Analytical Method Development and Validation for the Identification of Spiraeoside Using RP-HPLC in Pharmaceutical Gel Formulation

Vildan Tombayoglu[1], Ozlem Kenar[1], Arzu Palantoken[1], Ali Turkyilmaz[1]
[1]Sanovel Pharmaceutical, Research and Development Department, 34580 Silivri, Istanbul, Turkey

The aim of this study was to validate a Reversed-Phase High Performance Liquid Chromatographic (RP-HPLC) method for the qualitative determination of spiraeoside contained in a gel preparation. The chromatography column used was a stainless steel column (25 cm x 4.6 mm, dp=5 µm) packed with silica surface covered with cross-linked diol groups for polar selectivity (Luna HILIC). Mobile phase consisting buffer containing 1 mL of 10% Tetra methyl ammonium hydroxide aqueous solution (pH 2.5) and acetonitrile and isocratic system was used. The flow rate was 0.4 ml/min and the detective wavelength was 365 nm. Injection value for each time was 100 µl and separations were carried out isothermally at 35°C in a heated chamber and sample temperature was 25°C. System suitability parameters were studied by injecting the standard solution six times and results were well under the acceptance criteria. The proposed validated method could separate spiraeoside peak was confirmed using photo-diode array detector.

Introduction

Meadowsweet Filipendula ulmaria (L.) Maxim. (Rosacea fam.) is generally used as an analgesic, corroborant, anti-inflammatory, wound-healing, antiulcerogenic, hypoglycaemic, sedative, antihemorrhoid, astringent and diuretic drug in some European countries and in Russia. This plant is composed of flavonoids, tannins, salicylates, volatile oils, coumarin, mucilage, carbohydrates and ascorbic acid (vitamin C) and catechins [1–3]. From 3-4% in the flowering herb up to 6% in the fresh flowers contain flavonoids. Some flavonoids are spiraeoside (quercetin-4′-glucoside), hyperoside, other quercetin and kaempferol derivatives and kaempferol-4′-glucoside [4]. Many of these components have beneficial properties to human health.

The major and typical flavonoid of Filipendula ulmaria flowers is spiraeoside (quercetin-4′-glucoside) [5]. The chemical structures of the reported flavonoid glycosides from Filipendula ulmaria are shown in *Figure 1*. The efficacy of the fluid extract from onion is not yet fully elucidated. Therefore, the herbal preparation is regarded as the active substance in its entirity. The herbal preparation is composed of a complex mixture of constituents; it cannot be given a structural formula.

Background

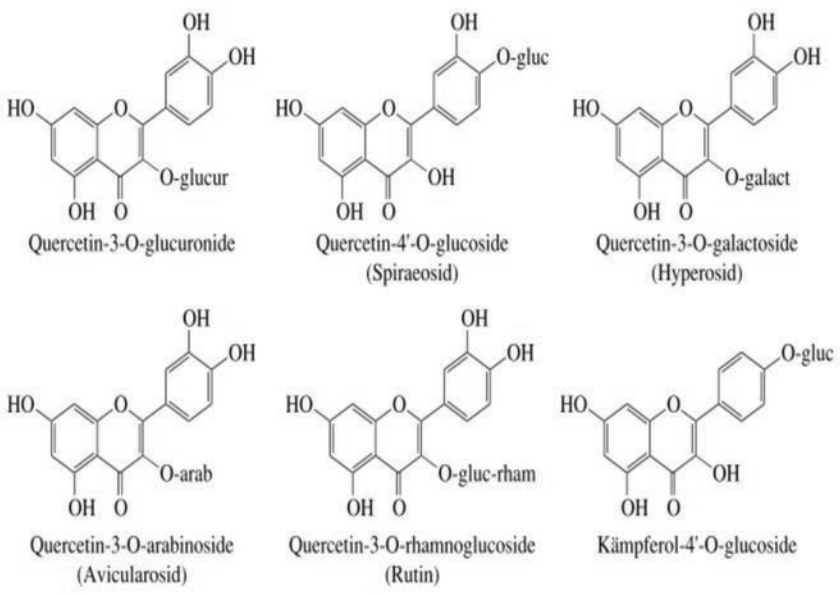

Figure 1. Chemical structures of the reported flavonoid glycosides from Filipendula ulmaria

Active ingredients of plant extracts are very difficult to identify in pharmaceutical dosage forms. Quantification of these compounds is usually performed by HPLC with UV detection [6]. The widely accepted technique for separating complex mixtures of plant extracts in pharmaceutical dosage forms is RP-HPLC. Derya Algul et al., reported this method for the quantitative determination of hyperoside in a cream preparation [7]. Geetha M. et al., reported to find out the quercetin and cyanidin present in red onion peel and big onion peel by using HPLC [8]. Xiuling Yang et al., established an RP-HPLC method for the simultaneous determination of four major constituents from Folium Rhododendri Micranthi [9]. An HPLC method for identification and determination of myricetin, quercetin, kaempferol and total flavonoids in ten herbal drugs of Macedonian origin is presented by Kulevanova et al. [10].

