Red flower oil is a popular remedy in China and Southeast Asia. It is used for the treatment of muscle and joint pain, sprains and bruising. It is prepared by blending several natural essential oils: principally wintergreen, clove, cinnamon, and turpentine plus other oils or herbal extracts in smaller quantities.

The most important chemical components in red flower oil are methyl salicylate (from wintergreen oil), eugenol (from clove or cinnamon), and α-pinene (from turpentine). This is recognized in the publication WS3-B-2699-97 from the Ministry of Health of the People’s Republic of China, which states that these three compounds must be quantified by a gas chromatography (GC) method before the product is approved for sale to the public. According to this document, the minimum contents of methyl salicylate and eugenol are 33.5% v/v and 38% v/v, respectively. Poor quality red flower oil typically has less eugenol, as clove and cinnamon essential oils are more expensive than wintergreen, and natural oil of wintergreen is more easily substituted by industrial methyl salicylate. Another possible source of poor quality is adulteration of the product by the addition of excess amounts of inert vegetable or paraffin oil.
Spectra were measured on a PerkinElmer® Spectrum™ GX FT-IR spectrometer using a ZnSe horizontal ATR accessory, co-adding 32 interferograms (for a measurement time of two minutes). The Spectrum 100 or 400 instruments and the 9-bounce diamond ATR accessory would be equally suitable for this application.

Due to the overlapping bands and the presence of unidentified components, PLS calibrations were used in preference to univariate regression. The calibration models were built using Spectrum QuAnT+ software. The full spectral range was used, with no pre-processing of the data apart from mean centering. In this case, the only inputs to the software were the spectra and concentrations; the default algorithm settings produced excellent results.

Results and discussion

Results of the PLS model development and validation are presented in Table 1 and Figure 1. There were no outlying samples that were judged to have a negative effect on the model; two samples with unusually low methyl salicylate both had high leverage, but low residuals, and the validation sample with low methyl salicylate was well predicted.

Experimental

Forty-eight commercial red flower oil samples were obtained from 9 manufacturers. Volume percentages of methyl salicylate, eugenol and pinene were determined by a GC method described in Reference 2. Twelve samples were set aside to use as a validation set, and the remaining 36 were used for calibration.

<table>
<thead>
<tr>
<th>Range (%)</th>
<th>Mean (%)</th>
<th>PLS factors</th>
<th>Cross-val SEP (%)</th>
<th>Val. SEP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl salicylate</td>
<td>21.9 - 73.4</td>
<td>56.5</td>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td>Eugenol</td>
<td>0 - 44.2</td>
<td>8.9</td>
<td>6</td>
<td>0.42</td>
</tr>
<tr>
<td>Pinene</td>
<td>0 - 32.7</td>
<td>15.0</td>
<td>6</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Figure 1. Cross-validation (top row) and independent validation (bottom row) results for the three red flower oil components.
The validation SEP values, which can be thought of approximately as the error standard deviations for predictions of new samples, are under 1% for eugenol and pinene and under 2% for methyl salicylate. This shows that FT-IR is capable of making a very precise measurement of the concentrations of the three most important constituents of red flower oil.

This full-spectrum approach relies upon the calibration set spanning the full range of variability expected to be present in the samples—the full ranges of concentrations of the main active ingredients, as well as all the other potential additives or diluents that are present in significant concentrations. Samples that have unexpected components will be automatically flagged by the software as outliers, and recommended for analysis by GC. These spectra can then be added to the PLS model so that, in future, similar samples can be predicted accurately by the IR method.

Conclusions

Infrared spectroscopy with ATR sampling can be used to obtain a complete, quantitative determination of the quality of red flower oil within two minutes. A PLS calibration against data obtained from GC measurements allows a very precise determination of methyl salicylate, eugenol and \( \alpha \)-pinene, so the quality of the product can be determined with respect to the specifications of the Pharmacopoeia.

References


3. Chen Jian-Bo, Zhou Qin, Sun Su-Qin, Ben Perston and Patrick Courtney Rapid Quality Control for the Traditional Chinese Remedy “Red Flower Oil” by FT-IR spectroscopy PerkinElmer 009319_01.