ANALYTICAL METHOD DEVELOPMENT OF NUTRACEUTICAL:

UMBELLIFERONE

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ABSTRACT
Coumarins, an old class of compounds, are naturally occurring benzopyrene derivatives. A lot of coumarins have been identified from natural sources, especially green plants. Coumarins have attracted intense interest in recent years because of their diverse pharmacological properties. Phytochemical investigation on the fruit of Aegle marmelos (Rutaceae) has resulted in the development of analytical method for Umbelliferone. The present study was undertaken to develop a validated, rapid, simple and low-cost ultraviolet (UV) spectrophotometric method for estimating Umbelliferone in dosage preparations. UV spectrophotometric analysis was performed spectrophotometrically at a pre-determined λ_max of 324 nm with distilled water (using ethanol as co-solvent) as diluent/blank. The method was validated for linearity, accuracy, precision, reproducibility, and specificity as per International Conference on Harmonization (ICH) guidelines. The method was also used in the determination of the content of umbelliferone in formulations of umbelliferone. The regression data for the calibration plots exhibited good linear relationship (R² = 0.9991) over a concentration range of 0.5–6.5 μg/ml and the linear Regression equation was y=0.169 x + 0.023. Mean recovery accuracy was 96.9 %, which was not significantly different from the expected value (p = 0.05), while coefficient of variation (CV) for both intra-day and inter-day was < 7 %. The method was specific for umbelliferone in the presence of common excipients, and when it was applied to formulations, umbelliferone content was 97.89 ± 3.08 and 99.05 ± 1.99 %, respectively, of calculated claim. The proposed method gave good validation results and the statistical analysis performed proved that the method is precise, accurate and reproducible, and hence can be employed for routine analysis of umbelliferone in bulk and commercial formulations.

Keywords: Umbelliferone, Spectrophotometric method, Validation, Dosage formulations, Quality control.

INTRODUCTION
Plant derived phenolic coumarins might play a role as dietary antioxidants because of their consumption in the human diet in fruits and vegetables. Umbelliferone (7-hydroxycoumarin), a derivative of coumarin, is a benzopyrone in nature and it is
present in the fruits of *Aegle marmelos* Correa and *Anethum graveolens* L. umbelliferone has also been reported to have antioxidant properties. The parent compound coumarin has been reported to reduce blood glucose level. Umbelliferone has reduced blood glucose and lipid peroxidation and elevated antioxidants? Status in plasma and liver of STZ-diabetic rats. Umbelliferone (7-hydroxycoumarin) and its derivatives are suitable for use photoactive agents, sunscreen composition, fluorescent probe and dyes and used in the synthesis of drugs especially anticancer.

Umbelliferone (7-Hydroxycoumarin) was isolated from the fruit of *Aegle marmelos*, for which the studies indicated that it possessed many biological effects. To detect the quantity of umbelliferone in plant and medicinal preparation, several analytical methods have been developed during the last decade. High performance liquid chromatography and thin layer chromatography are most widely used. These methods were developed to determine higher concentration of umbelliferone in the samples. A more rapid, more sensitive and validated detection method is necessary[1].

In the present study, a simple UV spectrophotometric method was developed and validated as per International Conference on Harmonization (ICH) guidelines [2, 3]. After fully validation, the method was successfully applied to in-vitro study of umbelliferone. The novelty of include simple method with low limit of detection, good precision and high recovery.

**MATERIALS AND METHODS**

**Determination of wavelength of maximum absorption**

Pure umbelliferone (5 mg) was dissolved in 50 ml of distilled water using ethanol as co solvent and diluted to the 50 ml mark with the same solvent to obtain a 100 μg/ml umbelliferone solution. Ten millitres of this solution was further diluted to 100 ml with the same solvent to obtain a 10 μg/ml umbelliferone solution, which was scanned spectrophotometrically in the wavelength region 190 to 400 nm to determine the wavelength of maximum absorption [4, 5].

**Linearity study**

The 100 μg/ml umbelliferone solution, used for the wavelength of maximum absorption determination was employed as a stock solution for linearity study. Aliquots in the range of 1 to 10 ml of this solution were taken and diluted to 2 to 100 ml
differently with the distilled water to obtain different concentrations within the range 0.5 – 6.5 μg/ml and used for the linearity calibration plot.

**Intra-day precision study**

Aliquots (1, 2 and 3 ml) of the 100 μg/ml umbelliferone stock solution were taken and respectively diluted to 100 ml with the distilled water to obtain three concentrations of 1.0, 2.0 and 3.0 μg/ml, respectively. Triplicate absorbance measurements of each were made and the mean, standard deviation and RSD calculated\[6\].

**Inter-day precision study**

The selected concentrations for the intra-day precision study were again analysed the following day and the mean, standard deviation and RSD calculated

**Recovery accuracy study**

This study was carried out using microsphere containing pure umbelliferone, and common excipients including sodium alginate and calcium chloride. The microsphere (50 mg) was then transferred into a 500 ml volumetric flask. Distilled water with ethanol was then added, shaken for 15 min using a vortex mixer and diluted to the 500 ml mark with the same solvent. The mixture was filtered to obtain sample stock solution. This stock solution (1 ml) was further diluted to 100 ml with distilled water and then assayed for the content of umbelliferone using the proposed method with a solution containing 5 μg/ml of pure umbelliferone as standard for comparison. All analyses were carried out in triplicate\[7\].

**Specificity in the presence of excipients**

This test was carried out using common excipients including sodium alginate and calcium chloride. Dummy microsphere devoid of the pure umbelliferone were prepared as in the recovery study above, their absorbance reading at 324 nm taken and compared with both that of the blank (distilled water) and that obtained for the recovery study.