Concerning literature data, there were many studies about the pharmacological effects of Filipendula ulmaria, yet only a few methods for flavonoid content in onions (Allium cepa L.). Possible alternatives to high-performance liquid chromatography (HPLC) analysis were investigated; these are spectrophotometery [11], thin layer chromatography [2], [12] and HPLC combined with mass spectrometric (LC/MS) detection [6]. However in 1992, Poukens-Renwart et al. quantified the spiraeoside-containing extract by HP-TLC densitometry after derivatisation with diphenylboric acid-2-aminoethylester [5].

According to Lombard et al., the spectrophotometer does not replace the precision achieved with HPLC for the separation and quantification of individual quercetin conjugates, the spectrophotometer represents a cheap and efficient method for analysis of high volumes of onions for total quercetin concentration [11].

It is claimed that used thin layer chromatography (TLC), which is more easily available chromatographic method for different laboratories and is more effective compared to HPLC by L. Pobłocka-Olech et al. [2] Brazilian Homeopathic Pharmacopoeia published Allium Cepa monograph which has a TLC method and visual control of colour after chemical reaction [12]

Pemp et al. utilised LC/MS in order to save time and detect flavonoids in the extracts without expensive sample purification [6]. In literature, there is no such an identification method for spiraeoside in gel formulation. The scope of the present study was to develop and validate a new identification method of Spiraeoside in liquid extract from onion which is contain in a gel formulation using a RP-HPLC method.

Experimental

Materials

Spiraeoside was purchased from Finzelberg (Germany). Tetra methyl ammonium hydroxide, phosphoric acid, sodium chloride, ethyl acetate were purchased from Merck Ltd. HPLC grade Acetonitrile was purchased from JT. Baker. High purity deionised water was obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system. Gel formulation (Sanovel, Turkey) was used.

Spiraeoside working standard was purchased from Phytolab.

Instrumentation

RP-HPLC system (Waters, USA) equipped with inbuilt autosampler and quaternary gradient pump with an on-line degasser was used. The column compartment having temperature control and photodiode array (PDA) detector was employed throughout the analysis. Chromatographic data was acquired using empower software.

Chromatographic conditions

Luna 5µ HILIC 200A 250x4.6 (Phenomenex, USA) column was used as stationary phase maintained at 35°C. The mobile phase involved a variable composition of (10 mL of 10% Tetra methyl ammonium hydroxide aqueous solution are removed in 1000 ml of water, adjusted to pH 2.5 with orthophosphoric acid) and 120 mL of this solution is taken up and stirred with 880 mL of acetonitrile. The mobile phase was pumped through the column with at a flow rate of 0.4 ml / min. Pure water, sodium chloride - ethyl acetate mixture solution and mobile phase solution; are used as dilution solution.

Injection volume was 100 µL and the optimum wavelength selected was 365 nm which represents the wavelength of maximum response for spiraeoside in gel. The samples were analysed using a PDA detector covering the range of 200–600 nm.

Preparation of Solutions

Dilution Solution (Sodium chloride - Ethyl acetate solution)

5.0 g of sodium chloride is weighed into a 150 mL volumetric flask. Add 30 mL of distilled water and stir until dissolved. 100 mL of ethyl acetate is added and the mixture is stirred for 10 min. mixed.

This solutions and mobile phase solution are used as a dilution solutions all of the preparation of samples.

Standard Solution

Take 0.5 mL of the Extractum Cepae working standard and transfer it to a 100 mL volumetric flask. Add 70 mL of distilled water and vortex for 15 minutes (min.) to mixed. Diluting it to volume with Sodium chloride - Ethyl acetate solution and stirred for about 30 min. After phase separation, take 1.0 mL of the upper phase and mix to volume with 10 mL volumetric flask of mobile phase.

Test solution

5.0 gr of gel was weighed into 100 ml volumetric flask. Add 70 mL of distilled water and vortex for 15 min. The ethyl acetate mixture was brought to volume with the solvent and stirred for 30 min. The completing volume with sodium chloride - ethyl acetate solution and stirred for 30 min. After phase separation, take 1.0 mL of the upper phase and mix to volume with 10 mL volumetric flask of mobile phase.

