ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 07 | July - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SIMULTANEOUS SEPARATION OF GALLIC ACID, PROTOCATECHUIC ACID, RUTIN, MANGIFERIN AND CHROMATOGRAPHIC PROFILING OF SOME INDIAN MEDICINAL PLANTS

Medicinal Plants

Pavan Kumar Mujawdiya — Department of Biological Sciences, Birla Institute of Technology and Science-Pilani, Hyderabad campus, Jawahar Nagar, Hyderabad

Pritee Pandey — Department of Biological Sciences, Birla Institute of Technology and Science-Pilani, Hyderabad campus, Jawahar Nagar, Hyderabad

Shashwat Sharad — Xcellence in Bio Innovations & Technologies Pvt. Ltd., BITS Pilani Hyderabad Campus, Jawahar Nagar, Shameerpet Mandal, Dist, Hyderabad, Telangana

Suman Kapur* — Department of Biological Sciences, Birla Institute of Technology and Science-Pilani, Hyderabad campus, Jawahar Nagar, Hyderabad *Corresponding Author

ABSTRACT

Background: Plants and plant-based extracts have been used across the globe for management of various ailments and disorders. The biological and pharmacological activities of plants and their extracts are primarily attributed to polyphenols, flavonoids, and other secondary metabolites. High Pressure Liquid Chromatography (HPLC) is a reliable, sensitive, and quick method to identify bioactive ingredients in complex plant extracts.**Methods:** In the present study, we have developed a quick and sensitive HPLC method for separation of four polyphenols, namely, gallic acid, protocatechuic acid, rutin, and mangiferin. Further, we used the developed HPLC method for the analysis of plant extracts obtained from Asparagus racemosus, Azadirachta indica, Capsicum annuum, Nigella Sativa, Phyllanthus niruri, Rosmarinus officinalis, Rheum palmatum, Withania somnifera, Withania somnifera, and Zingiber officinale. **Results:** The HPLC method developed in the present study was highly effective in separating gallic acid, protocatechuic acid, rutin, mangiferin. The method also provides excellent phytoconstituent fingerprinting of complex plant extracts of medicinal plants. **Conclusion:** The gradient mode HPLC method developed in the present study is highly useful for identification of plant polyphenols in complex plant-based extracts and generating HPLC chromatograms for complex plant extracts. The present method, thus, can be used for phytoconstituent profiling, quality control of herbal drugs, and assessing adulteration of plant extract or nutraceuticals.

KEYWORDS

HPLC, Plant Extracts, Polyphenols, Medicinal Plants, Nutraceuticals

INTRODUCTION

Plants are one of the major reservoirs of natural molecules and a primary source of naturally-derived therapeutic molecules. Molecules with complex structures are common in plants and therefore offer an unmatched opportunity to discover new drug molecules. A report by the World Health Organization estimates that more than 80% of the world population uses plants for complementary and alternative medicines (CAMs) [1]. CAMs are easily available to the masses, suit the local culture, and provide relief from a wide variety of ailments due to the synergistic action of CAM components. However, validation of CAMs through scientific approaches is essential to establish the therapeutic efficacy and confirm the long-held traditional therapeutic effects [2]. High performance liquid chromatography (HPLC) is a robust, advanced, and versatile analytical method for separation and identification of phytoconstituents present in plant extracts. In HPLC, various phytoconstituents/compounds show a different migration when passed through a common stationary phase using a common mobile phase in either isocratic or gradient mode [3]. HPLC is also a method of choice for separation and identification of flavonoids because it is performed at room temperature and therefore prevents the thermal decomposition of phytoconstituents, a common phenomenon observed at higher temperatures. The role of HPLC in quality control has gained considerable acceptance in recent times. As a quality control tool, it can help in quality control of herbal products and formulations that contain extracts taken from various plants. Therefore, development of novel, efficient, cost-effective, and optimized HPLC methods that help in simultaneous identification of several compounds can greatly benefit manufacturers involved in the production of herbal products, nutraceuticals, and other plant-based products [4]. HPLC also alleviates the need for derivatization of phytoconstituents, an essential requirement for Gas-chromatography, thus saves considerable time, chemicals, and manpower [5]. However, separation and identification of complex plant extracts using HPLC is a tedious and time-consuming job because optimization of HPLC conditions such as mobile phase, stationary phase, and other HPLC conditions (run time, column temperature, and mode of run) require extensive optimization. Moreover, each phytoconstituent/plant extract requires separate optimization to obtain a good resolution, thus making the whole process cumbersome. Therefore, a single optimized HPLC method that can separate several compounds or several plant extracts under common optimized HPLC conditions can significantly reduce run time, optimize precious resources, and save manpower. The present study is an attempt to optimize a single optimized HPLC protocol for ten plant extracts and four compounds.

