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Simultaneous determination of rosmarinic acid and ferrulic acid by high performance liquid chromatography

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ABSTRACT

A simple, rapidisocratic and sensitive high performance liquid chromatographic method with reverse-phase column for the simultaneous determination of Rosemarinic acid and Ferrulic acid developed.An ODS column(100mmx4.6mm ID,3µ)was used as the stationary phase and the mobile phase consist of ammonium hydrogen phosphate solution with pH-3.0±0.1& Acetonitrile(8:3). Flow rate 1.0ml/min ands UV absorbance was set at 320 nm. We found the linear curve 0.1-200 µg/ml for Rosemarinic acid and Ferrulic acid with goodresolution between two peaks. The Average recovery of Rosemarinic acid is 99.2%& 98.6% for ferrulic acid. The results for all compounds also showed good solutionstability up to 48hrs.. The Assay method was successfully applied to the determination of Rosemarinic acid content in rosemary leaves as well as extract. This method can be said to be more economical & rapid as compared to other methods reported in literature.

KEYWORDS

Rosmarinic acid;
Ferrulic acid**;**
HPLC-UV method;
UV-VIS detector;
Rosmarinus officinalis L.

INTRODUCTION

Rosmarinus officinalis L. (Lamiaceae),known as Rosemary, is used worldwide as afood-flavoring agentand in folk medicine. Anumber of important bioactivities have been assignedto Rosemary's extracts, such as antioxidant[1,2], antimicrobial[3], hepatoprotective[4], anti-inflammatory[5], antitumor[6] and antidiabetic[7] properties. These medicinal attributes can be relatedto its high content of phenolic compounds,mainly the caffeic acid derivatives suchas rosmarinic acid (RA)[1-3], which is one of themajor components of the plant and have beenconsidered its chemical marker. Considering thecomplex requirements from the phytomedicinesdevelopment, the quantitative determination ofthis important secondary metabolite is one ofthe first steps for standardization of Rosemaryphytopharmaceuticals[8]. Several analytical methods have been appliedfor quantitative determination of RA in Rosemary,including capillary electrophoresis[9], electrochemistry[8] as well as spectrophotometricand chromatographic methods[11-14]. Among thechromatographic techniques, the High PerformanceLiquid Chromatography (HPLC) coupledto photodiode array (PDA) detection is the mostemployed, mainly due to several advantagespresented by this technique such as high selectivity,sensitivity, specificity and accuracy[12,13,15]. However, most of these HPLC-PDA

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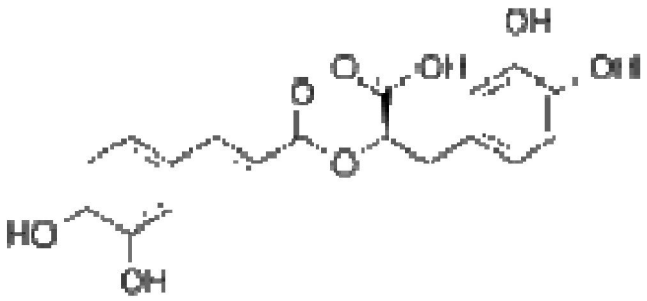

Figure 1 : Molecular Structure of Rosmarinic acid

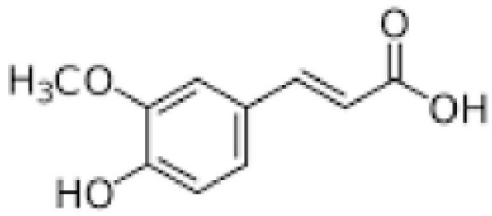

Figure 2 : Molecular Structure of ferrulic acid

methodsuse complex procedures for sample preparationand analysis, including excessive number ofsteps on the extraction procedures as well asthe use of mobile phase set at gradient flowrates,which may increase the consumption ofsolvents and analysis times and their costs[8]. Accordingly,undertaking a study in order to developa faster method for RA quantification, that alsopresents affordable costs and low environmentalimpact, is fully justified. The presentwork has been carried-out with the purpose ofsingle laboratory complete validation of a rapidand simple HPLC-UV method for Rosmarinic acid determinationin Rosemary Extract powder.

The objective of this study to develop method for the determination ofRosmarinic acid, ferrulic acidand its derivatives with short run time, which can also be used for its structurally related compounds analysis.

Pharmacognostic characterization

The powder moisture content was measuredfrom 0.5 g of sample employing a halogen lampanalyzer MB 35 (Ohaus Inc., USA). Total ashcontent, acid insoluble ash content and powdersize distribution were determined according tothe methodologies proposed elsewhere 16. Theresults were expressed as mean ± standard deviation(S.D) of three replicates.

