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Determination of caftaric acid in tincture and rose water obtained from *Rosae damascenae flores*

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Abstract. Polyphenolic compounds were determined from a pharmaceutical (tincture) and a cosmetic preparation (rose water), both obtained from the *Rosae damascenae flores*. Separation of the phenolic compounds was done by a HPLC method, using a Zorbax XDB or equivalent column C18, 250 mm x 4,6 mm; 5 µm. A gradient elution was performed with phosphoric acid and acetonitrile eluted under gradient conditions. The flow rate was 1.5 mL/min and the injection volume was 20 µL. HPLC method for determination of caftaric acid presented in this paper, has been validated. The results were statistically analyzed with SPSS 10 software.

Keywords: caftaric acid, polyphenols, *Rosa damascena*.

1. Introduction

Rosa damascena Mill. (Damask rose, Oil-bearing rose, Pink rose) is one of the most important species, producing a high-value aromatic oil, which is used in the pharmaceutical, flavourings and fragrance industries [1]. According to the literature, ”rose for sweetness” originates in Damask, the first capital of the Arab world. It is considered the national flower of Iran. The use of the essential oil of roses is known since the time of the Persian Empire and it is referred by physician and scientist Avicena, in the 10th century BC [2].

Some scientific papers specify the use of emollient ointments and cold creams containing rosewater [3]. It is also known therapeutic use of the rose oil in respiratory diseases, asthma, hay fever, wound healing and skin infections. The *Rosae damascenae flores* rose contains vitamins A, B3, C, D and E [4]. In Romania, the flowers of this species are used for obtaining essential oil of rose syrup and rose sweetness.

Rosae damascenae flores total polyphenols was determined by a group of researchers from Turkey who showed hepatoprotective activity on a residue from the distillation of the fresh rose flowers [5].

The flowers of the family Rosaceae species are known in the literature for their content in polyphenols and vitamin C. Polyphenols are known for their antioxidant, anti-infective, anti-inflammatory, anticancer, antimutagenic and antidepressant actions [6, 7].

Caftaric acid is the most abundant phenolic compound in many vegetal products [8 - 10]. The caftaric acid is used in cosmetic formulations for the treatment of dermatological disorders and for regulating skin pigmentation [11].

2. Experimental

2.1. Plant material

The flowers of *Rosa damascena Mill*. were harvested from Constanta on 1-3 June 2013.

The material was washed in quick water spurt and dried in warm air. It was performed identification of the species was kept in Pharmacognosy Laboratory, Faculty of Pharmacy, Ovidius University, Constanta.

2.2. Sample preparation

Tincture. A weighed amount (100 g) of the fresh rose flowers was extracted with 1000 mL of ethanol 70^0 at room temperature, for 14 days. The tincture was filtered through a 0.45m PTFE filter into a HPLC vial and capped.

Rose water. Rose water was obtained as follows: 70 grams of the fresh rose flowers was distilled with 1000 mL of water to a distillation apparatus for 3

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hours. The rose water was filtered through a 0.45m PTFE filter into a HPLC vial and capped.

2.3. HPLC analysis of phenolic compounds

For separation, identification and quantification of the phenolic compounds was adapted a USP30 HPLC method [12]. The phenolic composition was analyzed qualitatively and quantitatively by HPLC system (Agilent 1200) with quaternary pump, DAD, auto sampler. Separation was carried out on Zorbax XDB or equivalent column C18, 250 mm, 4,6 mm; 5 µm. A gradient elution was performed with solvent A (phosphoric acid) and solvent B (acetonitrile) as follows (**Table 1**). The flow rate was 1.5 mL/min and the injection volume was 20 µL.

The retention times and DAD spectra were compared to available authentic standards.

Table 1. The gradient elution

Time (minutes)	Solution A, mL %	Solution B, mL %
0-13	90	10
13	7	22
13	78	22
14	60	40
17	60	40
17.5	90	10
22	90	10

The concentration of the used standard solutions (70% methanol) were: E – resveratrol = 37 mg/mL, Z – resveratrol = 0.22 mg/mL. Z – resveratrol was obtained from E – resveratrol exposed 12 hours at UV 254 nmradiation (fig. 1.). The following concentrations were obtained: caffeic acid = 0.36 mg/mL, chlorogenic acid = 0.37 mg/mL, cinnamic acid = 0.58 mg/mL, vanillin = 0.42 mg/mL, gallic acid = 0.39 mg/mL, ferulic acid = 0.48 mg/mL, 3-methylgallic acid = 0.34 mg/mL, ellagic acid = 0.43 mg/mL, p-coumaric acid = 0.51 mg/mL, caftaric acid = 0.42 mg/mL (**Table 2**, **Fig. 2**).