MATERIALS AND METHODS

Procurement of Plant material:

All plant extracts used in the present study were purchased from a GMP certified manufacturer based in India.

Chemicals and Reagents:

HPLC mobile phase was prepared in HPLC grade solvents and buffers. Acetonitrile was purchased from SDFCL (HPLC grade) and Millipore water was used to prepare all buffers. Mobile phase was filtered using a 0.45µm filter (Cellulose acetate; Merck) and degassed in a sonicator for 10 minutes before it was used.

HPLC analysis of standards and plant extracts

The optimization of HPLC conditions were carried out in two separate phases. In the first phase, we optimized the separation conditions for standard polyphenols, namely, gallic acid, protocatechuic acid, rutin, and mangiferin. In the second phase, we used the optimized conditions for HPLC analysis of plant-extracts obtained from Asparagus racemosus, Azadirachta indica, Capsicum annuum, Nigella Sativa, Phyllanthus niruri, Rosmarinus officinalis, Rheum palmatum, Withania somnifera, Withania somnifera and Zingiber officinale. HPLC was performed on the Shimadzu HPLC system using Kinetex C_{18} column (150mm x 4.6mm, 100 Å, 5µm) in a gradient mode with a flow rate of 1.5ml/min and detection wavelength of 254 nm. The aqueous mobile phase was prepared by adding 68mg KH_2PO_4 and 250 µl ortho-phosphoric acid in 500ml Millipore water. Other standard operating procedures for HPLC were followed. The organic mobile phase contained 100% acetonitrile. Injection volume was 20 µl and the run time was 45 minutes. The gradient program was as follows: 0.01 min A: 70% B: 30%; 25 min A: 60% B: 40%; 28 min A: 4% B: 96%; 38 min A: 4% B: 96%; 43 min A: 70% B: 30%; 45 min A: 70% B: 30%.

RESULTS

HPLC separation of phytoconstituents and complex plant extracts using a binary solvent system is a preferred method of choice. For method development, we used a binary solvent system comprising acidified water and organic solvent acetonitrile. In the present study, we used a Kinetex C_{18} column (150mm x 4.6mm, 100 Å, 5µm) and the HPLC separation was performed in a gradient mode with a flow rate of

1.5ml/min and detection wavelength of 254 nm. We first separated four polyphenols, gallic acid, protocatechuic acid, rutin, and mangiferin, and then used the developed method to analyze the separation pattern of some of the most widely used plants in the complementary and alternative medicinal (CAM) system, namely, Asparagus racemosus, Azadirachta indica, Capsicum annuum, Nigella Sativa, Phyllanthus niruri, Rosmarinus officinalis, Rheum palmatum, Withania somnifera, Withania somnifera and Zingiber officinale.

An excellent separation was achieved for all polyphenols using the HPLC solvent system and parameters described above with a retention time of 2.71 minutes (gallic acid, Fig. 1), 7.31 minutes (mangiferin, Fig. 2), 8.8 minutes (rutin, Fig. 3), and 10.78 minutes (protocatechuic acid, Fig. 4). We obtained excellent separation of all four polyphenols during a combined run, suggesting that the developed method offers an excellent tool for separation and identification of four polyphenols without any overlapping peaks (Fig. 5). We then used the optimized HPLC conditions to generate the HPLC chromatograms for complex plant extracts rich in phytoconstituents. It was observed that all plant extracts used in the present study offered good separation of phytoconstituents and a chromatogram generated showed a variety of peaks of different areas under the curve (AUC) (Fig.6-Fig.15).

