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The Development of an LC-MS Method for the Identification of Ecdysteroids

Velislava Todorova[1,2], Stanislava Ivanova[1,2], Kalin Ivanov[1,2]

[1] Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria
[2] Research Institute, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria

INTRODUCTION & AIM

- Ecdysteroids are classified into three main groups according to their natural origin: phytoecdysteroids, zooecdysteroids, and mycoecdysteroids.
- Phytoecdysteroids are a class of biologically active molecules produced by plants as a defense against herbivorous insects.
- They accumulate in various plant organs, including fruits, seeds, flowers, leaves, and roots.
- The most commonly encountered and isolated phytoecdysteroids from these plants include 20-hydroxyecdysone, ayugasterone C, turkesterone, polypodine B, ponasterones A, B, and C, among others.
- Herbal preparations and dietary supplements from phytoecdysteroids-rich plants are available as adaptogens and for promoting muscle growth.

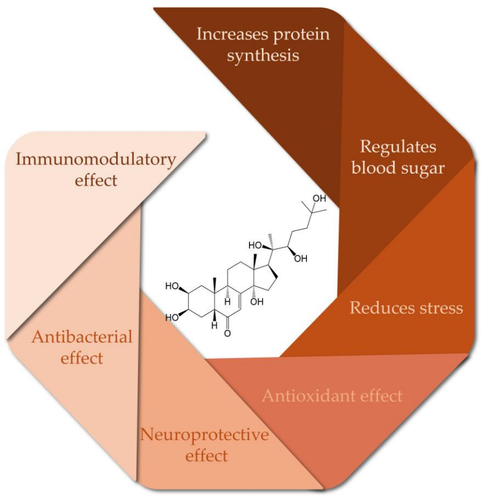


Figure 1. Biological effects of ecdysterone.

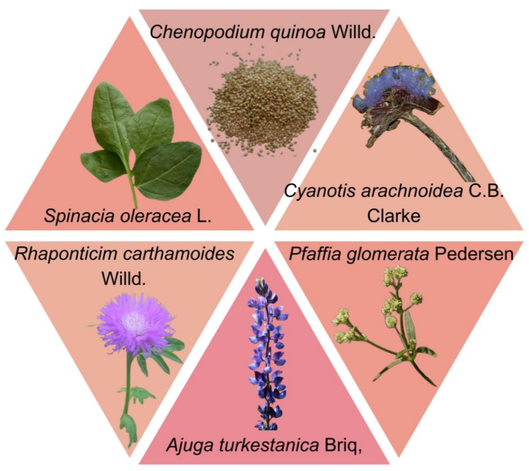


Figure 2. Plants containing ecdysterone.

METHOD

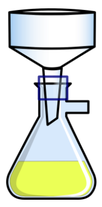
Preparation of Standard Solutions

Stock solutions of the standard substances 20-HE, PS, and TS were prepared in acetonitrile/water (50:50) at a concentration of 1 mg/mL.

Preparation of Extracts

Plants were ground in a mortar with pestle;

Preparation of the 50% methanolic plant extracts in ultrasonic for 30 min;



Filtered through a 0.45 µm membrane filter;

Stored in brown vials in 4°C.

Instrumentation

The method was developed using LC-MS: Shimadzu LCMS-2050 (Shimadzu, Kyoto, Japan). A C-18 column (150 mm × 4.6 mm, 3 µm, Shim pack, Shimadzu, Kyoto, Japan) was used.

Chromatographic Conditions

Gradient elution was applied to achieve better separation. The column temperature was maintained at 45 °C during analysis. The injection volume was 10 µL.

LC-MS

The mobile phase consisted of water with 0.1% formic acid and acetonitrile; the analysis time was 12 min. A single quadrupole MS detector was employed. Nebulizing gas flow was 2 L/min, drying gas 5 L/min, and heating gas 7 L/min. The desolvation temperature was 450 °C. A DUIS interface was used with a voltage of 3 kV. The mass range was 100–500 m/z. Single Ion Monitoring was used for quantitative analysis. Data acquisition and processing were performed using LabSolutions software (Shimadzu, Kyoto, Japan).

