

# AN IMPROVED HPLC/MS METHOD FOR THE ANALYSIS OF ESCINS IN COMMON HORSE CHESTNUT (*AESCULUS HIPPOCASTANUM*)

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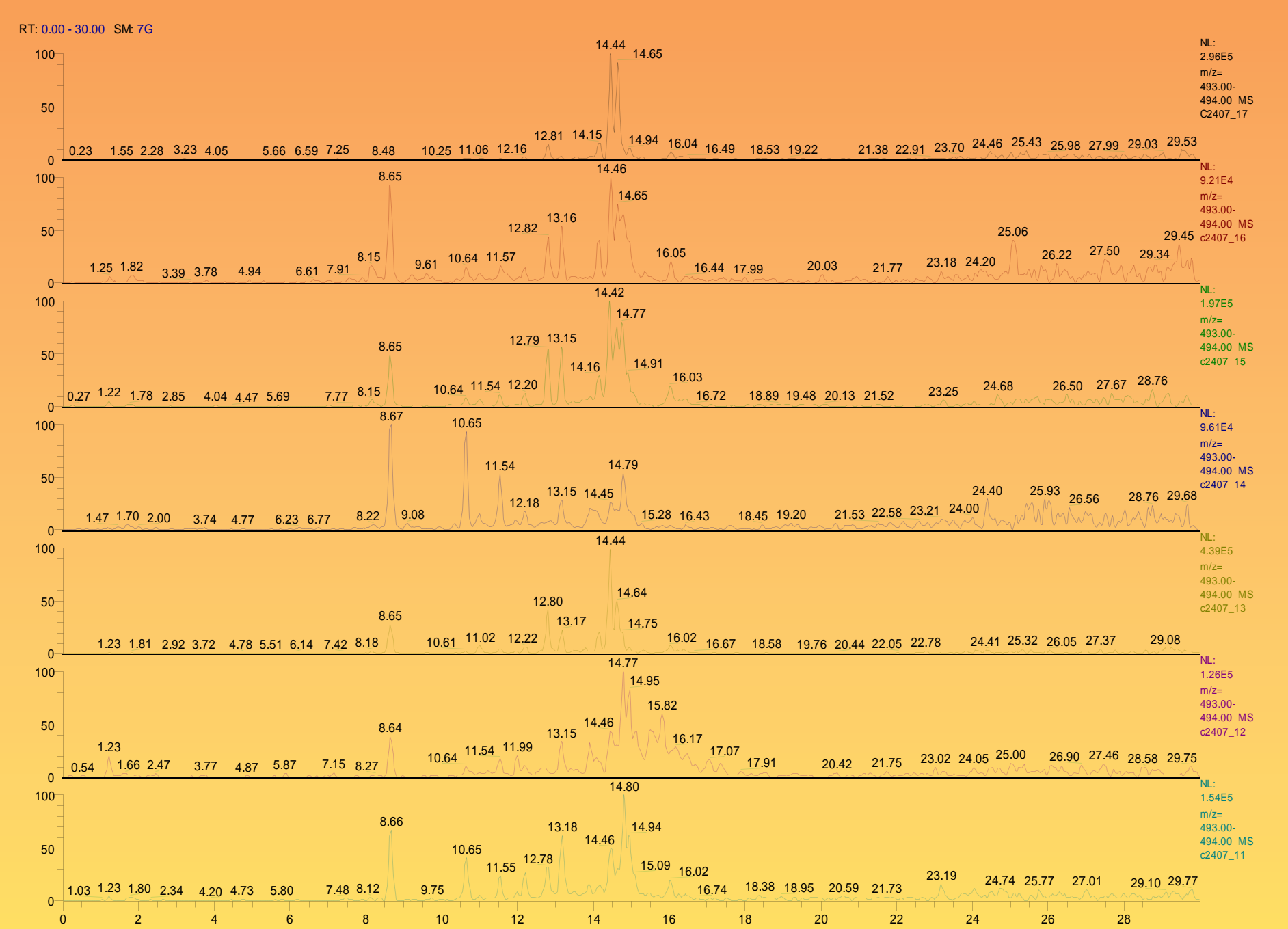