We also confirmed the presence of protocatechuic acid in Zingiber officinale (Fig.10) and gallic acid in Phyllanthus niruri (Fig.14). The results of the present study shows that the developed HPLC method can be useful in separation and characterization of polyphenols (gallic acid, protocatechuic acid, rutin, and mangiferin) in complex plant extracts and may help in both qualitative and quantitative analysis. The method can also be used for quality control and to detect the presence of contaminants in herbal formulations and nutraceuticals.

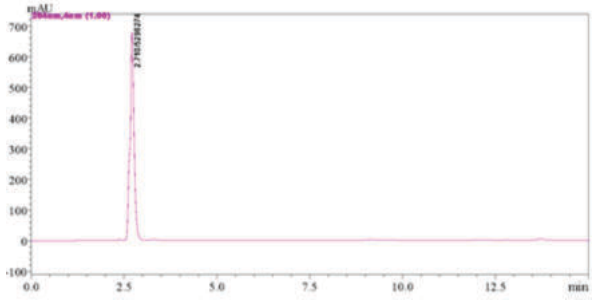

Figure 1: HPLC chromatogram of gallic acid at 100µg/ml concentration under binary gradient conditions at a flow rate 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

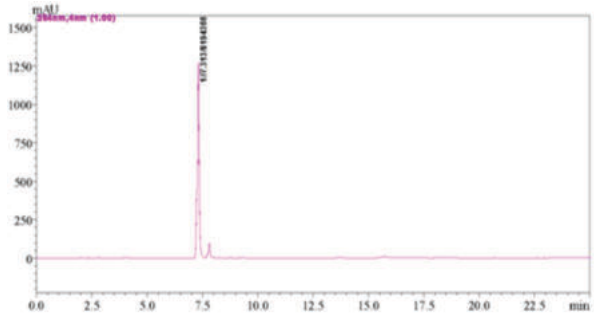

Figure 2: HPLC chromatogram of mangiferin at 100µg/ml concentration under binary gradient conditions at a flow rate 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

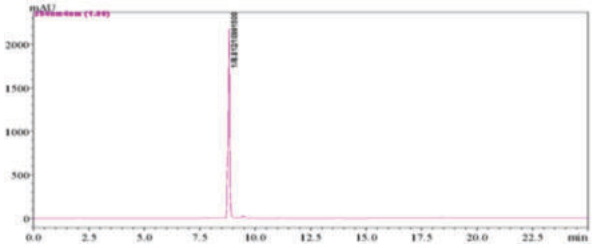

Figure 3: HPLC chromatogram of rutin at 100µg/ml concentration under binary gradient conditions at a flow rate 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

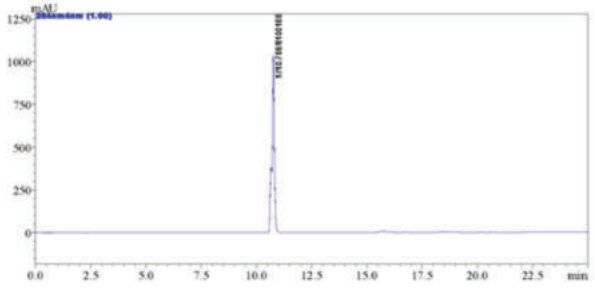

Figure 4: HPLC chromatogram of protocatechuic acid at 100µg/ml concentration under binary gradient conditions at flow rate 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