Method Validation

The developed method was validated in accordance with the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

CONCLUSION

The developed LC-MS method for the detection and quantification of 20-HE, TS, and PS is distinguished by its selectivity, speed, and sensitivity. It demonstrates high accuracy, precision, and robustness, while the use of a single-quadrupole mass spectrometer significantly reduces equipment costs and increases accessibility. These characteristics make LC-MS a particularly suitable method for the analysis of plant extracts containing ecdysteroids and an ideal solution for routine application.

RESULTS & DISCUSSION

METHOD DEVELOPMENT

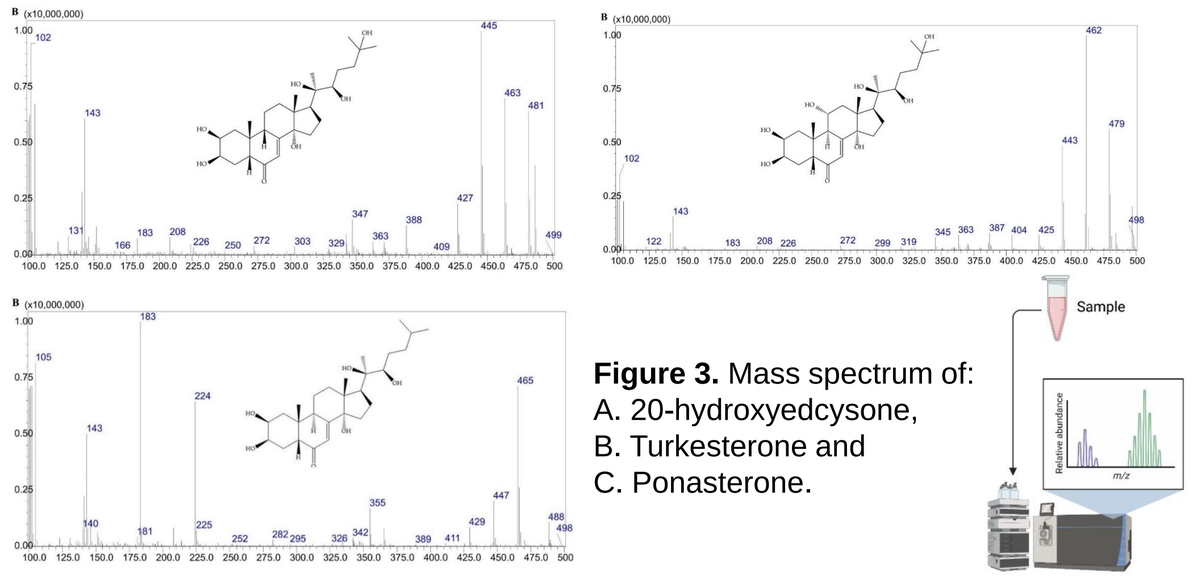


Figure 3. Mass spectrum of:
A. 20-hydroxyedcysone,
B. Turkesterone and
C. Ponasterone.

METHOD VALIDATION

Linearity		Compound	Limit of detection	
	R^2 = 0.9999	Ecdysterone	**LOD = 3.3×σ/S**	ng/mL
	R^2 = 0.9999	Turkesterone		ng/mL
	R^2 = 0.9998	Ponasterone		ng/mL
Accuracy (%)			**Limit of quantification**	
	✓	Ecdysterone	**LOD = 10×σ/S**	ng/mL
	✓	Turkesterone		ng/mL
	✓	Ponasterone		ng/mL
Precission (CV%)			**Robustness**	
	✓	Ecdysterone		✓
	✓	Turkesterone		✓
	✓	Ponasterone		✓

METHOD APPLICATION

Ecdysterone content in superfoods

Kaniwa – 670 µg/g d.w.

Asparagus – 189 µg/g d.w.

Red quinoa – 259 µg/g d.w.

Spinach – 252 - 455 µg/g d.w.

White qinoa – 310 µg/g d.w.