The method was validated in terms of linearity, precision, accuracy, and specificity, limit of detection and limit of quantification [13].

Table 2. The retention time of standards

Nr. Crt.	Compound	Retention time ± SD*
1	*E* - resveratrol	14.467 ± 0.017
2	*Z* – resveratrol	15.751 ± 0,058
3	Caffeic acid	4.598 ± 0.036
4	Chlorogenic acid	3.501 ± 0.015
5	Cinnamic acid	15.867 ± 0.007
6	Vanilin	6.919 ± 0.051
7	Gallic acid	0.990 ± 0.025
8	Ferulic acid	8.565± 0.058
9	Ellagic acid	15.303± 0.027
10	*p*-Coumaric acid	7.187± 0.019
11	3-*o*-Methylgallic acid	2.606± 0.008
12	Caftaric acid	3.013± 0.021

(*standard deviation for six injections)

2.4. Setting detection wavelength

In order to determine the wavelength of detection, a sample of caftaric acid solution, having a concentration of 80 ppm, was injected. After 10 minutes the absorption spectrum was recorded compared to the control (mobile phase).

Calibration curves of caftaric acid

The stock standard solution of each caftaric acid derivative was prepared as follows: about 4.2 mg compound was accurately weighed and placed into a 10 mL volumetric flask. Seventy percent ethanol in water was added and the solution diluted to volume with the same solvent.

Calibration curves were established on six data points covering the concentration range of 2-50 µg/mL.

Triplicate injections were made for each standard solution. Calibration curve was obtained by plotting the peak area.

2.5. Method validation

Linearity. The study of linearity was performed by the analysis of six standard solutions, in duplicate. Calculations were carried out by the least-squares method (analysis of variance with statistical F-test, including evaluation of the model lack-of-fit).