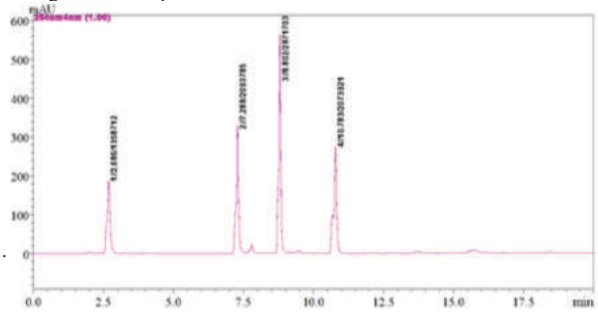

Figure 5: HPLC chromatogram a mixture of gallic acid, mangiferin, rutin, and protocatechuic acid at 100µg/ml concentration under binary gradient conditions at a flow rate 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

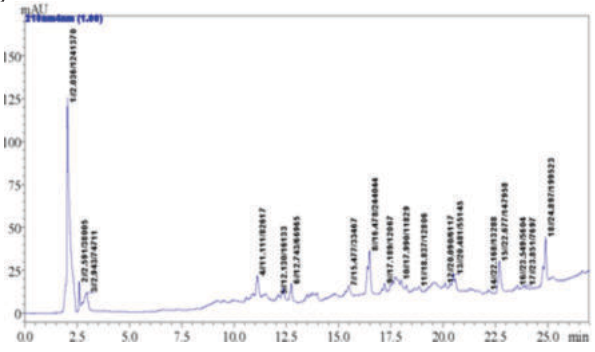

Figure 6: HPLC chromatogram of Withania somnifera at 100µg/ml concentration under binary gradient conditions at a flow rate of 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

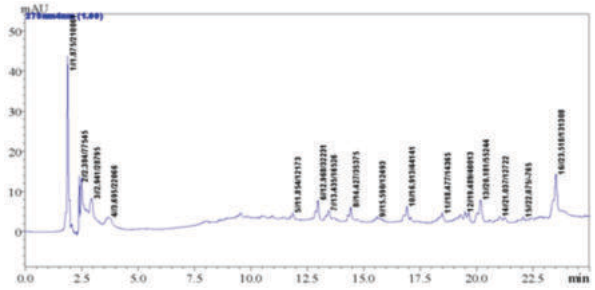

Figure 7: HPLC chromatogram of Azadirachta indica at 100µg/ml concentration under binary gradient conditions at a flow rate of 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water

and the organic mobile phase was 100% Acetonitrile.

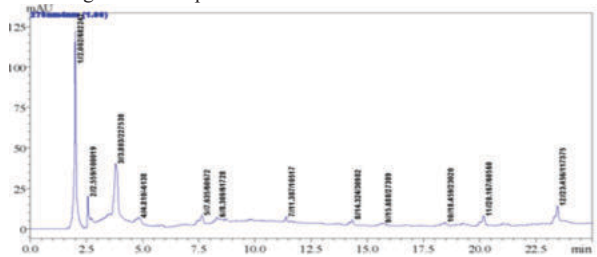

Figure 8: HPLC chromatogram of Asparagus racemosus at 100µg/ml concentration under binary gradient conditions at a flow rate 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

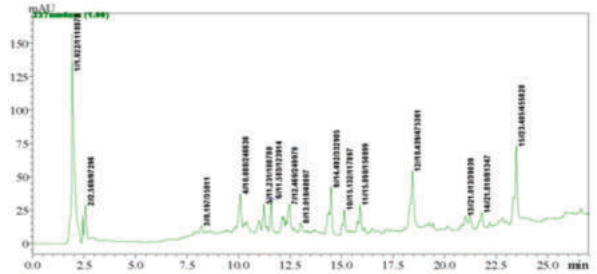

Figure 9: HPLC chromatogram of Withania somnifera at 100µg/ml concentration under binary gradient conditions at a flow rate of 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

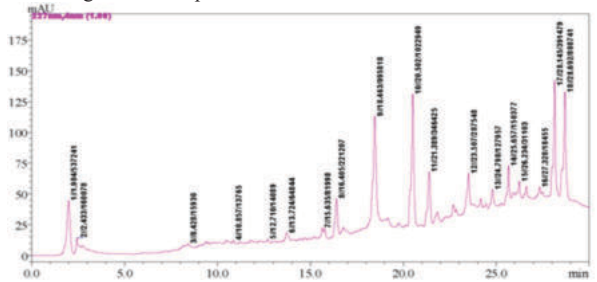

Figure 10: HPLC chromatogram of Zingiber officinale at 100µg/ml concentration under binary gradient conditions at a flow rate 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

