]. The content of kaempferol in Kaempferol – HSPC complex was found to be 79.32% and 79.19% at 365 nm and 265 nm. The good linearity was found to be within concentration range of 2-12 μg/ml.
The developed method was found to be simple, specific, economic, reliable, accurate, precise, and reproducible [Tables 2, 3] with correlation coefficient ($r^2$) >0.99 and regression equation of the curve was found to be $y = 0.0768x - 0.0148$ at 365 nm and $y = 0.0654x + 0.0307$ at 265 nm. The precision (intra-day and inter-day) data represents good reproducibility with % RSD lower than 2.0% which assured that method is precise.

Table 4: Results of intra- and inter-day precision (n=6)

<table>
<thead>
<tr>
<th>$\lambda_{max}$</th>
<th>Concentration (μg/ml)</th>
<th>Intra-day Absorbance (nm)±SD</th>
<th>% RSD</th>
<th>Inter-day Absorbance (nm)±SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>365 nm</td>
<td></td>
<td>265 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.7796±0.0002</td>
<td>0.0957</td>
<td>0.7796±0.0002</td>
<td>0.1461</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.7807±0.0014</td>
<td></td>
<td>0.7796±0.0002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.7983±0.0005</td>
<td></td>
<td>0.7993±0.0005</td>
<td></td>
</tr>
<tr>
<td>265 nm</td>
<td>10</td>
<td>0.7166±0.0003</td>
<td>0.058</td>
<td>0.7166±0.0003</td>
<td>0.0959</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.7203±0.0006</td>
<td></td>
<td>0.7172±0.0006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.7115±0.0002</td>
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</table>

RSD: Relative standard deviation

Table 5: Recovery study of kaempferol

<table>
<thead>
<tr>
<th>$\lambda_{max}$</th>
<th>Level of recovery (%)</th>
<th>Amount spiked recovery (μg/ml)</th>
<th>Amount recovered (μg/ml)</th>
<th>Recovery (%)</th>
<th>Mean recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>365 nm</td>
<td>80</td>
<td>8.75</td>
<td>98.12</td>
<td>99.70</td>
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<tr>
<td></td>
<td>100</td>
<td>10.15</td>
<td>101.50</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>120</td>
<td>11.94</td>
<td>99.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>265 nm</td>
<td>80</td>
<td>7.17</td>
<td>89.20</td>
<td>91.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>9.35</td>
<td>101.50</td>
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<tr>
<td></td>
<td>120</td>
<td>11.09</td>
<td>92.42</td>
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</table>

Table 6 Validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
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<tbody>
<tr>
<td>$\lambda_{max}$</td>
<td>365 nm</td>
</tr>
<tr>
<td>Beer’s law range (μg/ml)</td>
<td>2-12</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.9993</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.0768</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.0148</td>
</tr>
<tr>
<td>Accuracy</td>
<td>98.12-101.5%</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td>0.0957</td>
</tr>
<tr>
<td>Intra-day</td>
<td></td>
</tr>
<tr>
<td>Inter-day</td>
<td>0.1461</td>
</tr>
<tr>
<td>LOD (μg/ml)</td>
<td>0.015</td>
</tr>
<tr>
<td>LOQ (μg/ml)</td>
<td>0.0457</td>
</tr>
</tbody>
</table>

RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

Acknowledgment

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References