Development and application of a LC-MS/MS method for identification of polyphenols in propolis extract

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Introduction

The purpose of this study is to identify the most commonly polyphenols found in Romanian propolis and quantify their levels in various hydroalcoholic extracts. In this regard we have worked to develop an efficient and reliable method of analysis.

Methods

The LC-MS/MS analysis was carried out using a Q Trap 5500 Triple Quadrupole Mass spectrometer from Sciex with ESI/Turbo Ion Spray mode. In the chromatographic analysis, a Synergi C18 (Fusion-RP 80 Å, 50 x 2 mm, particle size of 4 µm) column was used with an injection volume of 5 µL. The solvents used were (A) formic acid (0.5 %) and (B) methanol. Gradient elution ranged from 2% to 98% B at 30°C, and elution flow was set at 900 µL/min. Elution time was 20 minutes.

The ionization source temperature of the MS was 500°C; mass spectra were recorded in the negative ion mode, between 50 m/z and 500 m/z using nitrogen as a collision gas. The pressure of the gas flux to the nebulizer was set at 1000 psi.

Results and discussion

Experimental parameters for each analyte were identified by direct injection in the MS module of individual standards, in the 0.001 - 0.1 µg/mL concentration range, resulting in the corresponding product ions.

Table 1 MS experimental characteristics of the investigated compounds

Compound	Parent Ion, Da	Precursor Ion, Da	DP[a], V	EP[b], V	CE[c], eV	CXP[d], V
Caffeic acid	178.9	134.9	-70	-10	-22	-13
p-Coumaric acid	162.9	118.9	-60	-10	-22	-9
Gallic acid	168.8	124.9	-65	-10	-20	-11
t-Ferulic acid	192.9	133.8	-70	-10	-22	-11
Kaempferol	284.9	92.9	-130	-10	-54	-7
Quercetin	300.9	135.8	-120	-10	-28	-11
Chrysin	253	208.9	-145	-10	-20	-17
Pinocembrin	255	212.8	-120	-10	-28	-28
Vanillin	150.9	135.8	-60	-10	-18	-9
CAPE	283	135	-120	-10	-72	-17
Gallangin	268.9	168.8	-105	-10	-36	-11

Ethanol calibration solutions were prepared (from a mixed working standard) in a 0.08 – 5 µg/mL range.

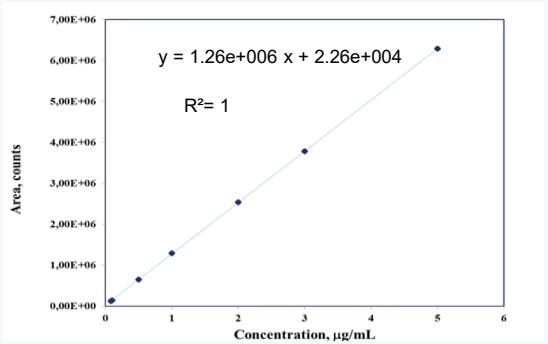

Figure 1 Calibration curve for t-ferulic acid

Selectivity has been investigated in terms of relative standard deviations of the retention times, Limit of Quantitation, LOQ, and Limit of Detection, LOD (Table 2), were evaluated as per ICH Guidelines.

Table 2 Validation characteristics

Analyte Name	Retention Time, min	RSD, %	LOD µg/mL	LOQ, µg/mL
Gallic Acid	0.262	0.020	0.17	0.52
Caffeic Acid	1.88	0.080	0.12	0.30
Vanillin	2.04	0.045	0.09	0.26
p-Coumaric Acid	2.32	0.024	0.16	0.49
t-Ferulic Acid	2.66	0.090	0.01	0.03
Quercetin	4.13	0.210	0.066	0.17
Kaempferol	4.64	0.070	0.08	0.24
Pinocembrin	4.86	0.120	0.12	0.37
CAPE	5.19	0.080	0.17	0.51
Chrysin	5.25	0.010	0.23	0.69
Gallangin	5.26	0.050	0.18	0.54

The method was applied for the analysis of Romanian propolis extracts. Figures 2 and 3 show typical LC-MS/MS chromatograms. The quantified levels of polyphenolics are collected in Table 3.

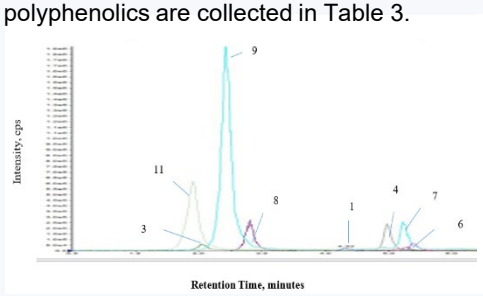

Figure 2 Chromatogram for ethanolic extract

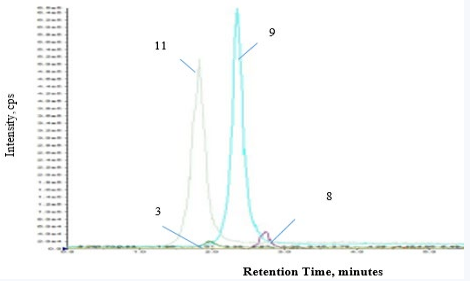

Figure 3 Chromatogram for aqueous extract

Table 3 Poliphenolics in ethanolics and aqueous extracts

Compound		Ethanolic extract		Aqueous extract	
Code	Name	Retention time, min	Concentration, µg/mL	Retention time, min	Concentration, µg/mL
1	Quercetin	4.311	0.834	-	-
2	Chrysin	-	-	-	-
3	Vanillin	2.04	3.589	1.964	0.292
4	Pinocembrin	4.978	10.502	-	-
5	Kaempferol	4.699	0.990	-	-
6	Gallangin	5.389	6.781	-	-
7	CAPE	5.276	4.579	-	-
8	*t*-Ferulic Acid	2.808	13.262	2.744	0.794
9	*p*-Coumaric Acid	2.427	10.802	2.34	1.261
10	Gallic Acid	-	-	-	-
11	Caffeic Acid	1.91	4.873	1.821	1.330

Conclusions

Experiments run at seven concentration levels, using at least two replicate injections for each concentration level gave linear regressions in terms of peak area, characterized by correlation coefficients larger than 0.9988, except chrysin, with a determination coefficient of 0.9822.

The use of the LC-MS analysis method and the determination of the working conditions proved to be effective in identifying 11 polyphenolics in aqueous and ethanolics extracts of propolis.

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