setting THE standard

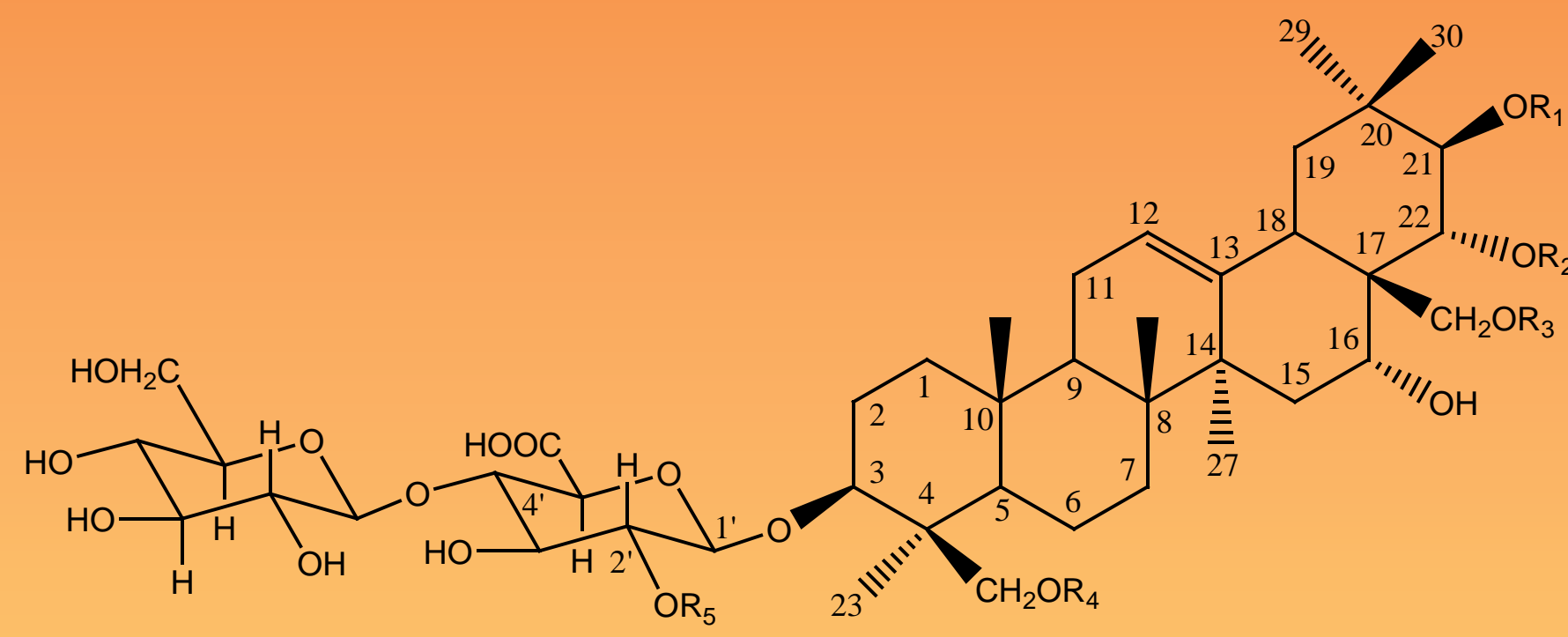
## ABSTRACT

The deciduous tree, *Aesculus hippocastanum* L. [Sapindaceae] known as the Common Horse-chestnut is native to a small area in the mountains of the Balkans, but is now widely cultivated in temperate regions around the world. The extract from the seed finds widespread use in the treatment of acute inflammation, chronic venous insufficiency, and edema. The components of the extract primarily responsible for the pharmacological effect are a group of saponin compounds collectively known as escins, which are comprised of over thirty glycosidic triterpene compounds derived from the aglycone protoaescigenin or barringtogenol C. Current methods used to quantify escins in herbal medications involves gravimetric analysis or colorimetric determination. Through the use of HPLC/MS, an analysis method has been developed which also allows for the determination of total escins. By selecting specific ionization fragments the percent total escins can be quantified reproducibly. A number of products on the market in addition to raw material were tested and the values were compared to specifications when available. In most cases, products achieved label claim, but not all. Extract procedures can also cause variation in products. This method is able to determine if the product is representative of the raw material extract, or if it has been changed due to extreme extract procedures.

Figure 3: Mixed Chromatogram Showing the Extracted Ion at  $m/z$  493.5.