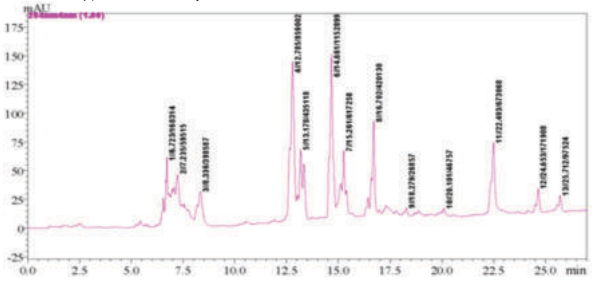

Figure 11: HPLC chromatogram of Rheum palmatum at 100µg/ml concentration under binary gradient conditions at a flow rate 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

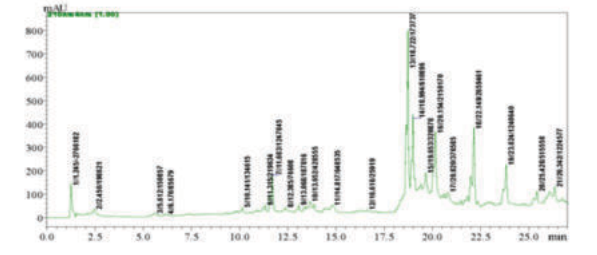

Figure 12: HPLC chromatogram of Rosmarinus officinalis at 100µg/ml concentration under binary gradient conditions at a flow rate 1.5 ml/min and run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

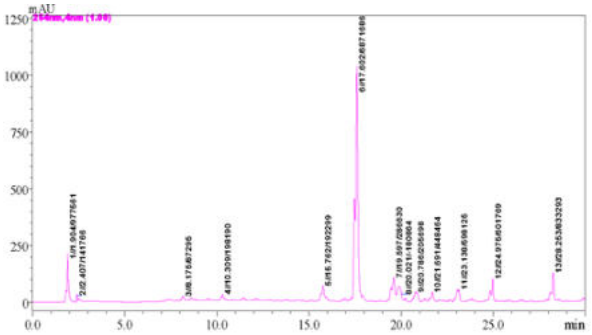

Figure 13: HPLC chromatogram of Capsicum annuum at 100µg/ml concentration under binary gradient conditions at a flow rate 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

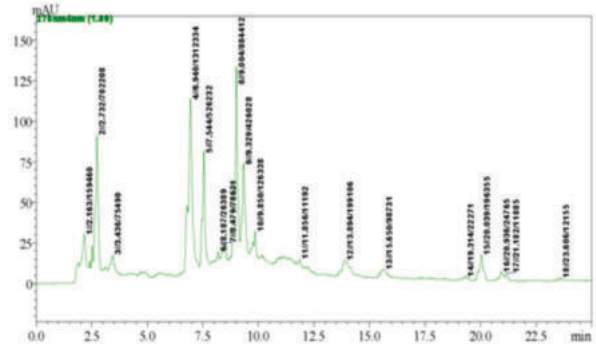

Figure 14: HPLC chromatogram of Phyllanthus niruri at 100µg/ml concentration under binary gradient conditions at a flow rate 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

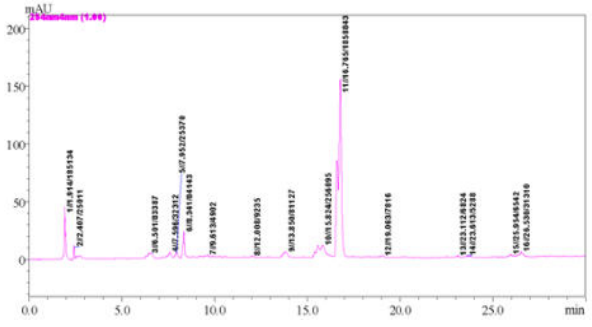

Figure 15: HPLC chromatogram of Nigella Sativa at 100µg/ml concentration under binary gradient conditions at a flow rate 1.5 ml/min and a run time of 45 minutes. The injection volume was 20µl. The aqueous mobile phase was 0.1% phosphoric acid in distilled water and the organic mobile phase was 100% Acetonitrile.

