



Comparison of two Spectrophotometric Methods for Quantifying Total Hydroxycinnamic Acids in Coneflower (*Echinacea purpurea*) Preparations

Hossein Mahdavi¹; Mohammad Reza Naderi^{2*}, Mohammad Hossein Esmaeili³

Phytotherapy, Department of Research and Development, Sina faravar herbal medicines company, Najaf Abad, Iran;

²*Crop ecology, Department of Research and Development, Sina faravar herbal medicines company, Najaf Abad, Iran;*

***Email:** naderi.mr@sinafaravar.com

³*Phytochemistry, Chemistry laboratory, Sina faravar herbal medicines company, Najaf Abad, Iran;*

ARTICLE INFO

Type: Original Research

Topic: Medicinal Plants

Received December 3th 2017

Accepted February 20th 2018

Key words:

- ✓ *Echinacea purpurea*
- ✓ *European pharmacopoeia*
- ✓ *Hydroxycinnamic acid*
- ✓ *Quality control*

ABSTRACT

Background & Aim: Hydroxycinnamic acids are one of the most important bioactive substances of *Echinacea* drugs. These compounds possess immuno-enhancing activity and thus, total hydroxycinnamic acids are mostly used as the main criterion for quality control of *Echinacea purpurea* and its drugs. Hence, the quality control of *Echinacea* requires to developing the reliable methods which are simple and also have a suitable accuracy in measuring total hydroxycinnamic acids. The current study was performed in order to sets a simple method for quantifying total hydroxycinnamic acids of *Echinacea* and then, comparison of this method which named as the AlCl₃ method with the method of European pharmacopoeia (EP).

Experimental: Total hydroxycinnamic acids of 10 coneflower samples were determined using AlCl₃ and EP methods and the amounts obtained by these two methods, were compared using statistical criterion.

Results: Accuracy of AlCl₃ method in measuring total hydroxycinnamic acids of *Echinacea* was not suitable, as the relative difference between amounts measured by this method and those measured by EP method was 50-130%. Comparison of this method with EP method indicated that performance of AlCl₃ method for quantifying total hydroxycinnamic acids of *Echinacea* drugs was very poor and low value of willmott index of agreement (d= 0.53) and high value of relative error (RE= 0.83) were obtained.

Recommended applications/industries: Due to non-selective reaction of Al(III) with hydroxycinnamic acids, the AlCl₃ method is not suitable for measuring the total hydroxycinnamic acids of *Echinacea* and its preparations.

1. Introduction

Phenolic compounds occur universally in the plant kingdom and are part of a large and complex group of

organic substances. Higher plants synthesize and accumulate a wide variety of phenolic compounds, which confer protection against the attacks of free

radicals, which are by-products from the process of photosynthesis, and against tissue injuries (Magnani *et al.*, 2014). The phenolic compounds can be classified into two groups: the group of simple phenolic compounds and the group of polyphenolic compounds (Burns *et al.*, 2001).

In the past few decades, dietary polyphenols, which are one of the most abundant classes of antioxidants in human diet, have received increasing attention (Ndhala *et al.*, 2010). Phenolic acids are an important group of secondary plant metabolites with powerful antioxidant capacities. These acids are usually divided in two main groups: benzoic acids, containing seven carbon atoms and cinnamic acids, consisting of nine carbon atoms (Awika and Rooney, 2004). These natural compounds exist predominantly as hydroxybenzoic and hydroxycinnamic acids (HCAs) that may occur either in their free or conjugated forms. Several types of hydroxybenzoic acids and HCA have been identified in the human diet, and are believed to play important roles due to their abundance and functional diversity (Razzaghi-Asl *et al.*, 2013).

Coneflower (*Echinacea purpurea*), a medicinal plant from asteraceae family, exhibits antioxidative, antibacterial, antiviral and antifungal activities, and affects various immune parameters of body. Because of its immuno-enhancing activity, it has been recently used in AIDS therapy (Zolgharnein *et al.*, 2010). It is used for treating influenza and cold. One of the main groups of biologically active substances in *Echinacea* includes hydroxycinnamic acid and related compounds (Zaporozhets *et al.*, 2003). Caffeic acid and its derivatives are most important hydroxycinnamic acids of various coneflower preparations. Hydroxycinnamic acids are potent antioxidant and anti-inflammatory agents, which can prevent renal dysfunction, cardiovascular disease, oxidative stress, diabetes, insulin resistance, body weight gain and dyslipidemia and can improve liver function (Alam *et al.*, 2016).

