Journal of PharmaSciTech
ISSN: 2231 3788 (Print)
2321 4376 (Online)
Research Article

Validated HPLC Methods for Determination of Echinacea Dry Extract in Pharmaceutical Formulations

Mohamed Aly Amin Ahmed Ibrahim[1]*, Mounir Zaky Saad[2], Alaa El Sayed Ahmed Amin[3]

[1]*Methodology senior, Mepaco Pharmaceutical Company, 15 Omar Ibn El khattab street, Hefna, Belbies, El Sharkia, Egypt*
[2]*Department of Analytical Chemistry, Chemistry Department, Faculty of Science, Zagazig University.*
[3]*Department of Analytical Chemistry, Dean of Faculty of Science, Benha University*
Correspondence: alichem2005@yahoo.com (002-01009560368)

Abstract

Two simple, precise and rapid HPLC methods were developed for estimation of Echinacea extract alone and in pharmaceutical dosage forms. The two chromatographic separations were conducted on Shimadzu (Prominence LC20 UFLC XR) connected with PDA detector; using column Phenomenex, Prodigy, ODS3, 5 μm, 100 A, (250 x 4.6 mm). However, two different isocratic mobile phases were used. First mobile phase consisted of 0.01 M KH2PO4 pH 2.5: acetonitrile in ratio of 85: 15 (v/v). The buffer solution of (0.01 M KH2PO4 pH 2.5) is composed of 1.3609 gm of potassium dihydrogen orthophosphate in 900 ml of water, adjusted to pH 2.5 with phosphoric acid 85%, diluted to 1000 ml with water and filtered through 0.45 µm nylon membrane filter. The mobile phase was delivered to the system at a flow rate of 2 ml/min. An injection volume of 10 μl was used for Echinacea extract. The detection was carried out by PDA detector 330 nm. The column temperature was at 40ºC. The calibration curve of Echinacea extract in mobile phase was linear with correlation coefficient (r^2) = 0.99928; over a concentration range of 200–2000 g/ml; with a retention time (Rt) of 2.634 and 10.690 min. The percentage recovery of Echinacea extract was 98.34, 98.46, 101.95, 98.65 and 101.39%. The relative standard deviation (RSD) was found to be less than 2.

The second isocratic mobile phase consisted of 0.1% formic acid: acetonitrile in ratio of (80: 20) (v/v). The mobile phase was delivered to the system at a flow rate of 1.5 ml/min. An injection volume of 10 μl was used for Echinacea extract. The detection was carried out by PDA detector 330 nm. The column temperature was at 40ºC. The calibration curve of Echinacea extract in mobile phase was linear with correlation coefficient (r2) = 0.999718; over a concentration range of 500–2000 g/ml; with a retention time of 3.547, 10.039 minutes. The percentage recovery of Echinacea extract was 101.20 and 99.81%. The relative standard deviation (RSD) was found to be less than 2. Both developed methods were validated and applied for determination of Echinacea extract in pharmaceutical dosage form, successfully.

Keywords: UFLC; Echinacea extract; PDA detector; method validation, pharmaceutical dosage form.

Introduction

Echinacea is a genus of flowering plants endemic to North America; species used medicinally include *Echinacea angustifolia, E. pallida* and *E. purpurea* [1]. These species and their extracts are most often used for the prevention and treatment of upper respiratory tract infections, such as cold, flu, and as an immune stimulant [2]. Phytochemical constituents include the polyphenols, alkamides, glycoproteins, and polysaccharides [3-4]. Root and aerial plant parts may be used raw or in formulations, while homeopathic remedies may include the whole plant [5]. Determination of major phenolic compounds in Echinacea as raw material and in finished products by High-Performance Liquid Chromatography makes it an important marker for possible standardization and QC purposes [3, 6-8].

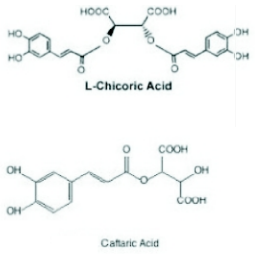

Figure 1. Chemical structure of polyphenols in Echinacea

Polyphenolic compounds in Echinacea play a critical role in stimulating the immune system as shown in Figure 1.These are often of most importance during the quantification and qualification of Echinacea products [2, 9-12].

