

HPLC-UV-MS STUDY OF POLYPHENOLS FROM *GLYCYRRHIZA GLABRA*

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Abstract

We have analyzed the polyphenols from roots of *Glycyrrhiza glabra* harvested from Syria. The presence of eighteen polyphenolic compounds was simultaneously assessed through a LC-UV-MS method, before and after hydrolysis. The compounds that we were observed were: p-coumaric acid, ferulic acid, sinapic acid, luteolin and apigenin.

Rezumat

Studiul prezintă analiza polifenolilor din rădăcinile de *Glycyrrhiza glabra* recoltate din Siria. Prezența celor 18 compuși polifenolici a fost realizată simultan printr-o metodă LC-UV-MS, înainte și după hidroliză. Compușii observați în probă au fost: acid p-coumaric, acid ferulic, acid sinapic, luteolină și apigenină.

Keywords: *Glycyrrhiza glabra*, HPLC-UV-MS, polyphenolic compounds

Introduction

Licorice, the root of the *Glycyrrhiza* species, is one of the most frequently employed botanicals in traditional medicine. The history of licorice, as a medicinal plant, is very old and has been used in many societies throughout the millennia. There are many useful compounds in licorice root such as, glycyrrhizin (saponin- like glycoside -50 times sweeter than sugar) and its aglycone, glycyrrhetic acid, which are clinically used for hyperlipidemia. Licorice flavonoid constituents mainly include flavones, flavonals, isoflavones, chalcones, bihydroflavones and bihydrochalcones. Pharmacological investigations indicate that they have antioxidant, antibacterial and anti-inflammatory activities [10, 11].

For polyphenols analysis from roots samples we used a HPLC-UV-MS method. Our analysis was based on an HPLC method already published in literature [1-10] with some modifications – the most important was the

replacement of the potassium phosphate with acetic acid from the mobile phase. The root samples contain only volatile compounds, so we can introduce the eluent from the chromatographic column directly into the mass spectrometer. The method was applied for the qualitative analysis (18 compounds) and the quantitative analysis (14 compounds).

Materials and methods

Standards: caffeic acid, chlorogenic acid, p-coumaric acid, kaempferol, apigenin, rutoside, quercetin, quercitrin, isoquercitrin, fisetin, hyperoside, myricetin (Sigma, Germany), ferulic acid, gentisic acid, sinapic acid, patuletin, luteolin (Roth, Germany), caftaric acid (Dalton, USA).

Plant material: The roots of *Glycyrrhiza glabra* were collected from Syria in four different regions: Bilekh, Rakka, Alpo-Azaz, Rass-Aenn.

Sample preparation: Samples of 5 g pulverized roots were extracted by refluxing with 25 mL methanol for 1 hour. In order to study the flavonoid aglycons that can be obtained by hydrolysis, a part of each extract of our samples were treated with an equal quantity of 2N hydrochloric acid and heated 40 minutes at 80 °C on a water bath. The resulting samples were properly diluted. We noted the samples: G1 (Bilekh), G2 (Rakka), G3 (Alpo-Azaz), G4 (Rass-Aenn).

Apparatus: HP 1100 series liquid chromatograph having a degasser, binary pump, autosampler, column thermostat, UV detector. The HPLC system was coupled with an Agilent 1100 MSD Ion Trap VL mass detector.

Chromatographic conditions: For the separation we used a reversed-phased Zorbax SB-C18 analytical column (100 x 3.0 mm i.d., 3.5 µm). The column was operated at 48°C. the mobile phase was prepared from methanol:acetic acid 0.1% (v/v), the elution begun with a linear gradient (started at 5% to 42% methanol for the first 35 minutes), followed by isocratic elution (with 42% methanol for the next 3 minutes). The flow rate was 1 mL/min and the injection volume was 5 µL. The detection was performed at 330 nm and 370 nm.

MS conditions: The MS was equipped with a Turbo-Ionspray (ESI - electrospray ionisation) interface, negative ion mode. ESI settings were: negative ionisation, ion source temperature 360°C, gas: nitrogen, flow rate 12 L/min, nebuliser: nitrogen at 70 psi pressure, capillary voltage 3000 V. The analysis mode was multiple reaction monitoring (MRM) and single ion monitoring (SIM).

Results and discussion

For the analysis of eighteen compounds from the plant material we have been developed a high performance liquid chromatographic method which allows the simultaneous analysis of different classes of polyphenols by a single pass column. Each sample was analysed before and after acid hydrolysis.

Polyphenols analysis by UV detection

Each class of compounds was detected based on of the wavelength corresponding to the maximum of absorbtion in UV spectrum. Thus, polyphenolic carboxylic acids were detected at 330 nm and flavonoids and their aglicons at 370 nm.

For the quantitative analysis, the calibration curves were realized for each compound in the 0.5-50 $\mu\text{g/mL}$ range. Because in the chromatographic conditions that we used there are two pairs of substances incompletely separated (caftaric acid-gentisic acid, respectively caffeic acid-chlorogenic acid) for these compounds we realized only the qualitative analysis based on the MS information. In Table I we present the retention times and calibration curves parameters of polyphenolic compounds analyzed, using UV detection.