Modern consumers increasingly demand products and foods with high quality. Allied to this, legislation is increasingly strict about quality and safety, which has challenged the industries (Nazer *et al.*, 2005). Therefore, establishment of a simple method that enables quantification of the total hydroxycinnamic acid in coneflower preparations is very important (Štefan *et al.*, 2015). For this purpose, global and nonspecific methods are more appropriate, both from the perspectives of time and cost. Among all techniques

available for quantifying total hydroxycinnamic acids, UV/Vis spectrophotometric methods are simple procedures which can be used for determining the total amount of hydroxycinnamic acids in order to evaluate quality of the raw plant material and to standardize phytopharmaceuticals (Fuentes *et al.*, 2012).

Zaporozhets *et al.* (2003) were introduced a very simple spectrophotometric method (from here, referred as AlCl₃ method) for quantification of total hydroxycinnamic acids in coneflower preparations. This method which was based on complex of hydroxycinnamic acids with Al(III) cation, has been used in a number of studies [e.g. 8, 17, 20] for quantification of total hydroxycinnamic acid of different medicinal plants. Thus, we used this method for determining total hydroxycinnamic acid in coneflower preparations. However, by using AlCl₃ method for determination of total hydroxycinnamic acid in coneflower, we faced on some challenges, which adjusting pH of complex to the optimum amount was most important of them. Hence, this method was set and then, its accuracy in quantifying total hydroxycinnamic acid in coneflower preparations was compared with the method of European pharmacopoeia.

2. Materials and Methods

Because caffeic acid and its derivatives are main hydroxycinnamic acids of coneflower, caffeic acid was used for evaluating the behavior of coneflower hydroxycinnamic acids and setting the AlCl₃ method.

2.1. Absorbance spectrum of caffeic acid, powdered coneflower aerial parts and powdered coneflower dry extract

Caffeic acid (1mg) was dissolved in 50 ml ethanol 50% and its absorbance spectrum was developed at wavelength of 270-400 nm. Ethanol 50% was used as blank.

For coneflower, 1 g of powdered aerial parts (passed through mesh No. 100) or 100 mg of powdered dry extract was dissolved in 10 ml ethanol 50%, and mixture was incubated at room temperature for 30 min. Then, each extract was separately passed through a whatman filter paper No. 40 and the volume of filtrates increased to 10 ml using ethanol 50%. 1 ml of each filtrate was diluted 10 (for coneflower dry extract) or

30 (for aerial parts) times using ethanol 50% and their absorbance spectrum were developed at wavelength of 270-400 nm.

2.2. pH Adjustment for complex of caffeic acid with aluminium trichloride

1 ml caffeic acid 100 ppm was placed into a 25 ml volumetric flask and 2.3 ml $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 100 mM (prepared by distilled water) was added to it. Then, pH of this complex adjusted to the desired value (3.5-5.5 as intervals of 0.5 units and 4.5-5.5 as intervals of 0.1 units) using sodium acetate trihydrate 10% (w/v, prepared by distilled water) and the absorbance spectrum of each pH was developed at wavelength of 270-400 nm. The blank solution was prepared by putting 1 ml ethanol 50%, 2.3 ml $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 100 mM and 100 μl sodium acetate 10% into a 25 ml volumetric flask and volume increased to the mark.

2.3. Adjustment of AlCl_3 concentration

After pH adjustment, effect of different concentrations of AlCl_3 on maximum absorbance of AlCl_3 -caffeic acid complex was evaluated. For this purpose, a 200 mM stock solution of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was prepared using distilled water as solvent. Then, 1.25, 2.5, 3.75, 5, 6.25 and 7.5 ml of stock solution were placed into separate 10 ml volumetric flasks and their volume was increased to the mark using distilled water. Therefore, the final concentration of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ in these solutions was 25, 50, 75, 100, 125 and 150 mM, respectively. Then, 1 ml caffeic acid 100 ppm was placed into separate 25 ml volumetric flasks and 2.3 ml from different concentrations of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added to each flask. pH of this complex was adjusted to 4.6 using sodium acetate trihydrate 10% and the absorbance of each complex was read at wavelength of 351 nm. For preparing the blank solution, 1 ml ethanol 50%, 2.3 ml from different concentrations of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ and 300 μl sodium acetate 10% were put into a 25 ml volumetric flask and volume was increased to the mark using distilled water.