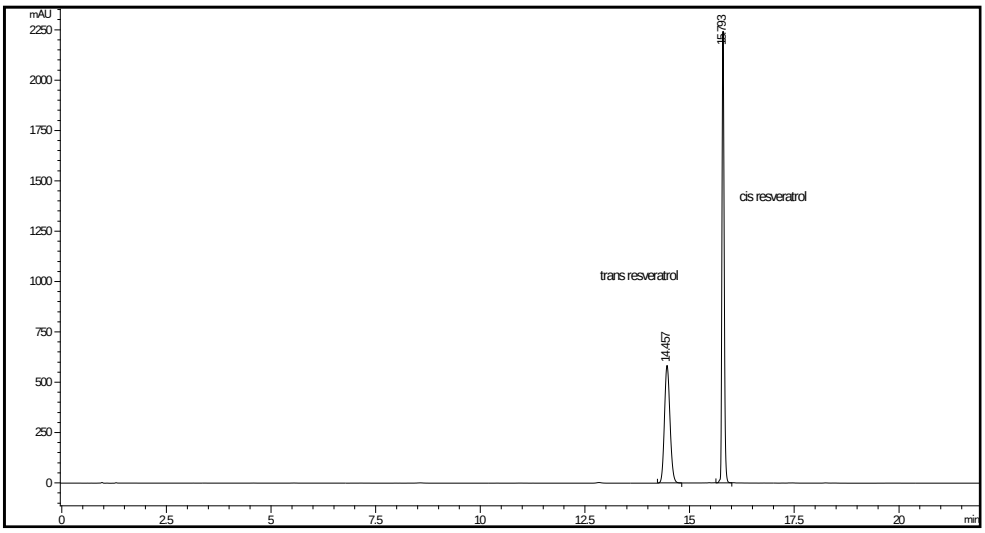

Figure 1. HPLC chromatogram of resveratrol after exposure UV 254 nm radiation

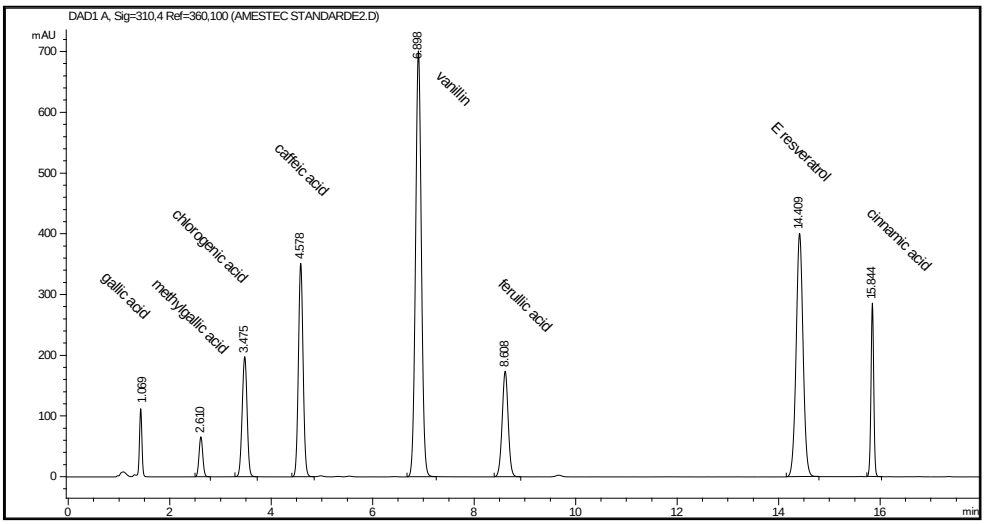

Fig.2. HPLC chromatogram of standards

Accuracy. The accuracy of the method was tested by determining recovery of caftaric acid added in tincture of *Rosae damascenae flores*, in known concentrations [14].

Precision of the applied method. *Intermediate precision.* This parameter was determined by repeating 10 times the same concentration of caftaric acid and tincture of *Rosae damascenae flores* injections for 3 consecutive days. Retention times and peak areas were statistically processed by determining the relative standard deviation (%R.S.D.).

Repeatability. The repeatability was calculated from 10 replicate injections of caftaric acid and tincture of *Rosae damascenae flores* samples, under constant operating conditions (laboratory, equipment, operator, and method) over a short period of time.

Limits of detection and quantification. Limits of detection (LOD) were calculated according to the

expression 3σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve. Limits of quantification (LOQ) were established by using the expression 10σ/S.

3. Results and Discussions

3.1. Setting detection wavelength

From the analysis of the absorption spectrum of the caftaric acid (**Fig. 3**) shows that it has an absorption maximum at a wavelength of 330 nm, which does not interfere with the absorption of mobile phase components.

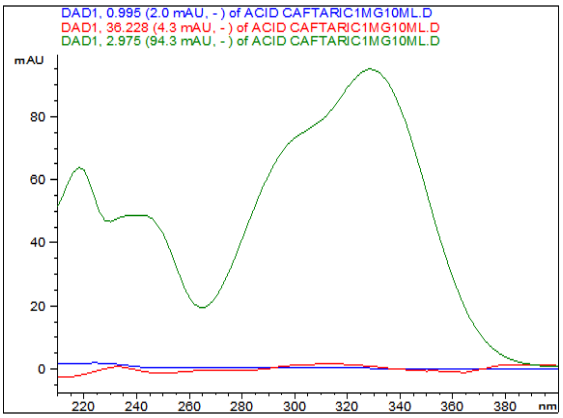

Fig. 3. Caftaric acid absorption spectrum

In conclusion, best results were obtained at the wavelength of 330 nm in all stages of the determination, separation and dispensing of caftaric acid.

3.2. Calibration curves of caftaric acid

Calibration curves were established on six data points covering the concentration range of 2-50 µg/mL (**Fig. 4** and **Fig. 5**).

3.3. Method validation

Linearity. The relationship between the peak area and the caftaric acid concentration was evaluated over the range 2 şi 50 µg/mL. Land was found linear (y = 10.685x - 5.3475; R^2 = 0.9993; n = 6).

Good linearity (correlation factor > 0.9960) was achieved in the concentration range 2 - 50 µg/mL for caftaric acid in ethanol 50% solution (**Fig. 6**).

ANOVA (analysis of variance) is used to validate the regression model used. In order to obtain the assurance that there is currently no alignment fault, it was applied F test (Fisher).

Fischer test is not significant, so there is a defect of alignment (**Table 3**).

