New simple spectrophotometric method for the determination of aescin from *aesculus hippocastanum*

Human heart is the most important muscle in the human body. The heart is a pump or two pumps in one. The right side receives blood from the body (Veins) and pumps it to the lungs. The left side (Arteries) does the opposite: it receives blood from the lungs and pumps it around the body. Veins, especially those in the legs, have to pump the blood “up hill” to the heart, against gravity. Inside the veins are one-way valves that help with pumping action and prevent blood from flowing backward. These valves allow blood to flow in only one direction, toward the heart. Varicose veins develop when the valves become weakened, damaged, or don’t work well. If not treated varicose veins cause aching, throbbing, fatigue and swelling. Severe cases lead to phlebitis, inflammation and ulceration. Aescin has been clinically proven to be beneficial for treatment of varicose veins, spider veins, hemorrhoids and related circulatory problems or "chronic venous insufficiency."

Abstract

Aescin is the major active principle from Aesculus hippocastanum (family Hippocastanaceae) the horse chestnut tree, a plant widely distributed all over the world because of its excellent resistance to environmental conditions. It is used in the treatment of varicose veins, spider veins, hemorrhoids and related circulatory problems or "chronic venous insufficiency". The proposed spectrophotometric method is based on the reduction of phosphomolybdotungstic mixed acid of the Folin-Ciocalteu (F-C) reagent by aescin in the presence of sodium carbonate giving rise to blue color product which could be measured at 720 nm. The method obeys Beer's law over the range of 8-60 μg ml⁻¹. Sandell's sensitivity and molar absorbptivity were 0.018 μg cm⁻² and 1.04 x 10⁴ mol⁻¹cm⁻¹ respectively. The color developed was stable up to 24 h. The methods can be successfully employed for the determination of aescin in presence of common pharmaceutical excipients.

Keywords: Aescin; Folin-Ciocalteu reagent; Aesculus hippocastanum; nutraceutical; spectrophotometry

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X.1. Introduction

Aescin is the major active principle from *Aesculus hippocastanum* (family *Hippocastanaceae*) the horse chestnut tree, a plant widely distributed all over the world because of its excellent resistance to environmental conditions. The horse chestnut grows in Northern India, Iran, Asia Minor, South-East Europe, from the Balkans to the Caucasus as well as in the USA [1]. Aescin is a natural mixture of triterpene saponins [2]. The aglycons are derivatives of protoascigenin acylated by acetic acid at C-22 and by either angelic or tiglic acids at C-21.

Aescin has been clinically proven to be beneficial for treatment of varicose veins, spider veins, hemorrhoids and related circulatory problems or "chronic venous insufficiency". Varicose veins not only are painful but on exposed body parts like legs and arms (particularly in women), are considered unattractive because of their bulging appearance. Hence, aescin is now being used in cosmeceutical preparations. The pharmacological profile of aescin has received significant contributions in recent years. At least three types of pharmacodynamic actions have been attributed to aescin: anti-oedematous properties; anti-inflammatory activities and venotonic properties. All of these appear to be due to a basic molecular mechanism, identified as a selective vascular permeabilization [3], allowing a higher sensitivity, for e.g. calcium channels, to molecular ions, resulting in increased venous and arterial tone [4]. These sensitizing effects to ions and other molecules, e.g. 5-HT, result probably in the enhanced

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Horse Chestnut (*Aesculus hippocastanum*)

A native of the Balkan peninsula (Greece-Albania), it is now cultivated in many countries for shade and ornament. Other members of the family are native trees and shrubs of the north temperate zones and of South America. It can reach 30 meters tall, and has striking candles of blooms in spring and early summer. Individual flowers have crumpled white petals with a yellow basal patch that changes to a dull red colour. The fruit has a lathery case covered with short pickles. The seed are used to play conkers. [www.wellness-gesund.info/heilpflanzen](http://www.wellness-gesund.info/heilpflanzen)
venous contractile activity, and as a consequence, in the anti-oedematous property of the molecule. Aescin is now, in fact, widely quoted in the literature as a pharmacological tool to assess the sensitivity of vascular tissues to different agonists in order to evaluate the mechanism of e.g. hypertension development in animal models [5].

A number of specific assays have been developed in order to quantitatively determine the aescin content of various products. The aescin content in ointments can be determined by TLC-densitometry, whereas an HPLC method has been developed for the separation and assay of aescin saponins in extracts and in pharmaceutical preparations [6]. A fingerprint of the aescin composition has been finally obtained by liquid chromatography-mass-spectrometry (LC-MS) using a thermospray (TSP) interface [7].