Table I
Retention times and calibration curves parameters
of polyphenolic compounds

No.	Phenolic compound	Retention time (min)	Calibration curve equation
1	Caftaric acid	2.10	qualitative
2	Gentisic acid	2.15	qualitative
3	Caffeic acid	5.6	qualitative
4	Chlorogenic acid	5.6	qualitative
5	p-coumaric acid	8.7	$A = - 0.325 + 33.23 x$
6	Ferulic acid	12.2	$A = - 1.016 + 39.55 x$
7	Sinapic acid	14.3	$A = - 0.236 + 37.10 x$
8	Hyperoside	18.6	$A = 0.107 + 19.29 x$
9	Isoquercitrin	19.6	$A = - 0.273 + 12.97 x$
10	Rutoside	20.2	$A = 0.226 + 13.47 x$
11	Myricetin	20.7	$A = -0.544 + 26.45 x$
12	Fisetin	22.6	$A = 0.241 + 19.19 x$
13	Quercitrin	23.0	$A = 0.047 + 10.69 x$
14	Quercetin	26.8	$A = -1.152 + 36.32 x$
15	Patuletin	28.7	$A = - 0.429 + 31.44 x$
16	Luteolin	29.1	$A = - 0.760 + 28.97 x$
17	Kaempferol	31.6	$A = - 1.270 + 30.15 x$
18	Apigenin	33.1	$A = - 0.908 + 20.40 x$

A = peak area mAUxs, x = concentration $\mu\text{g/mL}$

Polyphenols analysis by MS detection

The polyphenolic compounds that we analyzed contain in their molecule at least one phenolic function (and one carboxyl for polyphenolic acids) so, they can be transformed into negative ions (M-H) and they can be analyzed by negative ionization. The mass spectrometer is set to isolate the interest ions and then to fragmentize them, finally recording the corresponding mass spectra. In table II there are presented the MS analysis mode and the specific ions from the mass spectra of the eighteen polyphenols used as standards, and in table III the content in polyphenolic compounds ($\mu\text{g/mL}$).

Table II
MS analysis mode and the specific ions
for the identification from the mass spectra of polyphenolic compounds

No.	Phenolic compound	MS analysis Mode	Specific ions for identification Ion [M-H] > Ions of spectra
1	Caftaric acid	MRM*	311>148.6, 178.6
2	Gentisic acid	MRM	153>108.7
3	Caffeic acid	MRM	179.4>134.7
4	Chlorogenic acid	MRM	353.5>178.7, 190.7
5	p-coumaric acid	MRM	163> 118.7
6	Ferulic acid	MRM	193.2> 133.7, 148.7, 177.6
7	Sinapic acid	MRM	223.4>148.6, 163.6, 178.7, 207.7
8	Hyperoside	SIM**	463.1
9	Isoquercitrin	SIM	463.1
10	Rutoside	SIM	609.1
11	Myricetin	SIM	317.1
12	Fisetin	SIM	285.1
13	Quercitrin	SIM	447.1
14	Quercetin	SIM	301.1
15	Patuletin	SIM	331.1
16	Luteolin	SIM	285.1
17	Kaempferol	SIM	285.1
18	Apigenin	SIM	269.2

*MRM= multiple reaction monitoring, ** SIM= single ion monitoring

Table III
The content in polyphenolic compounds ($\mu\text{g/mL}$)

Polyphenols	G1		G2		G3		G4	
	NH	H	NH	H	NH	H	NH	H
Gentisic acid	*	*	-	*	-	*	-	*
Caffeic acid	*	*		*			*	*
p-coumaric acid	*	3.627		4.932	*	2.173	*	2.173
Ferulic acid	-	5.014	-	11.84	*	6.236	-	5.141
Luteolin	0.620	1.139	*	0.85	0.332	0.908	0.217	0.332
Apigenin	*	1.218	-	1.054	1.054	1.381	0.728	1.463
Sinapic acid	-	3.694	-	2.93	*	13.981	-	2.032

NH – non hydrolyzed sample; H – hydrolyzed sample

*MS qualitative determination; UV signal < LQ (limit of quantification) or interferences from other compounds

Results indicate the presence of caffeic, gentisic, p-coumaric, ferulic and sinapic acids in all samples, especially in esteric form. The samples collected from Alpo-Azaz have the highest content in the analyzed phenolic compounds. In the case of flavonoids, only the flavones luteolin and apigenin were present in all four samples as glycosides and free aglycons, except for the sample collected from Rakka which contains just glycosylated apigenin. None of the five flavonols (quercetin, patuletin, kaempferol, fisetin, myricetin) or four glycosides (hyperoside, quercitrin, isoquercitrin, rutoside) tested were found in any of the samples.

Conclusions

We analysed the polyphenols from *Glycyrrhiza glabra* harvested from 4 regions of Syria. The method HPLC-UV-MS was applied for the qualitative and quantitative analysis.

Our results allow a better characterization of the active principles from the roots of *Glycyrrhiza glabra* and represent data for future pharmacological determinations.

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