DISCUSSION

Plant extracts are highly complex in nature and contain a variety of phytoconstituents such as polyphenols, flavonoids, saponins, and alkaloids. Since these phytoconstituents possess several biological and pharmacological activities, their separation and identification has gained considerable attention in recent years [6]. It is pertinent to mention that the quality and efficacy of herbs largely depends on the geographical and climatic conditions. Further, time of collection and storage conditions can also change the herb quality. Thus, maintaining constant quality control in herb-based formulations is a challenging task [7]. A proper quality check at the production point also ensures safety and efficacy of plant-based products, which is highly essential for the therapeutic effects of plant extracts [8]. HPLC has emerged as a primary tool for obtaining fingerprint spectra of plant extracts due to

high sensitivity, ease of use, automation, and excellent resolution abilities [9]. Therefore, there is high demand for optimized HPLC methods that help in quick, reliable, and accurate identification of phytoconstituents. These optimized HPLC methods are extremely useful in herbal and food industries in quality control applications. In the present study, a simple reverse-phase HPLC protocol for qualitative and quantitative determination of four phytoconstituents (gallic acid, protocatechuic acid, rutin, and mangiferin) and ten plant extracts was developed. In the present method, the chromatographic separation was achieved using a Kinetex C_{18}column (150mm x 4.6mm, 100 Å, 5µm) in a gradient mode using phosphoric acid and acetonitrile as aqueous and organic mobile phases, respectively. The developed method is highly selective and separates four polyphenols with an excellent resolution. Moreover, the developed method has excellent resolution for ten plant extracts used in the present study with a minimum of 10 individual peaks observed for each plant extract. Therefore, the HPLC method developed in the present study can be used for quality control in pharmaceutical, food, and herbal industries. The developed method also holds promise in identifying adulteration in plant extracts. Further, the method can be employed for both qualitative and quantitative identification of gallic acid, protocatechuic acid, rutin, and mangiferin in plant extracts.

REFERENCES

[1] Boligon AA, Athayde ML. Importance of HPLC in Analysis of Plants Extracts. Austin Chromatogr. 2014;1(3): 2.
[2] Carmona F, Pereira AMS. Herbal medicines: old and new concepts, truths and misunderstandings. Revista Brasileira de Farmacognosia. 2013;23(2), 379-385.
[3] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 2011;8(1):1-10.
[4] Turova P, Rodin I, Shpigun O, Stavrianidi A. A new PARAFAC-based algorithm for HPLC-MS data treatment: herbal extracts identification. Phytochem Anal. 2020;31(6):948-956.
[5] Numonov SR, Qureshi MN, Aisa HA. Development of HPLC Protocol and Simultaneous Quantification of Four Free Flavonoids from Dracocephalum heterophyllum Benth. Int J Anal Chem. 2015;2015:503139.
[6] Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants (Basel). 2017;6(4):42.
[7] Zou HB, Du AQ, Zhang XL, Wei PH, Lu WJ, Yang GS, Hassan YA. Quality control methodology and their application in analysis on HPLC fingerprint spectra of herbal medicines. Chromatography Research International, vol. 2012, Article ID 851792, 12 pages, 2012. https://doi.org/10.1155/2012/851792
[8] Balekundri A, Mannur V. Quality control of the traditional herbs and herbal products: a review. Futur J Pharm Sci. 2020;6:67. https://doi.org/10.1186/s43094-020-00091-5
[9] Kalili KM, de Villiers A. Recent developments in the HPLC separation of phenolic compounds. J Sep Sci. 2011;34(8):854-76.