A = B-Escin; B = HCSE in Gel Cap; C = HCSE in Gel Cap; D = HCSE in Gel Cap; E = HCS; F = HCS in EtOH; G = HCSE in Gel Cap



Compound	R Groups					%
	R1	R2	R3	R4	R5	
Escin Ia	Tigloyl	Acyl	H	OH	Glucose	15
Escin Ib	Angeloyl	Acyl	H	OH	Glucose	23
Escin IIa	Tigloyl	Acyl	H	OH	Xylose	6
Escin IIb	Angeloyl	Acyl	H	OH	Xylose	9

Figure 1. The Basic Molecular Structures and Percentages of the Major Escin Isomers as Proposed by Wulff and Tschesche(3).

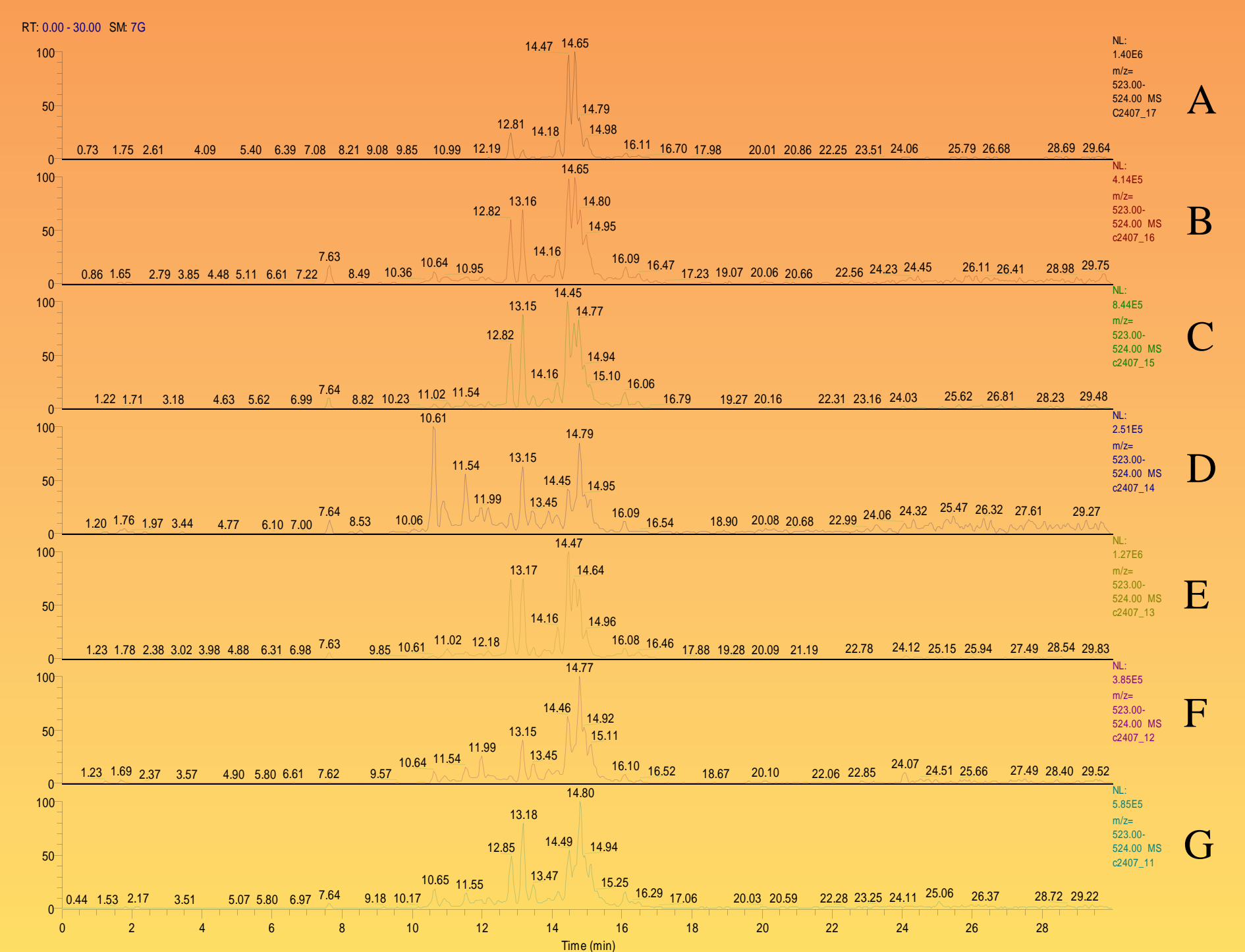
## INTRODUCTION

Extract from the seed of *Aesculus hippocastanum*, commonly known as horse chestnut, is the most widely prescribed medication in Germany for chronic venous insufficiency and edema (1). In China, the dried ripe seeds of *Aesculus chinensis* are used to normalize stomach function and relieve distension in the abdomen (2). The major bioactive compounds extracted from the horse-chestnut seed are collectively known as escins, which are comprised of over thirty glycosidic triterpene isomers derived from the aglycone protoaescigenin or barringtogenol C (3). Clinical studies involving the effects of escins have shown significant reduction in both leg volume and capillary filtration (4), resulting in a decrease in subjective patient complaints. The anti-inflammatory effects of escins are believed to be a result of the inhibition of histamine and serotonin induced vascular permeability (5). Horse chestnut seed extract has also been shown to reduce the activity of lysosomal enzymes in damaged veins, reducing the breakdown of mucopolysaccharides around the capillary wall, leading to a reduction of vascular permeability (6). Current methods used to quantify escins in herbal medications containing horse chestnut seed (HCS) or horse chestnut seed extract (HCSE) involve gravimetric analysis or colorimetric determination. Both of these techniques require multiple solvent extraction steps, which can be extremely time consuming. Therefore, an HPLC/ESI/MS method has been developed to quantify the total escin content in herbal products containing HCSE. The method was designed to be used in a quality control laboratory using commercially available reagents and common instrumentation. Commercially available  $\beta$ -escin was used as the reference standard, and in order to ensure the analysis of numerous samples in a short period of time, the extraction procedure was designed as simply as possible with no clean up procedure prior to LC-MS analysis.

