Short Communication

Densitometric evaluation of spiraeoside after derivatization in flowers of *Filipendula ulmaria* (L.) Maxim.*

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Introduction

European medicines have traditionally used aqueous extracts of *Filipendula ulmaria* flowers as an antiinflammatory, analgesic and diuretic drug [1–4]. The compounds of this plant are known to be flavonoids, tannins and salicylic acid derivatives [5]. Spiraeoside (quercetin-4'-glucoside) is the major [6] and most characteristic flavonoid of *Filipendula ulmaria* flowers and is not found in the leaves and stems. The extract was quantified by HPTLC densitometry after derivatization with diphenylboric acid-2-aminoethylester (cf. Fig. 1). The fluorescence of spiraeoside was measured at 330 nm.

Experimental

Materials

The spiraeoside chemical reference substance (CRS) was isolated and purified from flowers of *Filipendula ulmaria* following the method described by Stahl [7]. The IR and UV spectra and the melting point were similar to those of spiraeoside Extrasynthese (Genay, France) and corresponded to those described in ref. 7.

The solvents used were of analytical grade and were used without further purification. Ethylacetate, formic acid and dichloromethane were purchased from Merck (Darmstadt, Germany). Polyethylene glycol 400 (PEG 400)
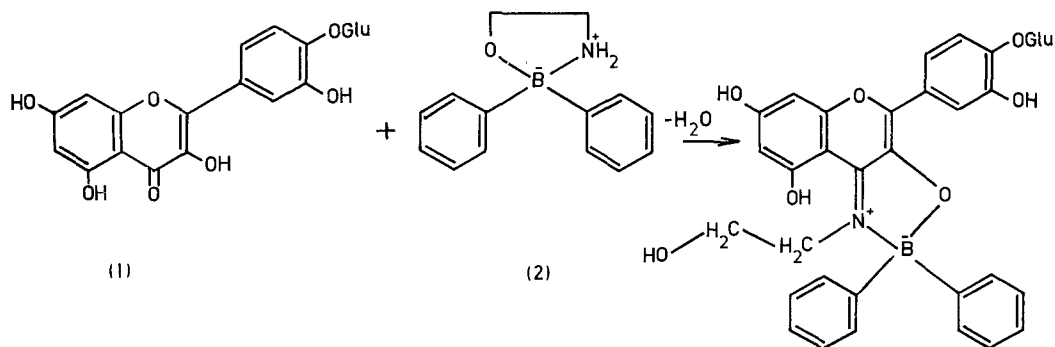
Figure 1
Structure of spiraeoside before and after derivatization. (1), Spiraeoside; (2), diphenylboric acid-2-aminoethylester.

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was obtained from UCB (Bruxelles, Belgium). For the derivatization reaction, diphenylboric acid-2-aminoethylester from Janssen (Geel, Belgium) was used.

Commercial batches of *Filipendula ulmaria* were obtained from Pharmaflore (Lessines, Belgium), Denolin (Braine-l'Alleud, Belgium) and Flandria (Gent, Belgium). They were authenticated by our department according to the monograph of the French Pharmacopoeia [8].

Instrumentation

The densitometric system consisted of a CD 60 Scanner (Desaga, Heidelberg, Germany). The solutions were spotted by means of a TLC Sampler AS 30 Desaga. A 20 × 10 cm Camag (Muttenz, Switzerland) dipping chamber was used for the dipping procedure.

Chromatographic conditions

Filipendula ulmaria flowers (250 mg) were extracted during 2 h with 25 ml methanol at 60°C. After filtration, the solution was evaporated, dissolved in 25 ml methanol and applied to the chromatoplate without further purification.

For the quantitative determination, a standard solution of spiraeoside CRS (4 mg in 10 ml of methanol) was prepared and different amounts were applied to the chromatoplate.

