1. Purpose

To determine the total phenolic contents in tea, grape products and echiancea products, et al., by UV spectrometric method.

2. Materials

The following materials are needed to carry out the analysis:

2.1. Agilent 8543 UV/Vis Spectrometer, and Biochem. Anal. Software for Agilent ChemStation or equivalent.
2.2. UV/Vis Spectroscopy cells or equivalent.
2.3. Multi-Wrist® Shaker, Model 3589.
2.4. Ultrasonic cleaner (Sonicate) (Altech).
2.5. Balance, accurate to 0.0001 gram.
2.6. Ethanol, HPLC grade (FisherBrand, P/N A995-4).
2.7. Water, HPLC grade (FisherBrand, P/N W5-4).
2.8. Sodium carbonate HPLC grade (FisherBrand, P/N S495-500).
2.9. Folin-Ciocalteu’s Phenol reagent (SigmaBrand P/N F-9252).
2.10. Gallic acid standard
2.11. 50 mL and 100 mL of volumetric flasks. class A.
2.12. 1 mL Reusable Class B volumetric pipets.

3. Preparation of Sample

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3.1. Weigh the appropriate amount of materials (see Table I) into 25 mL and record the weight.

Table I.

<table>
<thead>
<tr>
<th>Sample description</th>
<th>Amount of Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3% crude materials</td>
<td>250 mg</td>
</tr>
<tr>
<td>4% powder extracts</td>
<td>150 mg</td>
</tr>
<tr>
<td>10% native extracts</td>
<td>80 mg</td>
</tr>
<tr>
<td>20% native extracts</td>
<td>20 mg</td>
</tr>
<tr>
<td>40% native extracts</td>
<td>10 mg</td>
</tr>
<tr>
<td>80% native extracts</td>
<td>5 mg</td>
</tr>
</tbody>
</table>

3.2. 15 mL of extraction solvent (40% ethanol) is added. Mix and then sonicate for about 30 min and shake about 10 min. Allow the extracts cool down to room temperature and fill to full volume with extraction solvent. Mix well.

3.3. Filtrate the extracts with No. 1 Whatman paper and get at least 10 mL of clear solution for UV test.

3.4. From clear extraction solution prepared from sample preparation, transfer 1 mL to 100 mL volumetric flask with 60-70 HPLC grade water. Swirl contents to mix. Add 5 mL of Folin-Ciocalteu’s phenol reagent and mix again. After 1 min and before 8 min, add 15 mL of sodium carbonate solution (20 g in 100ml), record the time as time zero, and mix again. Make volume up to 100 mL exactly with HPLC grade water. Stopper the flask and mix thoroughly by inverting it several times. After 2 hours (to within 1-2 min) record the UV absorption range at 550-850 nm and maximum absorbance about 760 nm. Use same solution without the extraction solution as blank solution.

4. Preparation of Standard

4.1. Accurately weigh 20 mg of gallic acid into 25 mL volumetric flask, and record the weight for standard. 15 mL 40% EtOH was added into 25 mL volumetric flask and sonicate until no solid was present in the flask. Allow the solution cool down to room temperature and fill to full volume with extraction solvent. Mix well. Dilute the standard solution to 2, 4, 8, 16 and 32 times, if necessary.
4.2. Transfer 1 mL of standard solution to 100 mL volumetric flask with 60-70 HPLC grade water. Swirl contents to mix. Add 5 mL of Folin-Ciocalteu’s phenol reagent and mix again. After 1 min and before 8 min, add 15 mL of sodium carbonate solution, record the time as time zero, and mix again. Make volume up to 100 mL exactly with HPLC grade water. Stopper the flask and mix thoroughly by inverting it several times. After 2 hours (to within 1-2 min) record the UV absorption range at 550-850 nm and maximum absorbance about 760 nm. Use same solution without the extraction solution as blank solution.

6. Calculation

Calculation of content of Total phenols in percent, is based on gallic acid standard.

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\text{Total Phenols \%} = \frac{A_{\text{sample}} \times W_{\text{standard}} \times 50}{A_{\text{standard}} \times W_{\text{sample}} \times 50} \times 100\%
\]