The quantitative method was based on the signals generated by these two common polysaccharide fragments. The area counts for each fragment ion was determined by extracting the individual ion signal from the TIC chromatogram and integrating the observed peaks. The total area counts from each extracted ion were then combined to represent the total escin content. The quantitative method was shown to be linear within the concentration range of 0.01 to 0.19 mg/mL, with a correlation coefficient ( $r^2$ ) of 0.9967.

The sample results show that the powdered HCS (E) were above the limit specified by the USP-NF, and several samples were in close agreement with the label claim (A-C), but two of the samples had low results (E and F). The low results could be a result of low extraction efficiency, or possibly sample degradation. Although further development and validation are necessary, the results show that this method could be used to quantify escins in herbal products with high sensitivity in a short time.

Figure 2: Mixed Chromatogram Showing the Extracted Ion at  $m/z$  523.5.



A = B-Escin; B = HCSE in Gel Cap; C = HCSE in Gel Cap; D = HCSE in Gel Cap; E = HCS; F = HCS in EtOH; G = HCSE in Gel Cap

## EXPERIMENTAL

**HPLC:** Agilent 1100 series equipped with a vacuum degasser, an autosampler injection system, a thermostated column oven, and a binary pump with a quaternary low pressure mixing valve

**MS:** LCQ-Deca ion-trap mass spectrometer from Thermo-Finnigan equipped with an electrospray ionization source

**Column:** Phenomenex Gemini, 150 x 4.6 mm, 5  $\mu$ m particle size

**Mobile Phase:** A) 99:1:0.025  
H<sub>2</sub>O:IPA:TFA  
B) 99:1:0.025  
ACN:IPA:TFA

**Flow Rate:** 1.0 mL/min.

Gradient:	Time, min.	% A	% B
	0	95	5
	2	95	5
	20	5	95
	25	5	95
	30	95	5

**Detection:** ESI+  
**Temperature:** 30 °C  
**Injected:** Sample Volume: 10  $\mu$ L  
**Sample Prep:** Samples were sonicated in MeOH for 30 minutes and filtered prior to LC-MS analysis



Photos of *A. hippocastanum* (top) and seeds (bottom).

## DISCUSSION

- Figure 1 shows the basic molecular structure common to all escin isomers, as well as the R groups and percentages of the four major compounds found in the mixture
- Figure 2 shows a mixed chromatogram of the extracted ion at  $m/z$  523.5
- Figure 3 shows a mixed chromatogram of the extracted ion at  $m/z$  493.5 Table 1 shows a summary of the results obtained for the sample analyzed by the LC-MS method

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Table 1. Summary of Product Results

Sample	Type product	Dosage form	Label claim	Result
A	HCSE	Capsule	60 mg	64.3 mg
B	HCSE	Capsule	54 mg	53.3 mg
C	HCSE	Capsule	60 mg	47.4 mg
D	Powdered HCS	NA	NLT 3.0%	4.1%
E	HCS in EtOH	Liquid	15 mg	9.4 mg
F	HCSE	C		