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Simple Spectrophotometric Method For The Determination Of Aescin From *Aesculus Hippocastanum*

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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 phospho-molybdotungstic 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 720nm. The method obeys Beer's law over the range of 8-60μg ml^{-1}. Sandell's sensitivity and molar absorbtivity were 10.549μgcm^{-2} and 1.0439×104 $mol^{-1}cm^{-1}$ respectively. The color developed was stable up to 24h. The method can be successfully employed for the determination of aescin in presence of common pharmaceutical excipients.

KEYWORDS

Aescin;
Folin-ciocalteu reagent;
Aesculus hippocastanum;
Nutraceutical;
Spectrophotometry.

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 Northem 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 ag1icons are derivatives of protoascigenin acy1ated 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.

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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 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 720nm. 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.

EXPERIMENTAL

Apparatus

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

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 100mg of the sample in 2N sodium hydroxide solution and diluted to the mark with same solvent in a 100ml volumetric flask. Required standard solution of (100μg garcinol ml^{-1}) was prepared by diluting 100ml 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.

Procedure(Reduction with F-C reagent)

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

RESULT 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:

$3H_2O.P_2O_5.13\ WO_{3.5}\ MoO_3.l0\ H_2O$ and $3H_2O.P_2O_514\ WO_3.{}_4\ MoO_3.10H_2O$

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 1 shows the linear calibration ranges and equation parameters for this procedure.

TABLE 1 : Spectral data for determination of aescin

Parameters	
Color	Blue
λ_{max} (nm)	720
Stability (h)	24
Beer's law (μg ml^{-1})	8-60
Recommended ion concentration (μg ml^{-1})	28
Molar absorptivity (L $mol^{-1}cm^{-1}$)	1.04×10^4
Sandel's Sensitivity (μg cm^{-2})	0.010
Detection limit (μg ml^{-1})	5
Regression equation[a]	
Slope(a)	0.010
Intercept (b)	0.003
Correlation coefficient	1.005
Reaction time(min)	45
R.S.D[b] (n=5)	0.61

[a]y=ax+b where x is the concentration of AESCIN in μg ml^{-1}

[b]Relative standard deviation

TABLE 2 : Recovery of aescin in the presence of excipients and other substances

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 lauyrl sulphate	50	100.8±0.96
Vitamin C	10	>50<60**

*standard deviation(n=5)

**erratic values

TABLE 3 : Recovery studies using standard addition method

Aescin in excepient	Concentration of aescin in excepient (μgml^{-1})	Pure aescin added (μgml^{-1})	Total Concentration of aescin found (μgml^{-1})	% Recovery of pure aescin added*
	20.2	20.0	40.1	99.5±0.53
Cellulose	20.2	25.0	45.3	100.4±0.43
	20.2	30.0	50.2	100.0±0.71
	19.9	20.0	40.0	100.5±0.83
Talc	19.9	25.0	44.9	100.0±0.48
	19.9	30.0	50.0	100.3±0.56
	20.1	20.0	40.1	100.0±0.81
Starch	20.1	25.0	45.2	100.4±1.01
	20.1	30.0	50.1	100.0±0.83

* average of five determination±relative standard deviation.

Optimization of analytical variables

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

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_2CO_3+aescin gave less intensive and unstable color. While the orders (2) aescin +F-C reagent+Na_2CO_3 (3) aescin+Na_2CO_3+F-C gave more intense and stable color.

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^{0}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.

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 lauyrl sulphate did not interfere (TABLE 2).

APPLICATIONS

An accurately weighed 100mg of the drug with excepients(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 filterate 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 experi-

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ments were performed. The results are presented in TABLE 3.

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 procedure 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.

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