EXPERIMENTAL

Materials and methods

Solvents and organic modifiers used in the HPLC mobile phases were Acetonitrile HPLC grade purchased from SIGMA ALDRICH,Ortho phosphoric acid HPLC Grade (assaye"88%) purchased from RANKEM and Ammonium hydrogen phosphate purchased from RANHEM. The HPLC grade water used in analysis was purchased from RANKEM. The reference standardRosmarinic Acide" 95% were purchased from SIGMA ALDRICH.

Instruments

We have used HPLC system for our research work; conventional binary pump HPLC system 1525 made by Waters equipped with an UV-VIS detector 2489,and an Analytical Balance{Make-ESSAE Model#-GR202.(0.01/0.1mg d and having resolution 0.01mg.)

HPLC Analysis

Chromatographic conditions

The chromatographic separation was achieved on a C-18(ODS-3, 100mm x 4.6mm, 3µ) reversed-phase column. Column temperature 25±2°C, Theanalyte were analysed at single wavelength 320 nm forRosmarinic acid, ferrulic acid and its derivatives, Injection volume 20 µl,

Flow program

The mobile phase was pumped through the column at an isocratic flow rate of1.0 mL/min, for a run time of 10min. The output signal was monitored and processed using a EMPOWER System Software (Version 2.0). Peak areas were integrated and final concentrations were calculated in comparison with a known standard response.

Determination of Rosmarinic Acid & Ferrulic Acidby HPLC:

Preparation of mobile phase

Weigh 4.011g of Ammonium hydrogen phosphate, dissolve in 800ml HPLC Grade water, adjust the pH to 3.0 using 10% Orthohosphoric acid. Mix well 300ml of Acetonitrile HPLC Grade. Filter through 0.45µmmembrane filter paper and degassed by sonication.

Diluent

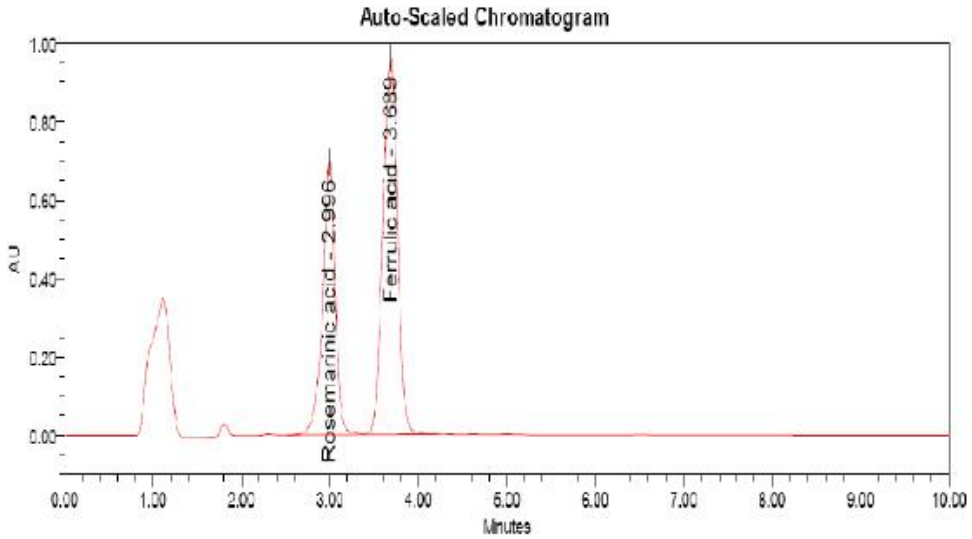

Figure 3 : HPLC chromatogram of resolution solution of rosmarinic acid and ferrulic acid

