



## Validation of an UV Spectrophotometric Method for Determining Diffractaic Acid from *Usnea sp.* in Inclusion Complexes with Hydroxypropyl- $\beta$ -Cyclodextrin

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**SUMMARY.** Diffractaic acid (DA) presents several biological activities. The goal of this study was to develop and validate a UV spectrophotometric method for determining diffractaic acid in inclusion complexes with hydroxypropyl- $\beta$ -cyclodextrin. Validation parameters were determined according to international guidelines for standardization. The linearity range of analytical curve was from 1 to 5  $\mu\text{g/mL}$  and the regression equation:  $C_{\text{DA}} = (\text{Area} - 0.0053)/0.1541$  ( $r^2 = 0.99998$ ;  $n = 3$ ). The intermediate precision indicated that the difference between the means was statistically insignificant ( $p < 0.05$ ). Accuracy revealed a mean recovery percentage of diffractaic acid in inclusion complexes of 100.1 %. The method was robust and the formulation excipients did not interfere on diffractaic acid quantification. Limits of detection and quantification of diffractaic acid were 0.03 and 0.08  $\mu\text{g/mL}$ , respectively. The proposed method proved to be accurate, precise and reproducible, thus being able to quantify diffractaic acid in raw material and inclusion complexes.

**KEY WORDS:** Diffractaic acid, Hydroxypropyl- $\beta$ -cyclodextrin, Inclusion complex, Validation method.

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