2.4. Calibration curve for standard concentrations of caffeic acid

A 100 ppm stock solution was prepared by dissolving 5 mg caffeic acid in 50 ml ethanol 50%. Then, 1, 2, 3, 4, 5, 6, 7, 8 and 9 ml from this stock solution were put into separate 10 ml volumetric flasks and volume of each flask was increased to the mark using ethanol

50%. The final concentration of caffeic acid in these solutions was 10, 20, 30, 40, 50, 60, 70, 80 and 90 ppm, respectively. 1 ml from each concentration was placed into separate 25 ml volumetric flasks, 2.3 ml $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 50 mM was added to each flask, pH was adjusted to 4.6 using sodium acetate 10% and volume was increased to 25 ml using distilled water. The absorbance of each solution was read at wavelength of 351 nm. For preparing the blank solution, 1 ml ethanol 50%, 2.3 ml $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 50 mM and 300 μl sodium acetate 10% were put into a 25 ml volumetric flask and volume was increased to the mark using distilled water.

2.5. Developing absorbance spectrum of coneflower drugs in complex with aluminium trichloride

For evaluating absorbance spectrum of powdered coneflower aerial parts and powdered coneflower dry extract in complex with AlCl_3 , 10 ml ethanol 50% was separately added to 40 mg powdered aerial parts (passed through mesh No. 100) and 10 mg powdered dry extract. The mixtures were incubated at room temperature for 30 min, filtered using whatman filter paper No. 40 and the volume of filtrates was increased to 10 ml using ethanol 50%. To 1 ml of each filtrate, 2.3 ml $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 50 mM was added, pH adjusted to 4.6 using sodium acetate 10% and volume was increased to 25 ml by distilled water. The absorbance spectrum of each solution was developed at wavelength of 270-400 nm. The blank solution was prepared by putting 1 ml ethanol 50%, 2.3 ml $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 50 mM and 300 μl sodium acetate 10% into a 25 ml volumetric flask and volume was increased to the mark using distilled water.

2.6. Comparison of AlCl_3 method with the method of European pharmacopoeia

After setting the AlCl_3 method, the performance of this method in determining hydroxycinnamic acid concentration of different coneflower drugs was compared to the European pharmacopoeia (EP) method, as the reference method. The coneflower drugs, were obtained from herbal archive of Sinifaravar® company. The method of EP for determining total hydroxycinnamic acid is as follow:

Stock solution: To 0.200 g of the powdered drug, add 80 ml of alcohol (50 percent V/V). Boil in a water-bath under a reflux condenser for 30 min. Allow to cool and filter. Rinse the filter with 10 ml of alcohol (50 percent V/V). Combine the filtrate and the rinsings in a

volumetric flask and dilute to 100.0 ml with alcohol (50 percent V/V) (European Pharmacopoeia, 2016).

Test solution: To 1.0 ml of the stock solution add 2 ml of 0.5 M hydrochloric acid, 2 ml of a solution prepared by dissolving 10 g of sodium nitrite and 10 g of sodium molybdate in 100 ml of water and then add 2 ml of dilute sodium hydroxide solution and dilute to 10.0 ml with water; mix (European Pharmacopoeia, 2016).

Compensation solution: Dilute 1.0 ml of the stock solution to 10.0 ml with water (European Pharmacopoeia, 2016). Measure the absorbance of the test solution, immediately at 518 nm, in comparison with the compensation liquid. Calculate the percentage content (w/w) of total hydroxycinnamic derivatives, expressed as caffeic acid, from the following expression (French Pharmacopoeia, 2007):

$$\frac{A \times 200}{m \times 300}$$

i.e: taking the specific absorbance of caffeic acid to be 300 at 518 nm.

A = absorbance of the test solution at 518 nm, m = mass of the herbal drug to be examined, in grams.

To evaluate the agreement between values obtained from AlCl₃ method and values of EP method, the following statistical indices were used: (I) the relative error (RE) (Stockle *et al.*, 2004) and (II) the Willmott index of agreement (d) (Willmott, 1982). Finally, the performance of the AlCl₃ method in determining total hydroxycinnamic acid of coneflower drugs was interpreted using the following Criteria (Gallardo *et al.*, 2011).