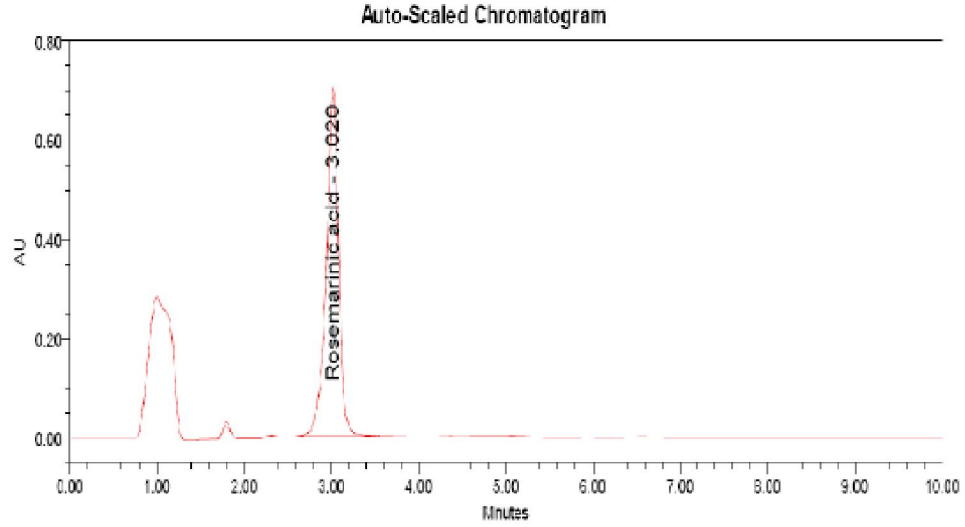

Figure 4 : HPLC chromatogram of rosmarinic acid standard

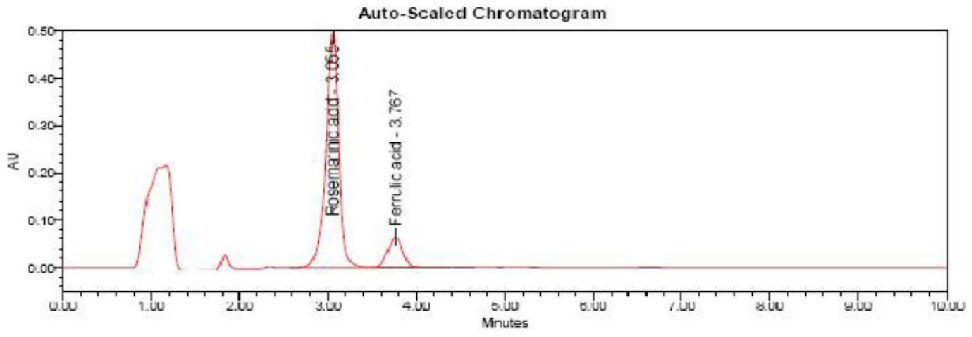

Figure 5 : HPLC chromatogram of rosmarinic acid sample

HPLC gradeMethanol.

Standard solution preparation

Weigh accurately 0.10g of Rosmarinic acidworking standard and transfer to a 50ml volumetric flask. Dissolve with 20ml of diluent and sonicate for 2-3min. Cool the sample and make up to the volume with diluent. Filter through 0.45µm Nylon syringe filter.

Standard solution preparation-2

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Weigh accurately 0.01g of Rosmarinic acid Reference standard and transfer into 50ml volumetric flask. Dissolve in 20ml of diluent and sonicate for 2-3 min. Cool the sample and make up to the volume with diluent. Filter through 0.45µm Nylon syringe filter.

Test solution preparation

Weigh accurately 0.10g of sample and transfer to a 50ml volumetric flask. Dissolve with 20ml of diluent and sonicate for 2-3min. Cool the sample and make up to the volume with diluent. Filter through 0.45µm Nylon syringe filter.

Quantification of areas for standardization

The quantification of Rosemarinic acid & ferrulic acid were performed by quantifying the areas of standardization, where [Sample] $g.mL^{-1}$ = Area standard × [default g.mL–1] / sample area. The results obtained in $g.mL^{-1}$ were expressed in%.

RESULTS AND DISCUSSION

Development of HPLC method

Different compositions of the mobile phase were tested, and the desired resolution of Rosmarinic acid and Ferrulic Acidwith symmetrical and reproducible peaks was achieved by using the mobile phase–Mixture of Ammonium Hydrogen Phosphate-Acetonitrile. Initially methanol and water were tried in various ratios Rosmarinic acid and its derivatives. Rosmarinic acid showed splitting peaknature along with large tailing factorand was unable to show clear Separation of peaks. Then both the drugs were tried with combination of methanol and water (1% acetic acid; adjusted to pH of 3.0 using 50% triethanolamine) at various ratios, still Rosmarinic acidwere unable to separate as a clear peak. Therefore, methanol was completely replaced with Acetonitrile: water(4.011g Ammonium Hydrogen Phosphate pH adjusted to 3.0 Using 10% Orthophosphoric acid) in the ratio 3:8 (v/v) which exhibited good peak nature and peaks were found to be symmetrical at 320 nm. Tailing factor for Rosmarinic acidwas less than 2% with good resolution.

Selectivity experiment showed that there is no interface or overlapping of the peak either due to excipients or diluents with main peaks of Rosmarinic acid and Ferrulic Acid, the assay was linear over a conc of 50mcg-150mcg/ml of Rosmarinic acid and Ferrulic Acid. Accuracy and precision % RSD of the method was found to be less than 1%. The ruggedness and robustness % RSD were found to be well within limits.

