P G03 DENSITOMETRIC EVALUATION OF SPIRAEOSIDE IN *FILIPENDULA ULMARIA* FLOWERS AFTER DERIVATIZATION

P. Poukens-Renvart, M. Tits, J.-N. Wauters and L. Angenot
Department of Pharmacognosy, Pharmaceutical Institute, University of Liège,
rue Fusch, 5, B-4000 Liège

In the traditional medicines of Europe, the water extract of *Filipendula ulmaria* flowers has been used as anti-inflammatory, analgesic and diuretic (1). The active principles of this plant are known to be salicylic acid derivatives and flavonoids (2). Spiraeoside is the major and characteristic flavonoid of *Filipendula ulmaria* flowers and, for this reason, we determined the amount of it by HPTLC densitometry. We measured the fluorescence of spiraeoside after derivatization by diphenylboric acid-2-amino-ethylester (3). The measurement was achieved by means of a TLC Scanner programmed to work in reflection-fluorescence at 360 nm (Mercury lamp; cut off filter 450 nm). We respected the following chromatographic procedure:
- Layer: HPTLC plates silicagel 60 Merck
- Mobile phase: Ethylacetate-Formic acid-Water: 6:1:1
- Standard solution (0.2 and 0.4 µl): 4 mg of spiraeoside SCR in 10 ml methanol
- Sample solution (0.2 µl): 0.250 g of *Filipendula* flowers were extracted by 25 ml methanol 60°C (2 hours). The solution was evaporated and dissolved in 10 ml methanol.

After linearisation, the concentration of spiraeoside was estimed by measurement of the different standards and samples mean areas. In our findings, the mean content of spiraeoside was 3.1 %.
The repeatability, reproducibility and the good linearity were confirmed by validation of the method.

References
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P G04 DENSITOMETRIC EVALUATION OF FRAXIN IN LEAVES OF *FRAXINUS EXCELSIOR*

P. Poukens-Renvart, M. Tits, J.-N. Wauters and L. Angenot
Department of Pharmacognosy, Pharmaceutical Institute, University of Liège,
rue Fusch, 5, B-4000 Liège

In France and Belgium, leaf extract from *Fraxinus excelsior* are especially used as anti-inflammatory (1). This property could be at least partially explained by the ability of some coumarins to inhibit the formation of leucotrienes in polymorphonuclear leucocytes (2). For this reason, we determined the amount of fraxin by HPTLC densitometry. Like other hydroxylated coumarin glucosides, fraxin shows a blue fluorescence under UV light (366 nm); this property was used for its quantitative estimation. The measurement was achieved by means of a TLC Scanner, programmed to work in reflection fluorescence at 350 nm (Mercury lamp; cut off filter: 450 nm). We respected the following chromatographic procedure:
- Layer: HPTLC plates RP 18 Silicagel 60 Merck with concentrating zones
- Mobile phase: Phosphoric acid 0.2 % - Acetonitrile (60:40)
- Standard solution: 5 mg of fraxin SCR were dissolved in 100 ml MeOH-H_2O (1:1)
- Sample solution: 0.250 g of *Fraxinus* leaves were extracted by 25 ml MeOH at 40°C (90 mins). The solution was evaporated and dissolved in 10 ml MeOH-H_2O (1:1)
- Applications of 1 and 2 µl for the standard and 2 µl for samples.

After linearisation, the concentration of fraxin was estimated by measurement of different standards and samples mean areas.
We are testing several commercial batches to establish the mean content of fraxin in *Fraxinus* leaves.
The accuracy, precision and good linearity were confirmed by validation of the method.