The samples were applied on HPTLC plates silicagel 60 F 254 (10 × 20 cm) Merck (Darmstadt, Germany) using an AS 30 autosampler. The application parameters were the following: plate 200 mm; 20 positions; length 2 mm; distance between samples 9 mm; start 10 mm. Volume by step 0.2 μl, 15 s μl^{-1}, 1 cycle, break 10 s. Application of 0.6, 0.8 and 1 μl for the standard and 0.4 μl for sample.

Plates were developed in an ascending mode, to a distance of 70 mm in a saturated chamber (30 min) at room temperature using ethylacetate–formic acid–water (6:1:1, v/v/v) [9, 10] as mobile phase. After development, plates were dried at 100°C for 10 min in a drying oven and derivatized by dipping successively in a solution of 0.5% diphenylboric acid-2-aminoethylester in ethylacetate and in a solution of 5% PEG 400 in dichloromethane [11].

Densitometric evaluation

Plates were scanned 30 min after derivatization by means of a CD 60 scanner supplied

with software operating via a personal computer under the following conditions: scanning mode, remission, fluorescence (mercury lamp); wavelength 330 nm (emission cut-off filter 450 nm); width of slit: 0.2 mm; height of slit: 3 mm; spot optimization; resolution 0.100 mm; number of measurements per position: 32, signal factor: 5 (cf. Fig. 2).

The results so obtained were compared by scanning the plates in absorbance under the following conditions: scanning mode; remission; absorbance at 437 nm; width of slit: 0.4 mm; height of slit: 3 m; spot optimization; resolution 0.100 mm; number of measurements per position: 32; signal factor: 20 (cf. Fig. 3).
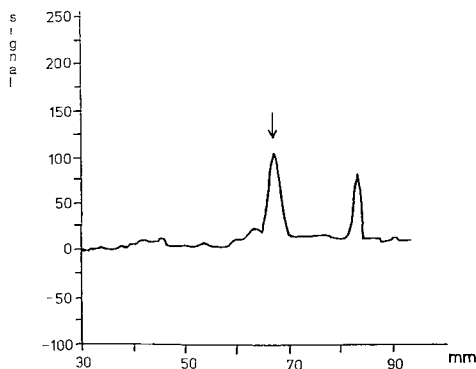
After linearization, the concentration of spiraeoside was estimated by measurement of the different standard and sample mean areas.

Results and Discussion

Filipendula ulmaria flowers contain a low amount of salicylates (*ca* 0.15%) that cannot
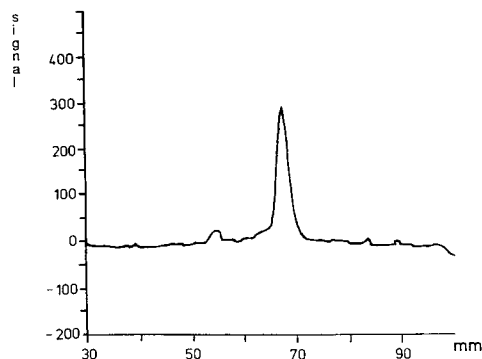
Figure 2
Densitometric scan of spiraeoside (fluorescence at 330 nm).

Figure 3
Densitometric scan of spiraeoside (absorbance at 437 nm).

Table 1
Linear regression equations and correlation coefficients of spiraeoside after derivatization at different wavelengths ($n = 9$)

Fluorescence wavelength (nm)	Equation	Correlation (r^2)
330	$y = 329.03 + 4952.44x$	0.9923
360	$y = -59.21 + 7854.67x$	0.9925
420	$y = 505.29 + 9676.63x$	0.9265
437	$y = -42.39 + 1774.49x$	0.9806

Concentration range 240–400 μg ml^{-1}.

