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

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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 detector 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, salkylates, volatile oils, coumarins, 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) [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 entirety. The herbal preparation is composed of a complex mixture of constituents, it cannot be given a structural formula.

Background

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 spectrophotometry [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-aminoethyl ester [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 solutions 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 Fisonsberg (Germany). Tetra methyl ammonium hydroxide, phosphoric acid, sodium chloride, ethyl acetate were purchased from Merck Ltd. HPLC grade Acetoniitrile was purchased from J.T. 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 (25 cm x 4.6 mm, 5 µm). The optimised chromatographic conditions were a isocratic study of buffer (10% methanol, 90% water) at a flow rate of 0.4 mL/min, with 10 min run time. The mobile phase was pumped through the column with 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.

Results and Discussion

Optimisation of the chromatographic conditions
Different chromatographic conditions have been tried to optimise HPLC parameters.

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, was used 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 organis.

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.

Optimisation of the chromatographic conditions
The optimised chromatographic conditions are a isocratic study of buffer (10% methanol, 90% water) at a flow rate of 0.4 mL/min flow rate, 35°C column temperature, 25°C tray temperature and 100 mL injection volume. The typical HPLC chromatograms (Figure 2) represent the spiraeoside peak could be detected.

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 min. The ethyl acetate mixture was brought to volume with the solvent and stirred for 30 min. The complete volume with sodium chloride - ethyl acetate solution and stored 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.

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 stored 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.

Validation of the Method
The developed method was validated 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.

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