

**LAPONITE-PILOCARPINE HYBRID MATERIAL:
EXPERIMENTAL AND THEORETICAL EVALUATION OF
PILOCARPINE CONFORMATION**

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SUPPORTING INFORMATION

The pilocarpine alkaloid was extracted from jaborandi leaves using the high performance liquid chromatography technique (HPLC, Shimadzu Prominence, AUTOSAMPLE SIL-10AF, CTO-20A, DGU-20A5, LC-6AD, CBM-20A, SPD-20A, Tokyo, Japan). Mass spectrometry was used to evaluate the purity and monoisotopic molecular mass of pilocarpine hydrochloride. The chromatographic conditions were set using the column LiChrospher 60 RP Select B (250 x 4.6 mm, 5 μ m). The reverse phase coated with cluster octyl is composed by the mobile phase in the following conditions: potassium phosphate 5% (v/v), adjusted at pH 2.5, flow rate 1 mL/min at isocratic mode (100%), the column oven was set to 50°C for a time run of 40 min to the further detection in a spectrophotometer at a single wavelength of 216 nm. Mass spectra were acquired in electron spray and ion trap, in a mass range of m/z 100 to 300 Da, 1.800 V, at 280°C in API source (Amazon SL from Bruker Daltonics (Bremen, Germany)). MS² was carried out in manual mode with fragmentation of the precursor ion by CID using He (helium) as the collision gas in mass range 30 to 230 Da. Precursor ions were selected within an isolation width of 2 u and scans were accumulated with variable RF signal amplitudes. The m/z scale of the mass spectrum was calibrated using the external calibration standard G2421A electrospray “tuning mix” from Agilent Technologies (Santa Rosa, CA, USA).

HPLC is a technique widely applied in the analysis of natural products including alkaloids and was used for pilocarpine applying a reverse phase (RP) system according to V eras *et al.*¹ This method, reduce the ion-exchange interaction between basic analytes and residual surface silanols. The chromatogram of pilocarpine (**Figure S1**) shows the 16.7 min retention time for the alkaloid, when eluted with potassium phosphate 5%

(v/v). The detection of 50 $\mu\text{g/mL}$ of pilocarpine hydrochloride reached one arbit. unit by UV-VIS detector. Hence, the HPLC data shows that the first peak is salt (hydrochloride) and the second peak (the major) is free pilocarpine, demonstrating that the procedure used to isolate pilocarpine was suitable to obtain a 98% pure molecule.

A previous process of extraction for pilocarpine using a liquid-liquid extraction with chloroform was necessary to further inject the sample into the mass spectrometer. Mass spectrum (**Figure S2**) shows, by Total Ions Count (TIC), the confirmation of the molecular mass of pilocarpine 209.1 Da $[\text{M} + \text{H}]^+$. Major fragments 122.1; 163.1; 181.1 and 191.1, in the range used, from parental ion (209.1 Da) were observed as well as the probable structural conformation. Therefore, with the data from HPLC and mass spectrometry it was possible to obtain the pure form of the alkaloid. Thus, the molecule can be used for further experiments such as the intercalation between the laponite clay layers.

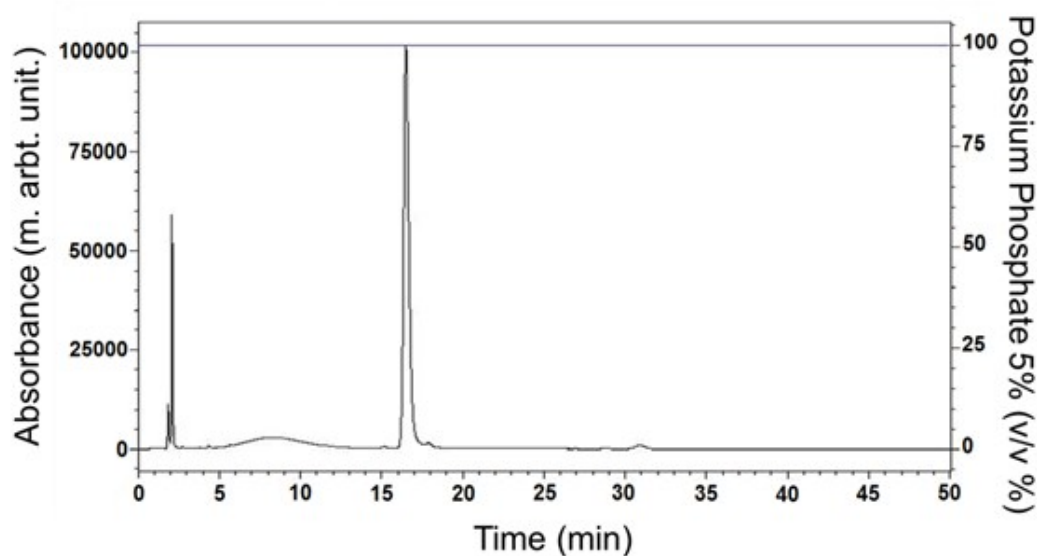


Figure S1: Chromatogram obtained from HPLC-UV of pilocarpine hydrochloride. Notice the retention time (16') for pilocarpine at 50 $\mu\text{g/mL}$ when a reverse phase column C_{18} (LiChrospher 60 RP, 250 x 4.6 mm) and potassium phosphate 5% (v/v) was used as eluent in isocratic gradient.