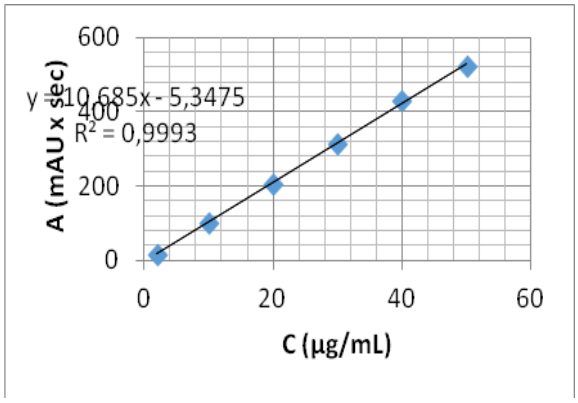

Fig. 4. Calibration curves of caftaric acid

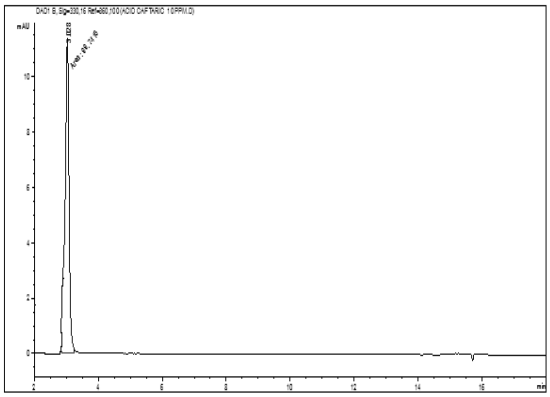

Fig. 5. HPLC chromatogram of caftaric acid

Accuracy. The accuracy of the method was verified by means of recovery assays. Adequate amounts of caftaric acid in *Rosae damascenae tincturae* in order to obtain added concentration 10 and 20 µg/mL. The recovery values af the added caftaric acid ranged from 97.54 % to 100.23% (98.74%±1.36; n = 3).

Precision of the applied method. Intermediate precision. The precision of the system was demonstrated by analyzing the 6 samples of the same concentration and of tincture of *Rosae damascenae flores*. Relative standard deviation obtained areas recorded RSD (%) = 0.5587 and relative standard deviation obtained record retention times, RSD (%) = 0.2906 is less than 2%. The RSD values obtained for the retention times ranged from 0.95% to 1.69% and the RSD values for peak areas ranged from

0.31% to 1.91% for real samples of tincture of *Rosae damascenae flores*. We can say that these data confirm the accuracy of the system.

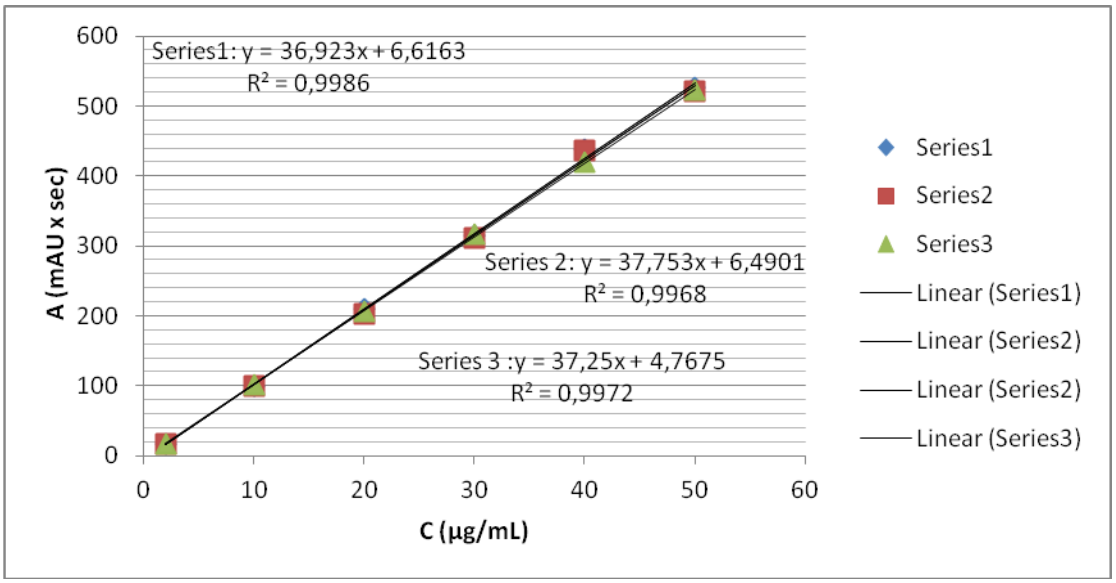

Fig. 6. The curves for each series of determinations

Table 3. Required parameters regression test Fischer

ANOVA[b]						
Model		Sum of Squares	df	Mean Square	F	Sig.
	Regression	190771.102	1	190771.102	4245.316	.000[a]
	Residual	179.747	4	44.937		
	Total	190950.849	5			
a. Predictors: (Constant), VAR00001						
b. Dependent Variable: VAR00002						