Table 1. Criteria of the AlCl₃ method performance in determining total hydroxycinnamic acid of coneflower drugs.

Criterion	Performance
$d \geq 0.95$ and $RE \leq 0.10$	Very good
$d \geq 0.95$ and $0.15 \geq RE > 0.10$	Good
$d \geq 0.95$ and $0.20 \geq RE > 0.15$	Acceptable
$d \geq 0.95$ and $0.25 \geq RE > 0.20$	Marginal

3. Results and discussion

3.1. Absorbance spectrum of caffeic acid, powdered coneflower aerial parts and powdered coneflower dry extract

The absorbance spectrum of caffeic acid, powdered coneflower aerial parts and powdered coneflower dry

extract are showed in Figure 1. As showed in this figure, the maximum absorbance of caffeic acid, powdered coneflower aerial parts and powdered coneflower dry extract was obtained at wavelength of 325, 326 and 324 nm, respectively. Therefore, *Echinacea* drugs have a maximum absorbance at wavelength of 324-326 nm, which is probably related to the caffeic acid and its derivatives.

Similarly, Zaporozhets *et al.* (2003) reported that absorption spectrum of caffeic acid solution is generally analogous to the spectra of chicoric acid and *Echinacea* extract. These authors concluded that the optical absorption of *Echinacea* preparations in this spectral region is mostly due to caffeic acid and its derivatives.

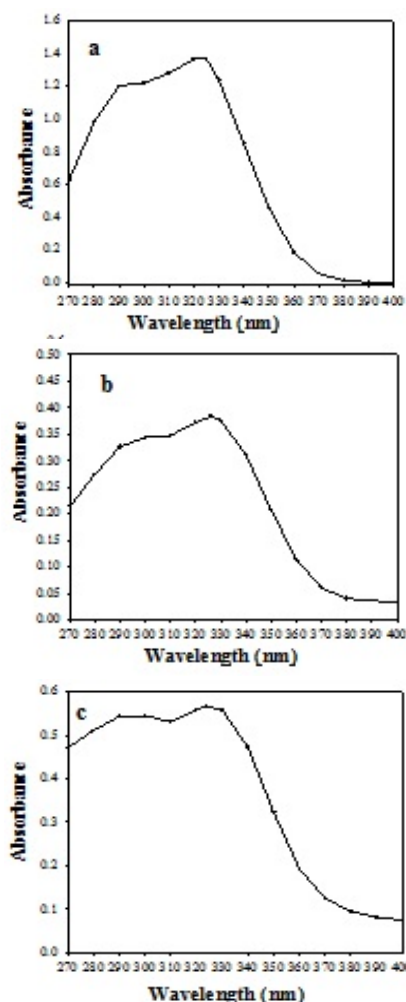


Figure 1. Absorbance spectrum of (a) caffeic acid, (b) powdered coneflower aerial parts and (c) powdered coneflower dry extract at wavelength of 270-400 nm.

3.2. pH Adjustment for complex of caffeic acid with aluminium trichloride

pH had a strong effect on formation of complex between hydroxycinnamic acids and AlCl_3 , as in $\text{pH} \leq 3$, caffeic acid did not form any complex with AlCl_3 and the maximum absorbance for the mixture of caffeic acid and AlCl_3 , was obtained at wavelength of 325 nm (Figure 2a), which is similar to the maximum wavelength of pure caffeic acid solution (without addition of AlCl_3). In pH more than 3, formation of complex between caffeic acid and AlCl_3 caused to increasing the maximum wavelength and maximum absorbance of the mixture and in pH 4.6, the maximum wavelength and maximum absorbance of caffeic acid- AlCl_3 complex was more than other pH (Figures 2b and 2d). In this pH (4.6), the maximum absorbance of caffeic acid- AlCl_3 was obtained at wavelength of 351 nm (Figure 2b) and thus, pH 4.6 and wavelength of 351 nm were set as the optimum pH and λ_{max} for the caffeic acid- AlCl_3 complex. Therefore, other analysis was performed at pH 4.6 and wavelength of 351 nm.