Table 2
Evaluation of within-day and between-day reproducibility: calculation of the RSD values for the results obtained for a spiraeoside concentration of 400 μg ml^{-1}

Fluorescence wavelength (nm)	Within-day reproducibility (%) ($n = 5$)	Between-day reproducibility (%) ($n = 10$)
330	0.98	1.49
360	1.05	2.17
420	2.69	3.02
437	1.35	1.74

justify the antirheumatic use of this plant [8, 12]. On the other hand, its antiinflammatory properties could be explained by the presence of polyphenols (e.g. flavonoids and tannins). It is one of the reasons why spiraeoside (major flavonoid of *Filipendula ulmaria* flowers) was chosen as a characteristic substance for the standardization of this medicinal plant drug. Different scanning wavelengths from 300 to 440 nm (Lambda stepwidth = 10 nm) were tested with a 450 nm emission cut-off filter. The validation data (Tables 1 and 2) and the fluorescence intensity obtained with the different methods were compared. The fluorescence intensity was maximal at 330 and 420 nm. The validation data obtained at 420 nm were not as good as those at 330 nm. The excitation wavelength of 360 nm, which is frequently used for the derivatized flavonoids, also gave good validation data but the fluorescence intensity of the signal was lower. For this reason 330 nm was preferred.

Validation of the method

Good linearity was obtained for the calibration curve of spiraeoside. By plotting the peak area vs the spiraeoside concentration, the following regression equation was found: $y = 329.03 + 4952.44x$ (330 nm; concentration range, 240–400 μg ml^{-1}, $r^2 = 0.9923$; $n = 9$).

The precision of the method was assessed by calculating the RSD values for the results obtained for a spiraeoside concentration of

400 μg ml^{-1}. At this concentration, the within-day reproducibility was 0.98% ($n = 5$) and between-day reproducibility was 1.49% ($n = 10$).

By comparing the fluorescence (330 nm) and absorbance (437 nm) measurements, we were able to confirm the accuracy of the method. The validation data in Tables 1 and 2 show good linearity and reproducibility for both methods. Table 3 shows that the results obtained for both methods are similar. The peak purity was confirmed by taking the absorbance spectra of spiraeoside CRS and spiraeoside in *Filipendula ulmaria* before (cf. Fig. 4) and after derivatization (cf. Fig. 5).

Amount of spiraeoside in samples of Filipendula ulmaria

The spiraeoside content in batches of *Filipendula ulmaria* flowers ranged from 3 to 4.3%. It is of note that these results are similar to those published elsewhere after HPLC analysis of other batches [6, 13].
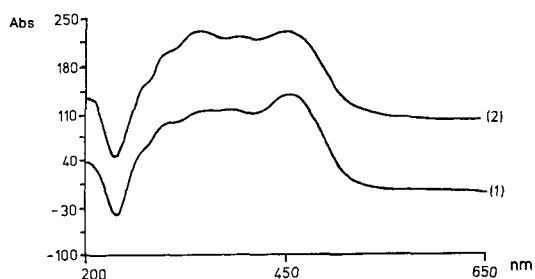
Table 3
Comparison of the results obtained with the two methods: fluorescence at 330 nm and absorbance at 437 nm

Filipendula ulmaria batch no.	Fluorescence (% spiraeoside)	Absorbance (% spiraeoside)
1	3.95	3.92
2	4.30	4.31
3	3.92	3.90
4	3.03	3.03
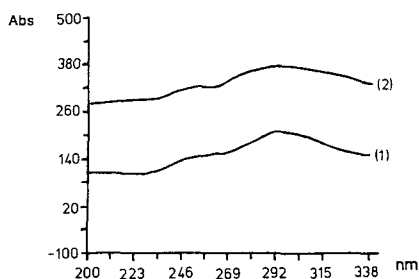
Figure 4
UV spectra of spiraeoside CRS (1) and spiraeoside in *Filipendula ulmaria* (2) before derivatization.

Figure 5
Spectra of spiraeoside CRS (1) and spiraeoside in *Filipendula ulmaria* (2) after derivatization.

Conclusion

The HPTLC method is a fast procedure that should be successfully used in the future for the quantification of spiraeoside in *Filipendula ulmaria* flowers and extracts.

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