Materials and Methods

Materials

All chemicals and reagents used were HPLC grade. Pure standards of Echinacea extract were obtained from Hetero Drugs, Hyderabad. Acetonitrile 200 far UV was HPLC grade from Romil. Ethanol absolute was HPLC grade from Fisher Chemical. Hydrochloric acid was HPLC grade from Fisher Scientific UK. Potassium dihydrogen orthophosphate was from EL Nasr Pharmaceuticals Chemicals. Formic acid solution was HPLC from Sigma Aldrich. Pharmaceutical products containing Echinacea Dry extract were purchased from the Egyptian pharmaceutical market. The pharmaceutical formulations were:

1) Immu-C sachets, Batch number 880414. Each 5 gm contains 200 mg Echinacea Dry extract, manufactured by Arab Company for Pharmaceuticals and Medicinal Plants (Mepaco- Medifood) (Cairo, Egypt).

2) Immulant capsules, Batch number 1360414. Each capsule contains 175 mg Echinacea Dry extract, manufactured by Arab Company for Pharmaceuticals and Medicinal Plants (Mepaco-Medifood) (Cairo, Egypt).

3) Immulant syrup, Batch number 130820. Each 100 ml contains 1670 mg Echinacea Dry extract, manufactured by Al-Debeiky for Pharmaceutical industries for Arab Company for Pharmaceuticals and Medicinal Plants (Mepaco- Medifood) (Cairo, Egypt).

4) Immunvita capsules, Batch number 9044001. Each capsule contains 210 mg Echinacea Dry extract, manufactured by PharaoniaPharmaceuticals (New Borg El-Arab City 3rd industrial Zone block 16 part 1) for EMA Pharm Pharmaceuticals.

5) Immuno Flu capsules, Batch number 4314. Each capsule contains 111.2 mg Echinacea Dry extract, manufactured by Global Napi Pharmaceuticals (2nd industrial Zone, 6th of October City- Egypt).

Analysis of Echinacea Extract in Pharmaceutical Dosage Forms

Chromatographic conditions

Both methods were conducted on Shimadzu LC prominence 20 (UFLC XR) connected with PDA detector. Shimadzu Lab solutions software was used for data acquisition. Column Phenomenex, Prodigy, ODS3, 5μ, 100A, (250$\times$4.6 mm) was used as a stationary phase. The column was maintained at 40ºC temperature.

The first method mobile phase was isocratic consisted of 0.01 M KH_2PO_4 pH 2.5: acetonitrile in ratio of (85: 15 v/v). 0.01 M KH_2PO_4, pH 2.5 is composed of 1.3609 g of potassium dihydrogen orthophosphate in 900 ml of water, adjusted to pH 2.5 with phosphoric acid 85%, diluted to 1000 ml with water and filtered through 0.45 µm nylon membrane filter delivered to the system at a flow rate of 2 ml/min. An injection volume of 10 μl was used for Echinacea extract. The detection was carried out by PDA detector 330 nm, run time was 13 min.

The second method mobile phase was isocratic consisted of 0.1% formic acid: acetonitrile in ratio of 80:20 (V/V). The mobile phase was delivered to the system at a flow rate of 1.5 ml/min. An injection volume of 10 μl was used for Echinacea extract. The detection was carried out by PDA detector 330 nm, run time was 17 min.

Preparation of Solvent

15 ml of concentrated hydrochloric acid was dissolved into 1000 ml distilled water.

Preparation of stock and working standard solution

A 1000 mg of Echinacea extract standard was weighed and transferred into a 100 ml clean and dry volumetric flask and 30 ml of the solvent (previously stated) was added. The volume was completed with absolute ethanol; and sonicated for 15 minutes until Echinacea extract was completely dissolved. Different dilutions were prepared with diluent to give concentration range of 200 – 2000 μg/ml for the first method and 500 – 2000 μg/ml for the second method.

Analytical Method Validation

Selectivity

Both methods provided an indication of the selectivity of the procedure. The method is to be selective, if the main peak retention time is well resoluted from any other peak by resolution of minimum 2. This was done by injecting placebo and comparing it with that of standard and the test samples. The peak purity was ascertained by using of PDA scanning.

Linearity

It is defined by the correlation coefficient, which should be found not less than 0.99, using peak area responses. Linearity for single point standardization should extend to at least 20% beyond the specification range and include the target concentration. This was performed by preparing seven different concentrations (for the first method) and five different concentrations (for the second method), and then making three replicates of each concentration. The linear working range was determined from the constructed standard calibration curve.

Intraday Precision

This study was conducted by performing multiple analysis on a suitable number of portions of a homogeneous sample. This was performed by assaying multiple aliquots with the same concentration starting from the first step to the final step of analysis. The analytical precision of the method was determined by the relative standard deviation.