The work described in this paper is part of our systematic investigations on the reaction based on the reduction of phospho-tungstic acid by aescin in presence of sodium carbonate to produce an intense blue color having maximum absorbance at 720 nm. Survey of the literature revealed that no spectrophotometric method has been reported for the determination of aescin. First-ever spectrophotometric method for the determination of aescin is reported. The proposed method is simple, sensitive and accurate.
X.2. Experimental

X.2.1. Apparatus

Specord 50 UV-vis spectrophotometer with 1.0-cm silica quartz match cell was used for measuring the absorbance.

X.2.2. Reagents and solutions

Aescin is gift sample from Samilabs Limited India, was used as received. F-C reagent, sodium carbonate and sodium hydroxide. (BDH, India). All other chemicals and solvents used were of analytical reagent grade. Double distilled water was used throughout.

Stock aescin solution (1000μg ml⁻¹) was prepared by dissolving 100 mg of the sample in 2N sodium hydroxide solution and diluted to the mark with same solvent in a 100 ml volumetric flask. Required standard solution of (100 μg garcinol ml⁻¹) was prepared by diluting 100 ml of standard aescin solution to 1000 ml with 2N sodium hydroxide.

F-C reagent 2N as supplied by S.D fine chem. India, Ltd. was used directly and aqueous solution of 20% (w/v) sodium carbonate solution was prepared in double distilled water and filtered.
X.2.3. Procedure (Reduction with F-C reagent)

Accurately measured aliquots of the standard aescin solution (2 to 80 μg ml⁻¹) and 1 ml each of 2N F-C reagent and 20 % (w/v) sodium carbonate solution were transferred to 25 ml volumetric flask. The mixture was stirred and allowed to stand for 45 min. The volume was completed with distilled water. The absorbance was measured at 720 nm against corresponding reagent blank.

X.3. Results and discussion

The color formation by the F-C reagent in the presence of aescin may be explained based on analogy with the reports of the earlier workers [8-10]. The mixed acids in the F-C preparation are the final chromogens and involve the following chemical species:

\[3 \text{H}_2\text{O}.\text{P}_2\text{O}_5.13 \text{WO}_3.5 \text{MoO}_3.10 \text{H}_2\text{O}\] and \[3 \text{H}_2\text{O}.\text{P}_2\text{O}_5.14 \text{WO}_3.4 \text{MoO}_3.10\text{H}_2\text{O}.

Aescin probably effects the reduction of 1, 2 or 3 oxygen atoms from tungstate and/or molybdate, producing one or more of several possible reduced species which have a characteristic blue color.

Table X.1 shows the linear calibration ranges and equation parameters for this procedure.
**Parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Blue</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>720</td>
</tr>
<tr>
<td>Stability (h)</td>
<td>04</td>
</tr>
<tr>
<td>Beer's law ($\mu$g ml$^{-1}$)</td>
<td>8-60</td>
</tr>
<tr>
<td>Recommended ion concentration ($\mu$g ml$^{-1}$)</td>
<td>28</td>
</tr>
<tr>
<td>Molar absorptivity (L mol$^{-1}$ cm$^{-1}$)</td>
<td>$1.04 \times 10^4$</td>
</tr>
<tr>
<td>Sandel’s Sensitivity ($\mu$g cm$^{-2}$)</td>
<td>0.010</td>
</tr>
<tr>
<td>Lower detection limit ($\mu$g ml$^{-1}$)</td>
<td>5</td>
</tr>
</tbody>
</table>

**Regression equation**

\[ y = ax + b \]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope (a)</td>
<td>0.010</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>0.003</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>1.005</td>
</tr>
<tr>
<td>Reaction time (min)</td>
<td>45</td>
</tr>
<tr>
<td>R.S.D$^b$ % (n=5)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

$^a y=ax+b$ where $x$ is the concentration of AESCIN in $\mu$g cm$^{-1}$.

$^b$ Relative standard deviation.

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**X.3.1. Optimization of analytical variables**

It was found that a F-C reagent in the range of 0.5-2.5 ml and 20% (w/v) aqueous solution of sodium carbonate in the range of 1.0-4.0 ml were necessary to achieve maximum color intensity and stability of the blue color. Hence, 1.0 ml each of F-C reagent and sodium carbonate were recommended.
**X.3.2. Order of addition**

The sequence of addition of aescin, F-C reagent and sodium carbonate was studied *via* the formation of the blue complex. The study indicated that the sequence of addition of reactants had profound influence on the intensity and stability of the color, for example; (1) F-C reagent+ Na$_2$CO$_3$ +aescin gave less intensive and unstable color. While, the orders (2) aescin +F-C reagent+Na$_2$CO$_3$ (3) aescin + Na$_2$CO$_3$ +F-C gave more intense and stable color.