Zaporozhets *et al.* (2003) reported the bathochromic shift of the absorption peak observed upon introduction of the metal ions is related to the formation of complexes between caffeic acid and Al(III) in solution. These authors found that the optimum pH interval for the determination of HCA and its derivatives is from 4.5 to 5.5. They reported the wavelength of 355 nm as the corresponding wavelength for maximum absorption of the caffeic acid- Al(III) complex. A slight difference observed between maximum wavelength reported by Zaporozhets *et al.* (2003) for caffeic acid- AlCl_3 complex and that obtained in this study which was probably due to additional purification of caffeic acid by recrystallization from hot ($T = 70^\circ\text{C}$) distilled water in the study of Zaporozhets *et al.* (2003).

In the method developed by Zaporozhets *et al.* (2003) for determining total hydroxycinnamic acid of *Echinacea* preparations, the ammonium chloride 1% is used for adjusting pH of caffeic acid- AlCl_3 complex to the desired amount (4.8) and then, the volume of this mixture is increased to 25 ml using distilled water. We examine this procedure, but adjusting pH of caffeic acid- AlCl_3 complex to 4.8 was obtained by applying about 75 ml of ammonium chloride 1%. Therefore, we substitute ammonium chloride 1% with sodium acetate 10% for adjusting pH of caffeic acid- AlCl_3 complex.

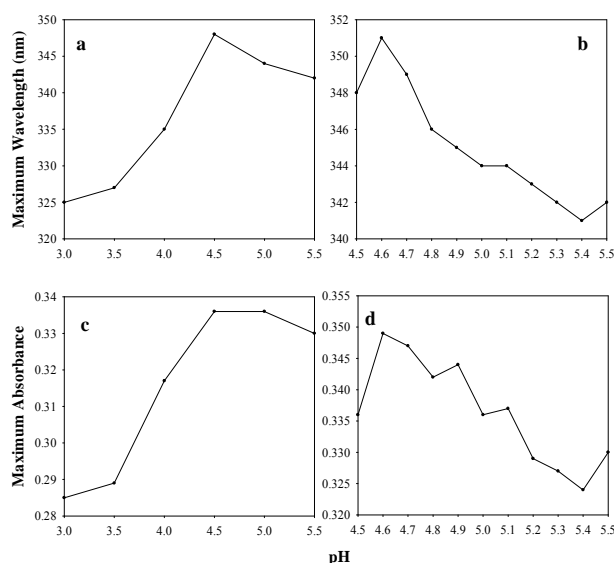


Figure 2. (a) maximum wavelength of caffeic acid- AlCl_3 complex at pH 3-5.5, (b) maximum wavelength of caffeic acid- AlCl_3 complex at pH 4.5-5.5, (c) maximum absorbance of caffeic acid- AlCl_3 complex at pH 3-5.5 and (d) maximum absorbance of caffeic acid- AlCl_3 complex at pH 4.5-5.5.

3.3. Adjustment of AlCl_3 concentration

After finding the optimum pH (4.6) for caffeic acid- AlCl_3 complex, the maximum absorbance of this complex was evaluated as function of AlCl_3 concentration. Results of regression analysis showed that maximum absorbance of caffeic acid- AlCl_3 complex was obtained using an AlCl_3 concentration of 50 mM and at higher concentrations, the maximum absorbance of caffeic acid- AlCl_3 complex did not show a significant change (Figure 3). Therefore, the concentration of 50 mM was set as the optimum concentration of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ for caffeic acid- Al(III) complex and in subsequent analysis, the AlCl_3 concentration of 50 mM was used.

Therefore, the AlCl_3 method for quantification of total hydroxycinnamic acids of *Echinacea* drugs was set as follow:

10 ml ethanol 50% was added to 40 mg powdered aerial parts (passed through mesh 100) or 10 mg powdered dry extract. The mixture was incubated at room temperature for 30 min, filtered using whatman filter paper No. 40 and the volume of filtrate was increased to 10 ml using ethanol 50%. To 1 ml of this extract, 2.3 ml $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 50 mM was added, pH adjusted to 4.6 using sodium acetate 10% and volume

was increased to 25 ml by distilled water. The absorbance of solution was measured at wavelength of 351 nm. The blank solution was prepared by putting 1 ml ethanol 50%, 2.3 ml $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 50 mM and 300 μl sodium acetate 10% into a 25 ml volumetric flask and volume was increased to the mark using distilled water.