Repeatability. The obtained RSD(%) value is 0.55 (below 2%) for standard solution while the RSD(%) value obtained for tincture of *Rosae damascenae flores* is 1.78%. Those values indicate excellent repeatability of the proposed method.

Limits of detection and quantification. LOD = 1.8820 µg/mL and LOQ = 6.270 µg/mL were established by the procedures described in the Section 2.

3.4. HPLC analysis of phenolic compounds.

The following compounds were detected: cinamic acid, gallic acid, caftaric acid, ferulic acid, elagic acid and 3-*o*-methylgallic acid, for tincture (**Table 4**, **Fig. 7**) and for the rose water (**Table 4, Fig. 8** and **Fig. 9**).

Table 4. Phenols compounds and their concentrations mg/100 mL sample

Sample	*Gallic acid mg%*	*3-o-Methylgallic acid mg%*	*Ferulic acid mg%*	*Ellagic acid mg%*	*Cinamic acid mg%*	*Caftaric acid mg%*
Tincture	876.037	33.328	2.897	1485.177	15.306	5.608
Rose Water	-	0.0986	-	-	0.832	0.13341

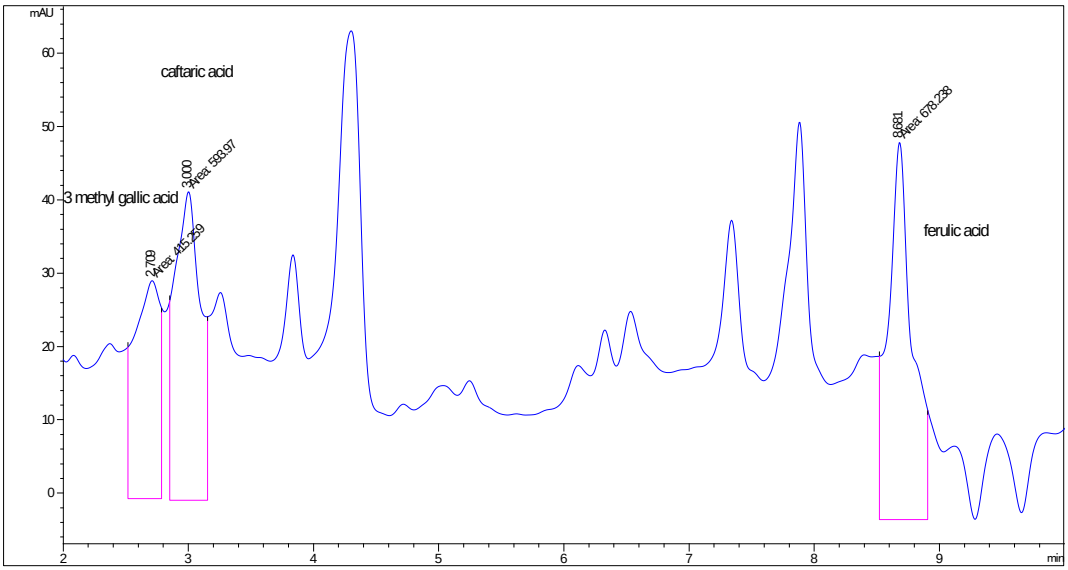