**Assay of content of umbelliferone in selected marketed brands**

This was carried out using the developed and validated method as follows.

**Sample preparation**

Accurately weighed microsphere, equivalent to 50 mg umbelliferone, was transferred into a 100 ml volumetric flask. An amount of distilled water (50 ml) was added, shaken for 15 min using a vortex mixer and diluted to the 100 ml mark with same
solvent. It was then filtered to obtain sample stock solution. One milliliter of the filtrate was further diluted to 100 ml with distilled water and then assayed for content of umbelliferone using the proposed method with a solution containing 5 μg/ml of pure umbelliferone as standard for comparison. All analyses were carried out in triplicate.

**Reference standard preparation**

Pure umbelliferone (5 mg) was accurately weighed and dissolved in 50 ml of distilled water using ethanol as co solvent. Out of this solution, 1 ml was further diluted to the 20 ml mark with the same solvent to obtain a 5 μg/ml umbelliferone standard solution. The absorbances of the sample preparation and reference standard solution were taken using distilled water as blank. The content of anhydrous umbelliferone in the microsphere was determined using Eqs 1 and 2.

\[
LH (\%) = \frac{(Ap \times Ws)}{(As \times Wp)} \times 100 \quad \ldots \quad (1)
\]


Hence, \( (L, \%w/w) = 0.976 \times LH \quad \ldots \quad (2) \)

Where L is the content of anhydrous umbelliferone;

\[
D = \frac{(L \times W20)}{(20 \times 0.5)} \quad \ldots \quad (3)
\]

Where D is % stated dose of anhydrous umbelliferone and W20 is the weight (g) of 20 tablets of generic sample.

**Statistical analysis**

Where applicable, results were expressed as mean ± SD and analysed statistically using Student t-test with the aid of Excel 2007. Differences were considered significant at the 95 % confidence limit.

**RESULTS AND DISCUSSION**

The wavelength of maximum absorption (\( \lambda_{max} \)) was 324 nm. The linearity parameter (Table 1) and the corresponding regression data, indicated excellent linear relationship (\( r^2 = 0.9993 \)) over the working concentration range (0.5 – 6.5 μg/ml). Table 2 presents the intra- and inter-day precision of the new method, confirming adequate sample stability and method reliability over a 24 h period. This is because for the three selected concentrations within the linearity range, the observed RSDs were all < 6 % and
calculated values of t were less than the tabulated value of t at 95% confidence limit. Mean analyte recovery of 98.11 % with RSD of 1.68 %, was not statistically different from the expected recovery as the calculated value of t (2.149) was substantially less than the tabulated value of 2.920 at the 95 % confidence level, thus indicating that the proposed method can satisfactorily be utilized to assay umbelliferone in dosage forms. This was confirmed when the new method was used to determine the percent absolute drug content of umbelliferone microsphere. The results indicated % label claims of 97.89 ± 3.08 and 99.05 ± 1.99 % respectively.

![Figure 1](image)

**Figure 1**
Calibration curve of umbelliferone using distilled water (ethanol as cosolvent) at $\lambda_{\text{max}}$ 324nm.

### TABLE 1: LINEARITY PARAMETER DATA

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>324</td>
</tr>
<tr>
<td>2</td>
<td>Beer’s law linearity range (µg/mL)</td>
<td>0.5-6.5</td>
</tr>
<tr>
<td>3</td>
<td>Regression equation</td>
<td>$y=0.169x+0.023$</td>
</tr>
<tr>
<td>4</td>
<td>Intercept (a)</td>
<td>0.023643</td>
</tr>
<tr>
<td>5</td>
<td>Slope (b)</td>
<td>0.169875</td>
</tr>
<tr>
<td>6</td>
<td>Correlation coefficient (r)</td>
<td>0.9991</td>
</tr>
<tr>
<td>7</td>
<td>N</td>
<td>12</td>
</tr>
</tbody>
</table>

www.pharmasm.com  IC Value – 4.01
TABLE 2: INTRA- AND INTER-DAY PRECISION DATA (N = 3, \( P = 0.05 \))

<table>
<thead>
<tr>
<th>Conc. (( \mu g/ml ))</th>
<th>Mean absorbance ± SD</th>
<th>Relative standard deviation (RSD, %)</th>
<th>Calculated value of t</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 1</td>
</tr>
<tr>
<td>1</td>
<td>0.201±0.011</td>
<td>0.189±0.015</td>
<td>0.378</td>
</tr>
<tr>
<td>2</td>
<td>0.413±0.009</td>
<td>0.409±0.014</td>
<td>0.943</td>
</tr>
<tr>
<td>3</td>
<td>0.516±0.008</td>
<td>0.499±0.013</td>
<td>0.478</td>
</tr>
</tbody>
</table>

* No significant difference between day 1 and day 2 mean values at 95 % confidence level considering the tabulated t value of 4.3000 at 4 degree of freedom

The developed method showed comparable accuracy. It was validated as per ICH guidelined and the results of the validation parameters were within acceptable limits. The observed linearity range fitted well Beer-Lambert’s law and the corresponding regression coefficient (\( r = 0.9991 \)) is an indication of a high degree of method sensitivity. Recovery accuracy was good; so also was the precision of the method since RSD (< 7 %) which is within the allowable limit of ≤15%. When the developed method was applied to new developed formulation, it demonstrated excellent reproducibility of the recovery accuracy and precision validation data; furthermore, the assay results were well within pharmacopoeial limits for tablets\(^5-7\).

CONCLUSION

The proposed UV spectrophotometric method is simple, rapid, selective, accurate, precise and highly sensitive. Therefore, it can be used for the determination of umbelliferone either in bulk or in their corresponding dosage forms without interference from commonly used excipients and related substances whose \( \lambda \)-max are not close to 324 nm.

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