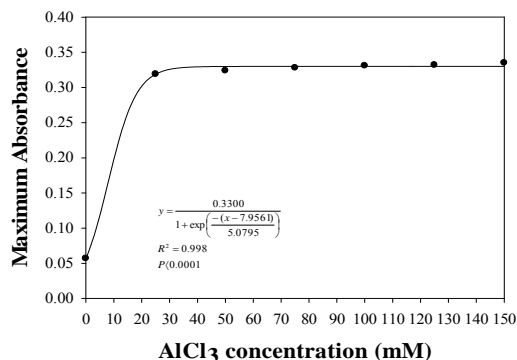


Figure 3. Maximum absorbance of caffeic acid- AlCl_3 complex as function of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ concentration.

3.4. Calibration curve for standard concentrations of caffeic acid

By using the AlCl_3 method, calibration curve for standard concentrations of caffeic acid was developed at wavelength of 351 nm (Figure 4). According to the calibration curve, at standard concentrations of 0-100 ppm caffeic acid, the absorbance of caffeic acid- AlCl_3 complex was linearly increased (Figure 4). The regression coefficient for linear function obtained from calibration curve was about 1, which this indicates the high accuracy and suitability of the fitted linear function for determining total hydroxycinnamic acid based on equivalent concentration of caffeic acid.

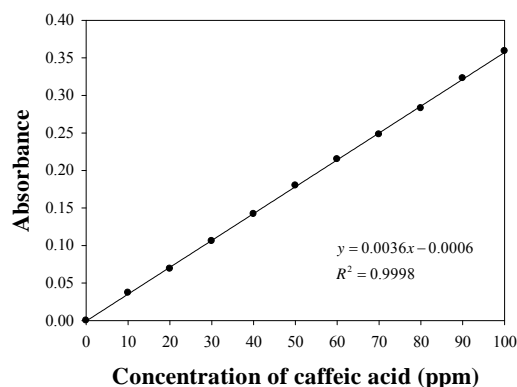


Figure 4. Calibration curve for standard concentrations of caffeic acid.

3.5. Absorbance spectrum of coneflower drugs in complex with aluminium trichloride

Evaluating the absorbance spectrum of AlCl_3 -coneflower drugs complex showed that despite the AlCl_3 -caffeic acid complex which its maximum absorbance was obtained at wavelength of 351 nm, the maximum absorbance of AlCl_3 -coneflower drug complexes was at wavelength of 365 nm (Figure 5). This indicates that in coneflower drugs, the caffeic acid and its derivatives are not the only compounds which complex with Al(III) and thus, despite the statements of Zaporozhets *et al.* (2003) who concluded that the Al(III) can selectively interact with hydroxycinnamic acids, the Al(III)-hydroxycinnamic acids complex is not a selective reaction. The *Echinacea purpurea* herb contains the flavonols such as quercetin, kaempferol, nicotiflorin and rutin (Kurkin *et al.*, 2010). These compounds can form complexes with AlCl_3 (Peğal *et al.*, 2014) and this can be the reason of shifting maximum wavelength of AlCl_3 -coneflower drug complexes from 351 nm. However, for assurance about this conclusion, in the next section of this study, the performance of the AlCl_3 method was compared to the European pharmacopoeia method.

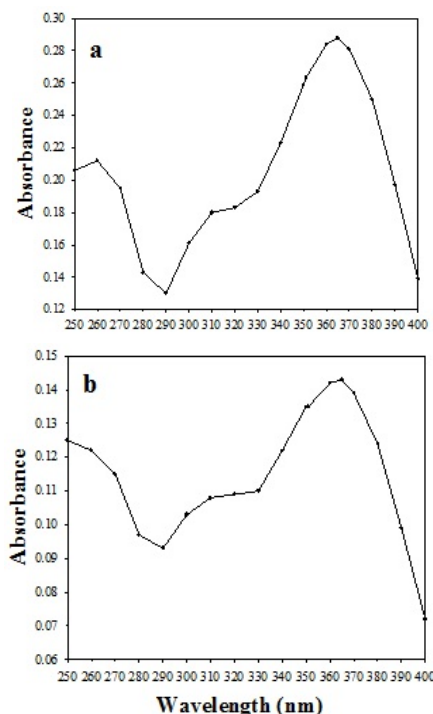


Figure 5. Absorbance spectrum of coneflower drugs: (a) powdered coneflower aerial parts, (b) powdered coneflower dry extract.