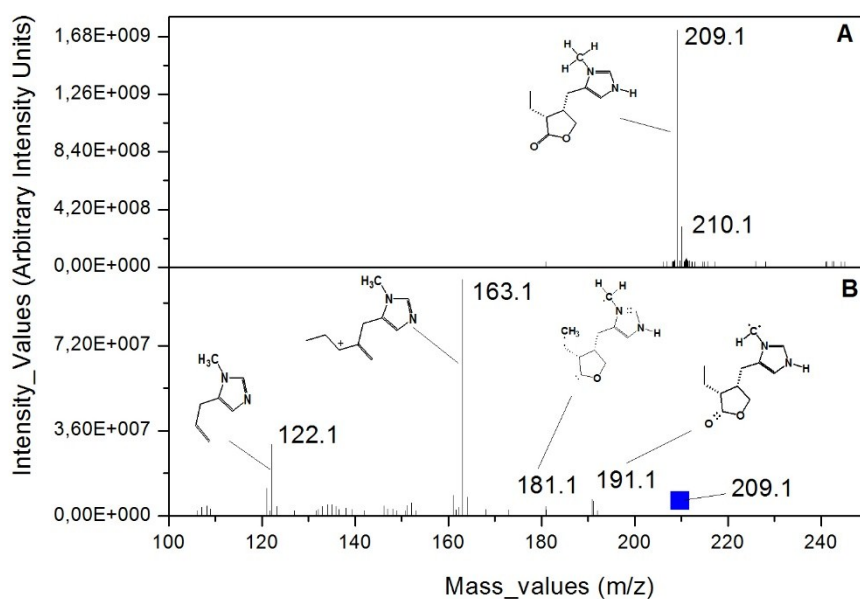


Figure S2: Mass spectra obtained through ESI, Ion Trap (AmaZon SL, Bruker) of (A) pilocarpine and (B) MS² from pilocarpine. Also shown its characteristic fragments and generated probable structural conformation.

Figures S3 and S4 show TG and DSC curves of Lap and Pilo, respectively.

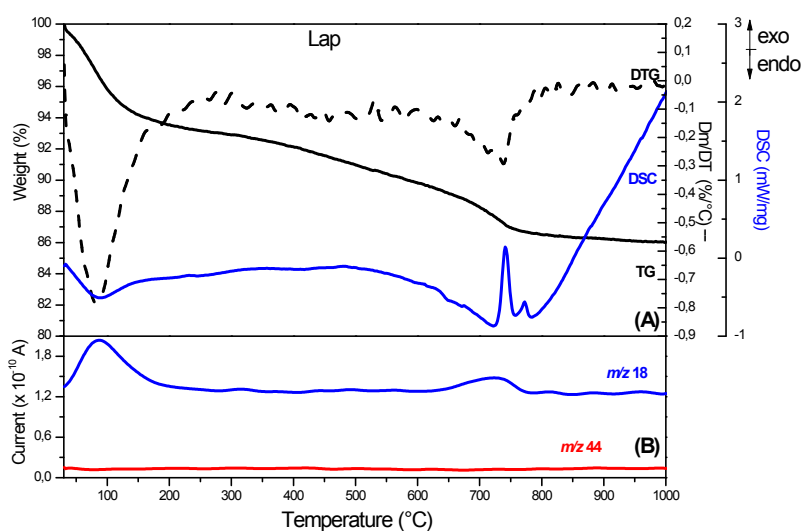


Figure S3: (A) TG-DSC (solid line) and DTG (dashed line) and (B) MS curves of Laponite.