Fig. 7. HPLC profile of phenolic compounds from *Rosa damascenae tincturae*

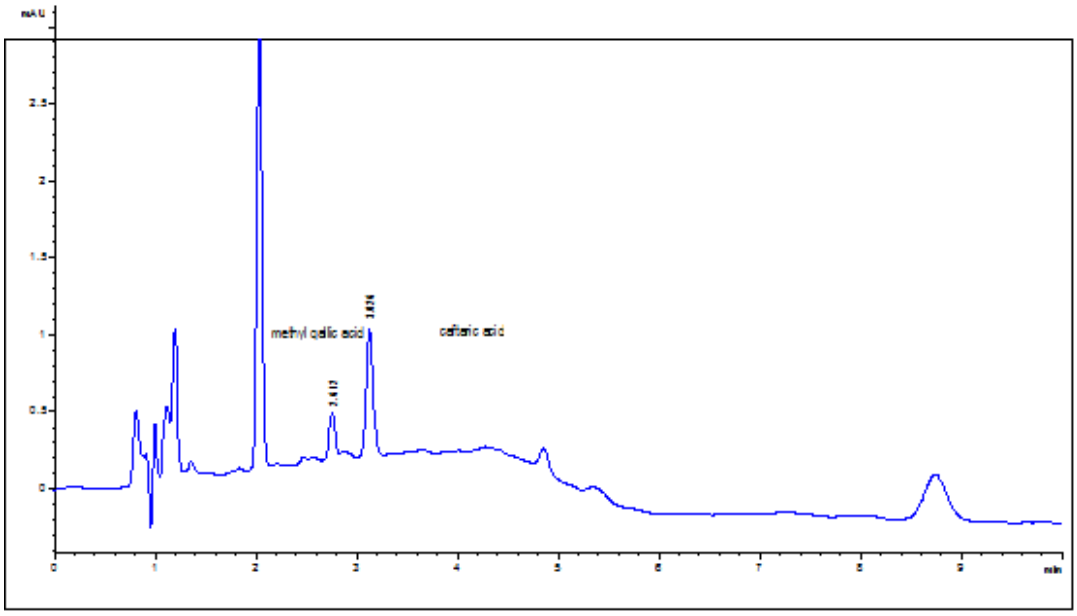

Fig. 8. HPLC profile of phenolic compounds from *Rose Water*

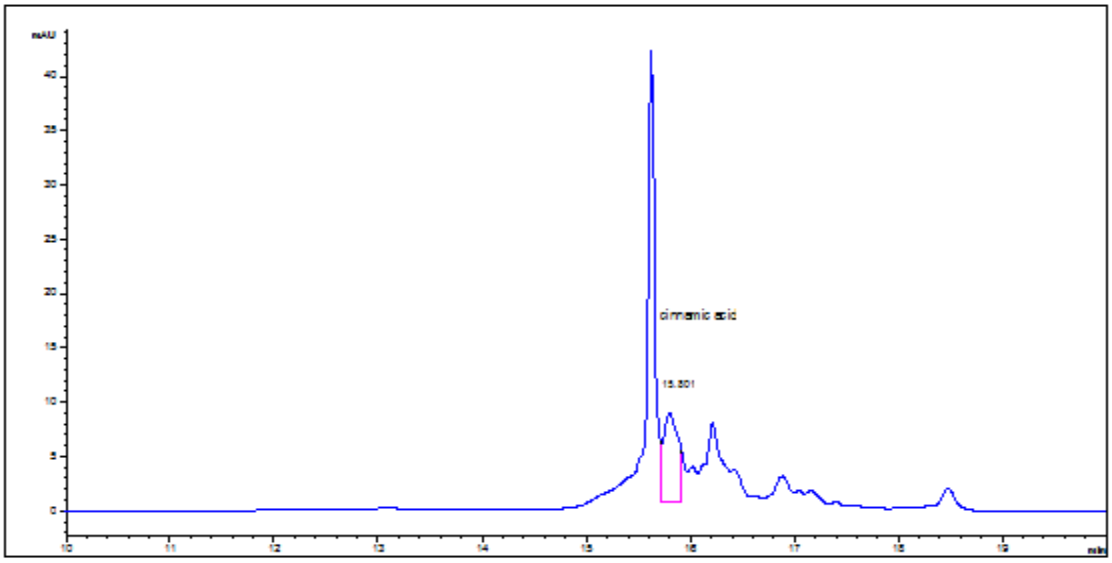

Fig. 9. HPLC profile of phenolic compounds from *Rose Water*

4. Conclusions

The proposed method was considered adequate, with regard to the linearity, precision, repeatability and accuracy of the results. Thus, it can be employed in the analysis of vegetal product.

In tincture were determined polyphenols known for their antioxidant action. Tincture of *Rosae damascenae flores* could afford health benefits by preventing unwanted free-radical-induced oxidative reactions.

The main active principle identified in tincture and in rose water is caftaric acid.

Since it is known the use of caftaric acid for regulating skin pigmentation, its presence in rose water can demonstrate the utility in cosmetic preparations.

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