Inter-day Reproducibility (Method Ruggedness)

It is the degree of reproducibility determined by analysis of samples from homogeneous lot of materials, under different but typical test conditions. The method is to be rugged, at any item if the pooled %RSD of the total number of replicates that have been made in this item is within the acceptance criteria. Three replicates of a single sample of powder material are used for each determination. On the first day, three replicates while on the second day, three replicates; then finally on third day, another replicates of freshly prepared test from the same sample are analyzed, using the same conditions.

Accuracy and Recovery

Accuracy was evaluated by spiking standard solution. The measurements are made at a concentration of standard mix, which is found to be the target concentration, and at suitable intervals around this point. The test samples was spiked with known quantities of standard Echinacea extract using three determinations over three concentrations level covering the specified range. Relative recoveries of Echinacea extract used in the standards were evaluated by comparing their peak area with those obtained from the calibration curve equation.

Results and Discussion

Both proposed HPLC methods required fewer reagents and materials, and were simple and very rapid. They could be used in quality control test in pharmaceutical industries. The chromatogram of *Echinacea* extract using the first method was shown in Figure 2. (Retention time: 2.634 and 10.690 min); while its chromatogram using the second method as shown in Figure 3 (Retention time: 3.547 and 10.039 min).

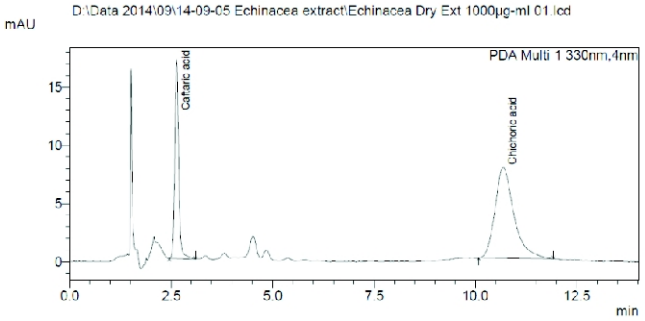

Figure 2. HPLC chromatogram of Echinacea extracts (first method)

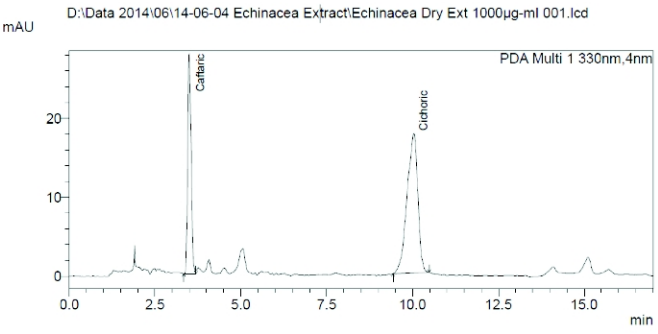

Figure 3. HPLC Chromatogram of Echinacea extracts (second method)

Specificity

The PDA chromatograms of the Echinacea extract in standard and sample were recorded. In the chromatograms of the formulations, some additional peaks were observed which may be due to excipients present in the formulations. These peaks however did not interfere with the standard peaks,which demonstrate that both assay

methods were specific. Furthermore, the purity of the peaks was studied by peak purity studies. The results revealed that the peak is free from interferences, which shows that both HPLC methods are specific.

Linearity

The response for the detector was determined to be linear over the range of 200-2000 g/ml (200, 300, 500, 800, 1000, 1600 and 2000) for Echinacea extract as shown in Figure 4.

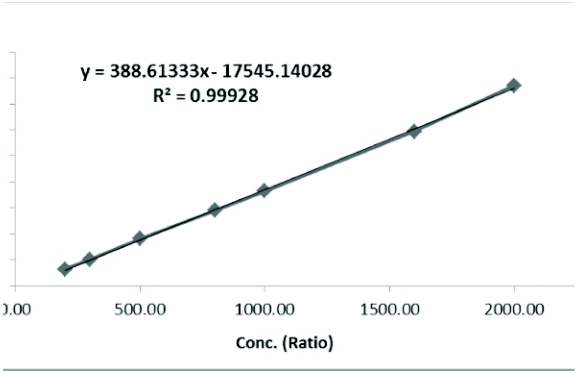

Figure 4. Calibration curve of standard polyphenols using first method

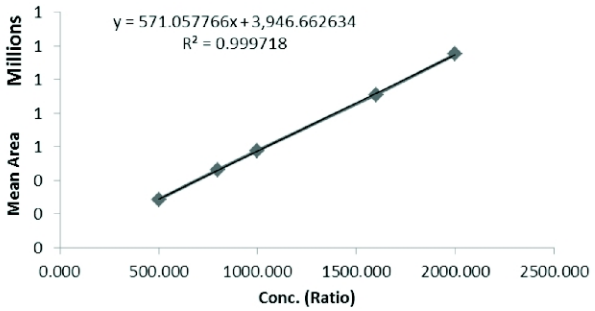

Figure 5. Calibration curve of standard polyphenols using second method

The response for the detector was determined to be linear over the range of 500-2000μg/ml (500, 800, 1000, 1600 and 2000) for Echinacea extract as shown in Figure 5. Each of the concentrations was injected in triplicate to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. They were represented by the linear regression equation:

$Y_{\text{Echinacea extract}} = 388.61333x - 17545.14028$ ($r^2 = 0.99920$) (First method)

$Y_{\text{Echinacea extract}} = 571.057766x + 3946.662634$ ($r^2 = 0.9997$) (Second method)

Slopes and intercepts were obtained by using regression equation ($Y = mx + c$) and least square treatment of the results used to confirm linearity of the method developed.

Quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (3:1) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (10:1). The LOD of Echinacea extract found to be 33.33 μg/ml. The LOQ of Echinacea extract found to be 100 g/ml.

Solution stability

In this study, the mobile phases, the standard solutions, and the sample solution were subjected to long term (3 days) stability studies. The stability of these solutions was studied by performing the experiment and looking for changes in separation, retention, and asymmetry of the peaks which were then compared with the pattern of the chromatogram of freshly prepared solutions

System suitability

The resolution, capacity factor, theoretical plates/meter, Rt values and peak symmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of the above drug combinations System suitability parameters might be fall within $\pm$ 3% standard deviation range during routine performance of the methods.

Conclusion

Both methods are simple, specific, precise, selective, and easy to perform and require short time to analyze the samples. Low limit of quantification and limit of detection makes this method suitable for use in quality control. This method enables determination of Echinacea extract because of good separation and resolution of the chromatographic peaks. Both methods were found to be accurate, precise, linear, and rugged.

Acknowledgement

The authors are grateful to Mepaco-Medifood Pharmaceutical Company (El Sharkia, Egypt) for her ultimate support for Research and Development team.

Conflict of interest

The authors declare no conflict of interest.

References

[1].Binns SE, Baum BR, Arnason JT. A taxonomic revision of Echinacea (Asteraceae: Heliantheae). Syst Bot Syst Bot 2002; 27:610–32.

[2].Upton R, Graff A. Echinacea Monograph. American Herbal Pharmacopoeia and Therapeutic Compendium, Scott's Valley, CA: AHP, 2005.

[3].Bauer R. Chemistry, analysis and immunological investigations of Echinacea phytopharmaceuticals, in: Immunomodulatory Agents from Plants, Wagner H (Eds.), Switzerland, 1999. pp. 41–88.

[4].Lopes-Lutz D, Mudge E, Ippolito R, Brown P, Schieber A. Purification of alkylamides from Echinacea angustifolia (DC.) Hell. roots by high-speed countercurrent chromatography. J Agric Food Chem 2011; 59:491–94.

[5].Bauer RJ. Echinacea sp. Herbs Spices Medicinal Plants. 1999; 6: 51–62.

[6].Pellati F, Benvenuti S, Magro L, Melegari M, Soragni F. Analysis of phenolic compounds and radical scavenging activity of Echinacea spp. J Pharm Biomed 2004; 16; 35:289–301.

[7].Thygesen L, Thulin J, Mortensen A, Skibsted LH. Antioxidant activity of cichoric acid and alkamides from Echinacea purpurea, alone and in combination. Food Chem 2007; 101:74–81.

[8].Gilroy CM, Steiner JF, Byers T, Shapiro H, Georgian W. Echinacea and truth in labeling. Arch Intern Med 2003; 163: 699–704.

[9].NSF International. Phenolics in Echinacea by HPLC: 2004 INA method 106.000, http://www.nsf.og/business/ina/echinacea.asp?program=INA, Accessed on January 9, 2009.

[10].Luo X, Chen B, Yao S, Zeng J. Simultaneous analysis of caffeic acid derivatives and alkamides in roots and extracts of Echinacea purpurea by high-performance liquid chromatography-photodiode array detection-electrospray mass spectrometry. J Chromatogr A 2003; 986:73–81.

[11].Perry, N.B., Burgess, E.J. and Glennie V. Echinacea standardization: analytical methods for phenolic compounds and typical levels in medicinal species. J Agric FoodChem 2001; 49:1702–06.

[12].Bergeron C, Livesey JF, Awang DVC, Arnason JT, Rana J, Baum BR, Letchamo W. A quantitative HPLC method for the quality assurance of Echinacea products on the North American market. Phytochem Anal 2000; 11:207–15.