Solution stability

The short term stability studies were carried out at ambient laboratory temperature protected from sun light (22-25 °C) and at refrigerated temperature (2-8°C) for 48hours in solvent. % RSD for solution of Rosmarinic acid and Ferrulic Acidconcentrations during solution stability experiments was within 1%. No significant changes were observed for the chromatograms of standard solution and the experimental solution. Further, absence of degradation peaks confirmed that the sample is stable in solvent used during the assay for 48 hours.

Limit of quantification& limit of detection

The LOD and LOQ were determined at a signal-to-noise (S/N) ratio of 3 and 10 respectively. Linearity was established over the range of 10-200 µg/mL using the weight least square regression analysis. Accuracy of the method was determined by recovery experiments. 10µg/mL, 50µg/mL 100µg/mL,150µg/mL and 200 µg/mL. The average recovery obtained from six injections was reported as percentage nominal of the analyzed concentration.

CONCLUSION

Results showed that the method was selective, sensitive, precise, accurate, and linear over the concentration range between 2.5-50 µg. mL–1. The main advantages of the method were the easiness and promptness of sample analysis, as well as equipment convenience. These results allowed us to conclude that it can be successfully applied on the routinely quality control of Rosemary phytopharmaceuticals inputs. Moreover, it would be useful to perform other validation studies between different laboratories in order to make it a

pharmacopeical method.

A simple isocratic HPLC method has been developed for the simultaneous determination of Rosmarinic acid and Ferrulic acid its derivatives. The advantages of the proposed method over then previously reported ones is the use of a UV-VIS detector which is widely available in the ordinary laboratories, with no need for the more sophisticated mass or fluorescence detectors. Another noted advantage is its stability-indicating power and itsability to selectively analyze the studied drugs in the presence of their forced degradation products. This method can be said to be more economical as compared to other methods reported in literature. The proposed RP-HPLC method may be utilized for other derivatives of rosmarinic acid and ferrulic acid and it is subject of further studies.

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REFERENCES

[1] Yesil O.Celiktas, G.Girgin, H.Orhan, H.J.Wichers, E.Bedir, Vardar F.Sukan; Eur.Food Res.Technol., **224**, 443-51**(2007)**.

[2] A.Ibarra, J.Cases, A.Bily, K.He, N.Bai, M.Roller et al.; J.Med.Food, **13**, 1-9 **(2010).**

[3] P.J.Tsai, T.H.Tsai, S.C.Ho; Food Chem., **105**, 311-6 **(2007).**

[4] R.Gutiérrez; J.L.Alvarado, M.Presno, O.Pérez-Veyna, C.J.Serrano, P.Yahuaca; Phytother.Res., **24**, 595-601 **(2010).**

[5] J.P.Benincá, J.B.Dalmarco, M.G.Pizzolatti, T.S.Fröde; Food Chem., **124**, 468-75 **(2011).**

[6] S.Cheung, J.Tai; Oncol.Rep., **17**, 1525-31 **(2007)**.

[7] T.Bakirel, U.Bakirel, O.Ü.Keles, S.G.Ülgen, H.Yardib; J.Ethnopharmacol., **116**, 64-73 **(2008).**

[8] D.Gonçalves, R.O.Couto, E.C.Conceição, N.S.Reis, E.S.Gil; Quim.Nova, **34**, 330-4 **(2011).**

[9] Y.Peng, J.Yuan, F.Liu, J.Ye; J.Pharm.Biomed.Anal., **39**, 431-7 **(2005).**

[10] Validated HPLC-PDA method for rosmarinic acid quantification in rosemary renê O.COUTO*, Edemilson C.CONCEIÇÃO, Luiza T.Chaul, Ezequiane M.S.Oliveira, Suzana F.Alves, Kênnia R.Rezende, Maria T.F.Bara & José R.PAULA Faculdade de Farmácia, Universidade Federal de Goiás, CP, Goiânia, GO, Brazil, **131**, 74001-970.

[11] M.Kivilompolo, T.Hyötyläinen; J.Chromatogr.A, **1145**, 155-64 **(2007).**

[12] H.Wang, G.J.Provan, K.Helliwell; Food Chem., **87**, 307-11 **(2004).**

[13] N.Troncoso, H.Sierra, L.Carvajal, P.Delpiano, G.Günther; J.Chromatogr.A, **1100**, 20-5 **(2005)**.

[14] L.Almela, Sánchez B.Muñoz, Fernández J.A.López, M.J.Roca, V.Rabe; J.Chromatogr.A, **1120**, 221-9 **(2006).**

[15] J.C.Luis, C.B.Johnso; Span.J.Agric.Res., **3**, 106-12 **(2005).**