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# *Equisetum giganteum*: OPTIMIZATION OF FLAVONOID HETEROSIDES HYDROLYSIS AND VALIDATION OF LC METHOD FOR DETERMINATION OF CORRESPONDING AGLYCONES

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**Introduction:** *Equisetum giganteum* L. (Equisetaceae, common name: "horsetail"), is a native specie of South America used in folk medicine for treating kidney ailments due to their adstringent, diuretic and remineralizant properties. However, few phytochemical studies on this species have been reported[1]. In our previous work, we reported some flavonoids glycosides in *E. giganteum* extracts, which yielded the quercetin and kaempferol aglycones after acid hydrolysis.
**Objective:** Thus, the aim of this investigation was to optimize the hydrolysis conditions for flavonols glycosides in *E. giganteum* raw material and to develop and validate a LC method for the determination of the corresponding aglycones, quercetin and kaempferol.
**Materials and Methods:** The powder (< 0.355 mm) of dried aerial stems of *E. giganteum* was used as raw material. The hydrolysis procedure was optimized using Central Composite Rotational Design (CCRD) and Response Surface Analysis (RSA) for investigate the effects of HCl concentration and hydrolysis time (**Table 1**). This step was carried out in 50% aqueous methanol and refluxing at 90°C. The corresponding aglycones were extracted with ethyl acetate and analyzed by LC method. The analysis was performed using a Luna C18(2) column (4.6x250mm, 5µm) with a gradient solvent system composed of $H_2O$ (A) and MeOH (B), both acidified with formic acid 0.3% (v/v), flow rate of 0.9 mL/min and UV detector at 370 nm. The obtained responses were processed by Minitab® to evaluate the significance of the effects and to find the optimum hydrolysis condition. The LC method was validated in the following parameters: specificity, linearity, accuracy, repeatability and intermediate precision, detection (LOD) and quantification limits (LOQ), calculated by signal/noise. The Plackett-Burman experimental design was used for robustness evaluation.
**Results and Discussion:** The optimum condition found for hydrolysis in RSA was 1.18M HCl for 204.9 min. Using these conditions, the concentrations of 0.147±0.002 mg/g of quercetin and 1.923±0.011 mg/g of kaempferol were founded in *E. giganteum* (dried drug). In LC method validation, the standard curves were linear over the range 0.175 to 43.7 µg/mL and 0.222 to 55.6 µg/mL for quercetin and kaempferol, respectively, with $r^2 > 0.999$. The method showed good precision for intra-day (RSD < 4.6%) and inter-day (RSD < 4.3%) tests. Spiked samples of hydrolyzed extract showed recovery rates between 96.3 and 100.3%. The LOD/LOQ was of 0.047/0.151 µg/mL for quercetin and 0.043/0.205 µg/mL for kaempferol. The peak purity of the analytes was confirmed by diode array detector analysis. The method was not rugged enough for other column and flow rate variation.
**Conclusions:** This is the first report regarding quercetin and kaempferol quantification in *E. giganteum*. The optimum condition for hydrolysis of the corresponding flavonols glycosides was 1.18M HCl for 204.9 min. The validation revealed that the proposed LC method is specific, linear, accurate and precise to quantify quercetin and kaempferol in hydrolyzed extract. The proposed analytical methodology can be used in quality control of the *E. giganteum* raw material.

**Table 1.** Factors and levels of CCRD experimental conditions.

| | factors | |
|---|---|---|
| **level** | **[ ] HCl (M)** | **Time (min.)** |
| **-1.414** | 1.02 | 35.1 |
| **-1** | 1.6 | 60 |
| **0** | 3 | 120 |
| **1** | 4.4 | 180 |
| **1.414** | 4.98 | 204.9 |

*References:* 1. J.F. Ovalles, *et al.*, *Rev Fac Farm Univ Andes*, **32**, 2 (1996).
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