# Micellar Electrokinetic Chromatography of Coumarins in *Dipterix odorata* seed extracts

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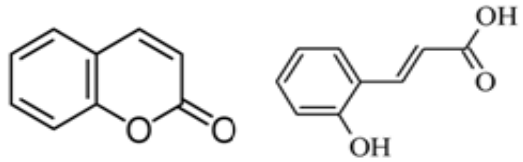
## Introduction

Coumarins are lactones derived from o-hydroxy-cinnamic acid, widely distributed in plants. In these compounds is attributed a variety of biological activities, such as antimicrobial, antiviral, anti-inflammatory, antispasmodic, antitumor and antioxidant[1]. Its main application is in the flavoring industry as substitute for vanilla. Tonka bean (*Dipterix odorata*) is a widely used tree in the north of Brazil. Its seeds have high commercial value, mainly due to the high content of coumarin (3 to 10%) present on its seeds[2]. As coumarin is a neutral molecule, it may be analyzed by capillary electrophoresis in micellar electrokinetic chromatography (MEKC) mode[3].

In this work we developed a methodology using MEKC to analyze coumarins in crude extracts of tonka bean seeds extracts.

**Figure 1.** Chemical structure of coumarin and o-coumaric acid.




## Results and Discussion

We developed and optimized a MEKC methodology for the separation of coumarin, o-coumaric acid, umbelliferone, scopoletin, trans-coumaric acid and 6-methoxy-coumaric acid, using as buffer 20 mM sodium tetraborate + 50 mM SDS + 5% MeOH. Instrumental conditions were: voltage 25 kV, with injection of 3s. Analyses were conducted using detection by UV/DAD absorption in 3 wavelengths: 210, 280 and 330 nm. We tested different buffer compositions, varying the concentration of sodium tetraborate, SDS and methanol. The voltage was varied from 15 to 25 kV, so as to obtain the best separation of standards. 5g of tonka bean seeds were crushed and sieved and subjected to ultrasonic extraction for 15 min, using 100 mL of MeOH. After, the extract was filtered, evaporated to dryness and reconstituted in MeOH: $H_2O$ (1:1). Extraction process was performed in triplicate and the extract was filtered again in Millex (0.22 µm) and injected directly into the capillary electrophoresis. The method was specific and allowed to separate coumarin and phenolic acids. It was been identified coumarin and o-coumaric acid in tonka bean extract by addition of standard and comparison of the spectra in the DAD detector. The method was validated being constructed a calibration curve for quantification of coumarin and o-coumaric acid in the range of 1; 10; 100; 500; 1000 and 2000 $mgL^{-1}$ of coumarin and 2; 10; 20; 30; 40 and 50 $mgL^{-1}$ of o-coumaric acid. The concentrations determined are shown in Table 1.

**Table 1.** Quantification of coumarin and o-coumaric acid in tonka bean extract.

| | Concentration ($mg.L^{-1}$) |
|---|---|
| Coumarin | 1840,0 |
| o-Coumaric acid | 16,0 |

## Conclusions

The MEKC methodology allowed the separation and quantitation of coumarin and o-coumaric acid in crude extracts of tonka bean seed, showing specific and selective.

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