3.6. Comparison of AlCl₃ method with the method of European pharmacopoeia

Štefan et al. (2014) were evaluated the accuracy of European pharmacopoeia method for measuring the total hydroxycinnamic acids in Lamiaceae species. These authors concluded that good quality of the measurements at the lower analyte level, excellent resolution of blank and analyte signals, homogenous data material, ideal linear calibration and analytical evaluation functions, very low limit of detection and limit of quantification, and high precision and accuracy confirmed the high quality of investigated procedure as valuable tools in total hydroxycinnamic acids analysis (Štefan et al., 2014). Thus, we used the European pharmacopoeia method as the reference procedure for evaluating the performance and accuracy of AlCl₃ method in quantifying total hydroxycinnamic acids of coneflower drugs.

The total hydroxycinnamic acids of different *Echinacea* drugs measured by both AlCl₃ and EP methods are presented in Table 1. For all *Echinacea* drugs, the AlCl₃ method overestimated the total hydroxycinnamic acids in comparison with the amounts obtained using European pharmacopoeia method. The relative difference between amounts obtained from AlCl₃ method and amounts obtained using EP method was 50-130% (Table 2) and this reveal that accuracy of AlCl₃ method in measuring total hydroxycinnamic acids of *Echinacea* drugs is not suitable. Furthermore, since the willmott index of agreement (d) was less than 0.95 and the relative error (RE) was more than 0.25, the performance of AlCl₃ method in measuring total hydroxycinnamic acids of *Echinacea* drugs was very poor (Table 2).

Table 2. Comparing the amount of total hydroxycinnamic acids measured in different *Echinacea* drugs using AlCl₃ and European pharmacopoeia (EP) methods.

<i>Echinacea</i> drug	Total hydroxycinnamic acids (%)		Relative difference (%)*
	AlCl ₃ method	EP method	
Dry extract	3.7	2.4	54
Aerial parts	1.8	1.2	50
Dry extract	4.6	2.4	91
Flower	1.8	1.0	80
Aerial parts	2.7	1.4	92
Aerial parts	2.6	1.5	73
Aerial parts	3.5	1.8	94
Aerial parts	3.7	2.2	68
Aerial parts	2.8	1.5	86
Aerial parts	2.3	1.0	130
d			0.53
RE			0.83
Performance			Very poor

*Calculated as: $((\text{value of AlCl}_3 \text{ method} - \text{value of EP method}) / \text{value of EP method}) \times 100$

4. Conclusion

In current study, a simple method based on formation of complex between hydroxycinnamic acids and Al(III) was set in order to measuring total hydroxycinnamic acids of *Echinacea* drugs. Comparison of this method (AlCl₃ method) with European pharmacopoeia method (EP method) showed that accuracy and performance of AlCl₃ method in quantifying total hydroxycinnamic acids of *Echinacea* drugs was not suitable. Probably,

this can be due to non-selective reaction of hydroxycinnamic acids with Al(III), because coneflower flavonols such as quercetin, kaempferol, nicotiflorin and rutin also can form complex with Al(III) cation and this resulted in amounts of total hydroxycinnamic acids measured by AlCl₃ method be about 50-130% higher than those measured using EP method. In conclusion, the AlCl₃ method which its general principles was firstly developed by

Zaporozhets et al. (2003) and then used in a number of studies, is not a suitable and accurate method for measuring total hydroxycinnamic acids and quality control of *Echinacea* drugs.