**X.3.3. Stability**

The resultant product of the proposed method was studied at different temperatures. The result indicated that the absorbance values remained constant in the temperature range 5-70°C. At higher temperatures the absorbance values decreased indicating the dissociation of the products on prolonged heating. The colored product was stable up to 24 h at room temperature.

**X.3.4. Interference**

The interference by various substances that often accompany aescin in pharmaceutical preparations was studied. It was found that commonly encountered pharmaceutical additives and excipients such as glucose, lactose, dextrose, starch, sodium alginate and sodium lauryl sulphate did not interfere (Table X.2).
Material | Amount (mg) | % Recovery of aescin ± SD*  
--- | --- | ---  
Glucose | 50 | 101.2 ± 0.88  
Lactose | 50 | 100.8 ± 0.78  
Dextrose | 50 | 99.2 ± 0.62  
Starch | 50 | 98.6 ± 1.08  
Sodium alginate | 50 | 101.4 ± 0.84  
Sodium lauryl sulphate | 50 | 100.8 ± 0.96  
Vitamin C | 10 | >50<60**  

* standard deviation (n=5);  
** erratic values

Table X.2  
Recovery of AESCIN in the presence of excipients and other substances

X.3.5. Applications

An accurately weighed 100 mg of the drug with excipients (50% aescin in cellulose, 50% aescin in talc and 50% aescin in starch) were dissolved in 2N sodium hydroxide and filtered through a Whatman No.42 filter paper. The filtrate was made up to 100-ml in a volumetric flask. A suitable volume of the filtrate was accurately diluted with 2N sodium hydroxide so as to obtain a sample of required concentration. An aliquot of this solution was analyzed by the proposed method. Known amount of aescin was added to the same solution and recovery experiments were performed. The results are presented in Table X.3.
Table X.3: Recovery Studies using standard addition method

<table>
<thead>
<tr>
<th>Aescin in excepient</th>
<th>Concentration of aescin in excepient (µg/ml)</th>
<th>Pure aescin added (µg/ml)</th>
<th>Total Concentration of aescin found (µg/ml)</th>
<th>% Recovery of pure aescin added *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>20.2</td>
<td>20.0</td>
<td>40.1</td>
<td>99.51 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>20.2</td>
<td>25.0</td>
<td>45.3</td>
<td>100.40 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>20.2</td>
<td>30.0</td>
<td>50.2</td>
<td>100.02 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>19.9</td>
<td>20.0</td>
<td>40.0</td>
<td>100.51 ± 0.83</td>
</tr>
<tr>
<td>Talc</td>
<td>19.9</td>
<td>25.0</td>
<td>44.9</td>
<td>100.03 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>19.9</td>
<td>30.0</td>
<td>50.0</td>
<td>100.30 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>20.1</td>
<td>20.0</td>
<td>40.1</td>
<td>100.04 ± 0.81</td>
</tr>
<tr>
<td>Starch</td>
<td>20.1</td>
<td>25.0</td>
<td>45.2</td>
<td>100.40 ± 1.01</td>
</tr>
<tr>
<td></td>
<td>20.1</td>
<td>30.0</td>
<td>50.1</td>
<td>100.03 ± 0.83</td>
</tr>
</tbody>
</table>

*average of five determination ± relative standard deviation
X.4. Conclusion

The resurgence of phytochemicals in varied fields demands development of simple and sensitive methods for the assay. The present trend is in the direction of improvement of physico-chemical methods of analysis. It is envisaged that simple methods based on spectrophotometry will become an accepted analytical tool for the assay and evaluation of phytochemicals. The procedures described in this paper meets most of the demands of analytical chemists namely selectivity, sensitivity, simplicity, reliability and cost of analysis. A value-addition of this method is achieved, if the procedure is combined with on-line or at-line system and this is currently under investigation.

Health in focus

Horse chestnut leaves have been used by herbalists as a cough remedy and to reduce fevers, to reduce pain and inflammation of arthritis and rheumatism, used topically to treat skin ulcers and skin cancer. Other uses include the internal and external application for problems of venous circulation, including varicose veins and haemorrhoids. Aescin also possesses anti-inflammatory properties and has been shown to reduce oedema (swelling with fluid) following trauma, particularly following sports injury, surgery, and head injury. A topical aescin preparation is very popular in Europe for the treatment of acute sprains during sporting events. Horse chestnuts also contain flavonoids, sterols, and tannins.

Double-blind and preliminary clinical trials have shown that oral horse chestnut extracts reduce the symptoms of chronic venous insufficiency, including swelling and pain.

[http://www.numarkpharmacists.com/Herb/Horse_Chestnut.htm]
Chapter X

Reference