Results and Discussion

Optimisation of the chromatographic conditions

Different chromatographic conditions have been tried to optimise HPLC parameters.

To optimise the reverse phase HPLC parameters and a good separation, several chromatographic conditions were tried.

Column selection

Different columns for injections were used to achieve best partition of spiraeoside with other blank and placebo peaks. The appropriate peak shape, retention time, tailing factor, and column efficiency were good with Luna HILIC 200A (25 cm x 4.6 mm, 5 µm).

Mobile phase composition

Different proportions of mobile phase were tried to obtain enough selectivity and retention time of spiraeoside at the gel sample. Tetramethylammonium hydroxide aqueous solution, the inductive effect (+I) is very high when compared with other ionising. The basis of peak shape, symmetry, retention time and peak tailing, 10% Tetramethylammonium hydroxide aqueous was decided as the buffer preparation to be used. Different percentage of 10 Tetramethylammonium hydroxide and organic solvents were analysed and according to experiments with acetonitrile and methanol, excellent retention time, column pressure and peak tailing were observed with methanol. For this reason, acetonitrile was selected as an organic modifier. After many trials, based on the peak shape, peak symmetry, retention time and peak tailing, at 0.4 ml/min flow rate were selected.

Detection of Wavelength

UV spectrum of spiraeoside and their pacebo peaks were scanned between 200 nm – 600 nm by photo-diode array detector. Wavelength at 365 nm was found to be optimum for all analysed peaks.

pH adjustment of the buffer

Different tests on pH of the Tetramethylammonium hydroxide buffer were made to achieve the optimum pH at which all peaks related with APIs and placebo separated well. Based on peak shape, peak tailing and theoretical plate count, suitable pH of the buffer was found as 2.5.

The optimised chromatographic conditions are a isocratic study of buffer (Tetra methyl ammonium hydroxide, at pH 2.5) and acetonitrile at 365 nm as detection wavelength, 0.4 mL/min flow rate, 35ºC column temperature, 25ºC tray temperature and 100 mL injection volume. The typical HPLC chromatograms *(Figure 2)* represent the spiraeoside peak could be detected.

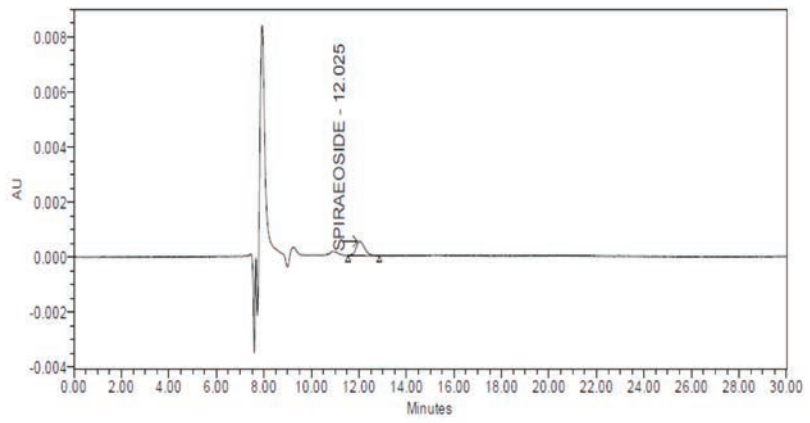

Figure 2. HPLC chromatogram of gel sample

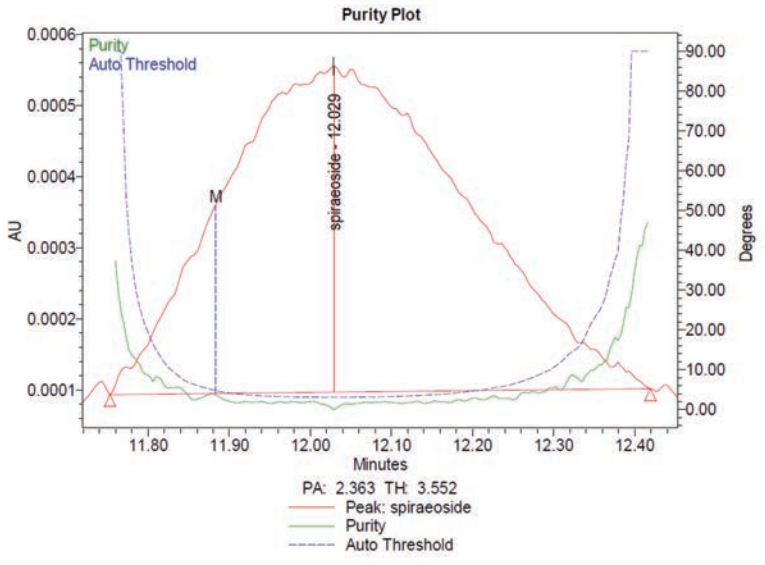

Figure 3. HPLC peak purity plot of gel sample

Validation of the Method

The developed method was validated for as per ICH Q2 (R1) guidelines [13] and validation of compendial procedures from USP [14] for various parameters such as specificity, filter effect and carry over effect.