5. References

- Alam, A., Subhan, N., Hossain, H., Hossain, M., Reza, H.M., Rahman, M. and Obayed Ullah, M. 2016. Hydroxycinnamic acid derivatives: a potential class of natural compounds for the management of lipid metabolism and obesity. *Nutrition & Metabolism*, 13(27): 1-13.
- Awika, J.M. and Rooney, L.W. 2004. Sorghum phytochemicals and their potential impact on human health. *Phytochemistry*, 65(9): 1199-1221.
- Burns, J., Gardner, P.T., Matthews, D., Duthie, G.G., Lean, M.E. and Crozier, A. 2001. Extraction of phenolics and changes in antioxidant activity of red wines during vinification. *Journal of Agriculture and Food Chemistry*, 49: 5797-5808.
- European Pharmacopoeia (Volume IV). 2016. Rosemary Leaf. pp. 351-352.
- French Pharmacopoeia. 2007. Galium Aparine. pp. 1-3.
- Fuentes, E., Báez, M.E., Bravo, M., Cid, C. and Labra, F. 2012. Determination of total phenolic content in olive oil samples by UV-visible spectrometry and multivariate calibration. *Food Analytical Methods*, 5: 1311-1319.
- Gallardo, M., Giménez, C., Martínez-Gaitan, C., Stockle, C.O., Thompson, R.B. and Granados, M.R. 2011. Evaluation of the VegSyst model with muskmelon to simulate crop growth, nitrogen uptake and evapotranspiration. *Agricultural Water Management*, 101: 107-117.
- Gregoris, E., Lima, G.P.P., Fabris, S., Bertelle, M., Sicari, M. and Stevanato, R. 2013. Antioxidant Properties of Brazilian Tropical Fruits by Correlation between Different Assays. *BioMed Research International*, 2013: 1-8.
- Kurkin, V.A., Akushskaya, A.S., Avdeeva, E.V., Velmyaikina, E.I., Daeva, E.D. and Kadentsev, V.I. 2010. Flavonoids from *Echinacea purpurea*. *Russian Journal of Bioorganic Chemistry*, 37(7): 905-906.
- Magnani, C., Isaac, V.L.B. Correa, M.A. and Salgado, H.R.N. 2014. Caffeic acid: a review of its potential use in medications and cosmetics. *Analytical Methods*, 6: 3203-3210.
- Nazer, A.I., Kobilinsky, A., Tholozan, J.L. and Dubois-Brissonnet, F. 2005. Combinations of food antimicrobials at low levels to inhibit the growth of *Salmonella* sv. typhimurium: a synergistic effect?. *Food Microbiology*, 22: 391-398.
- Ndhlala, A.R., Moyo, M. and Van Staden, J. 2010. Natural antioxidants: fascinating or mythical biomolecules? *Molecules*, 15: 6905-6930.
- Pełkal, A. and Pyrzynska, K. 2014. Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. *Food Analytical Methods*, 7: 1776-1782.
- Razzaghi-Asl, N., Garrido, J., Khazraei, H., Borges, F. and Firuzi, O. 2013. Antioxidant Properties of Hydroxycinnamic Acids: A Review of Structure-Activity Relationships. *Current Medicinal Chemistry*, 20: 4436-4450.
- Štefan, M.B., Rodríguez, J.V., Blažeković, B., Kindl, M. and Vladimir-Knežević, S. 2014. Total Hydroxycinnamic Acids Assay: Prevalidation and Application on Lamiaceae Species. *Food Analytical Methods*, 7(2): 326-336.
- Stockle, C.O., Kjelgaard, J. and Bellocchi, G. 2004. Evaluation of estimated weather data for calculating Penman-Monteith reference crop evapotranspiration. *Irrigation Sciences*, 23: 39-46.
- Vanzani, P., Rossetto, M., De Marco, V., Sacchetti, L.E., Paoletti, M.G. and Rigo, A. 2011. Wild Mediterranean Plants as Traditional Food: A Valuable Source of Antioxidants. *Journal of Food Science*, 76(1): 46-51.
- Willmott, C.J. 1982. Some comments on the evaluation of model performance. *Bulltin of American Meteorological Society*, 63: 1309-1313.
- Zaporozhets, O.A., Krushinskysya, E.A., Barvinchenko, V.N., Lipkovskaya, N.A. and Pogorelyi, V.K. 2003. Spectrophotometric determination of hydroxycinnamic acid and related compounds in *Echinacea* preparations. *Pharmaceutical Chemistry Journal*, 37(12): 632-636.
- Zaporozhets, O.A., Krushynska, O.A., Lipkovska, N.A. and Barvinchenko, V.N. 2004. A New Test Method for the Evaluation of Total Antioxidant Activity of Herbal Products. *Journal of Agriculture and Food Chemistry*, 52: 21-25.
- Zolgharnein, J., Niazi, A., Afuizadeh, S. and Zamani, K. 2010. Determination of Cichoric Acid as a Biomarker in *Echinacea purpurea* Cultivated in Iran Using High Performance Liquid Chromatography. *Chinese Medical Journal*, 1: 23-27.