Specificity

The peak purity indices for the gel solutions were determined with PDA detector under optimised chromatographic conditions. Peak purity indices were found as (purity angle < purity threshold) *(Figure 3)* indicating that no additional peaks were co-eluting with the placebo sample. Baseline resolution was achieved for all investigated compounds.

Conclusion

The proposed RP-HPLC method for identification of spiraeoside was found specific and selective according to validation studies. The method was validated as per ICH guidelines [13] and validation of compendial procedures from USP [14]. The developed method can be used for the routine analysis of identification of spiraeoside in pharmaceutical gel formulations.

References

1. *K. Busia, Fundamentals of Herbal Medicine: Major Plant Families, Analytical Methods ... - Kofi Busia - Google Kitaplar. 2016.*
2. *L. Pobłocka-Olech, DANIEL GŁÓD1, MARIA E. EBROWSKA2, MAŁGORZATA SZNITOWSKA2, and MIROSLAWA KRAUZE-BARANOWSKA1, "TLC determination allium cepa," Acta Pharm, vol. 66, pp. 543–554, 2016.*
3. *G. I. Vysochina, T. A. Kukushkina, and E. S. Vasfilov, "Biologically Active Substances in Filipendula ulmaria (L.) Maxim. Growing in the Middle Urals," Chem. Sustain. Dev., vol. 21, pp. 369–374, 2013.*
4. *"Meadowsweet." [Online]. Available: http://www.centerchem.com/products/downloadfile.aspx?fileid=6994.*
5. *P. Poukens-Renwart, M. Tits, J. N. Wauters, and L. Angenot, "Densitometric evaluation of spiraeoside after derivatization in flowers of Filipendula ulmaria (L.) Maxim.," J. Pharm. Biomed. Anal., vol. 10, no. 10–12, pp. 1085–1088, Oct. 1992.*
6. *E. Pemp, G. Reznicek, and L. Krenn, "Fast quantification of flavonoids in Filipendulae ulmariae flos by HPLC/ESI-MS using a nonporous stationary phase," J. Anal. Chem., vol. 62, no. 7, pp. 669–673, 2007.*
7. *Y. Algul, Derya; Uzuner, "RP-HPLC ANALYSIS OF HYPEROSIDE IN A CREAM FORMULATION Derya Algul * , Yasemin Uzuner," Indo Am. J. Pharm. Res., vol. 6, no. 5, 2016.*
8. *M. Geetha, M. Saravanakumar, and P. S. Devi, "Hplc Analysis of Quercetin and Cyanidin From Onion Peel (Allium Cepa L .)," Am. J. Pharmtech Res., no. November 2011, 2011.*
9. *L. Yang, Xiuling; Zhang, Xiaowei ; Yuan, Zhifang ; Li, Xiaona; Zhang, Lantong ; Fan, "Simultaneous Determination of Myricitrin, Hyperin,Quercitroside, and Quercetin in Folium Rhododendri Micranthi by RP–HPLC," J. Chromatogr. Sci., vol. 47, pp. 714–717, 2009.*
10. *S. Kulevanova, M. Stefova, T. K. Panovska, and T. Stafilov, "HPLC identification and determination of myricetin , quercetin , kaempferol and total flavonoids in herbal drugs," Maced. Pharm. Bull., vol. 30, no. 2002, pp. 25–30, 2003.*
11. *K. A. Lombard, E. Geoffriau, and E. Peffley, "Flavonoid Quantification in Onion by Spectrophotometric and High Performance Liquid Chromatography Analysis," POSTHARVEST Biol. Technol. HORTSCIENCE, vol. 37, no. 374, pp. 682–685, 2002.*
12. *"Brazilian Homeopathic Pharmacopoeia," 2011.*
13. *"International Conference on Harmonization (ICH) Q2 (R1): Validation of Analytical Procedures—Test and Methodology," 2005.*
14. *"Validation of compendial procedures." [Online]. Available: https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/c1225.pdf. [Accessed: 23-Jun-2017].*