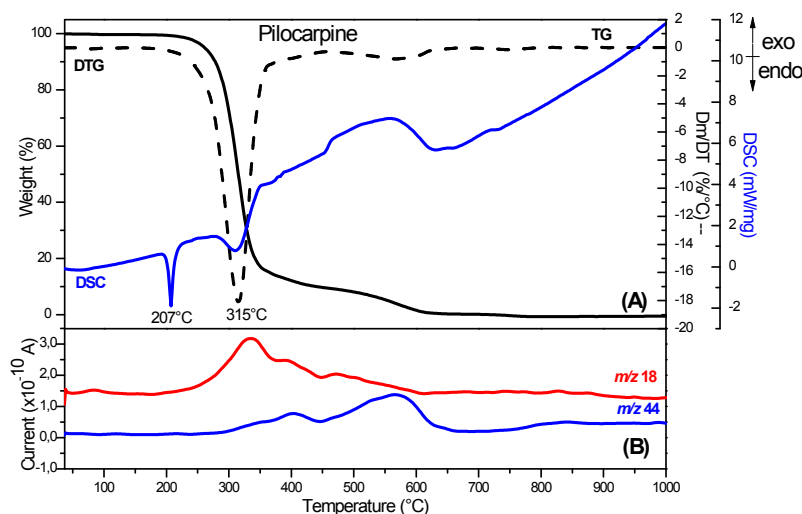


Figure S4: (A) TG-DSC (solid lines) and DTG (dashed line) and (B) MS curves of PiloHCl.

In order to evaluate the chemical environments in Raman spectra of the theoretical cationic pilocarpine dispositions different situations for the molecule were simulated: *i*) positively charged in vacuum; *ii*) positively charged in water environment using the PCM model; *iii*) neutral condition by the presence of chloride anion (close to the imidazole nitrogen) in vacuum. In all cases, the total relaxation geometry was performed, using the same basis set, 6-311G(d,p), and exchange correlation function (B3LYP) discussed in the main text. **Figure S5** shows the results obtained in all these cases. The vertical green dashed lines are located in a couple of bands in Pilo-Lap and can be correlated to the bands of the charged in water system of Pilo⁺ planar disposition, compared to Pilo⁺ distorted in water and vacuum environments. Vertical red dashed lines are signaling characteristic peaks in the PiloHCl spectrum, and can be seen better correspond to the charged in water Pilo⁺ distorted situation. It can also be noticed in **Figure S5**, that the chloride in water system exhibits the largest discrepancy with both experimental situations.

The Raman spectra general characteristic differences between the two theoretical atomic dispositions (Pilo⁺ planar and Pilo⁺ distorted) are reproduced under all studied environment conditions. However, in order to mimic the interlayer region, the one chosen for the present study (and discussed in the main text) is the simulation of the charged molecule under water environment.

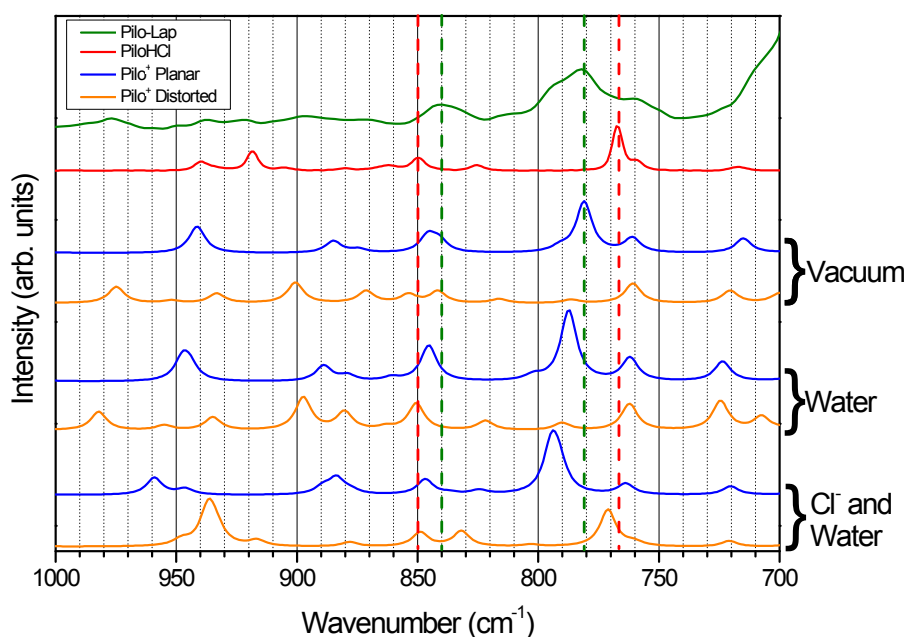


Figure S5: Experimental FT-Raman spectra obtained for Pilo-Lap (green line) and PiloHCl (red line). Theoretical spectra for Pilo⁺ planar (blue line) and Pilo⁺ distorted (orange line), obtained in different chemical conditions. The dashed green and red lines are references for the experimental bands of Pilo-Lap and PiloHCl, respectively.

Figure S6 shows the FTIR and Raman spectra of Pilo-Lap in the range 1950-550 cm⁻¹. The broad band at around 1010 cm⁻¹ in the FTIR spectrum and 1087 cm⁻¹ in the Raman spectrum are attributed to ν Si-O of the Laponite.

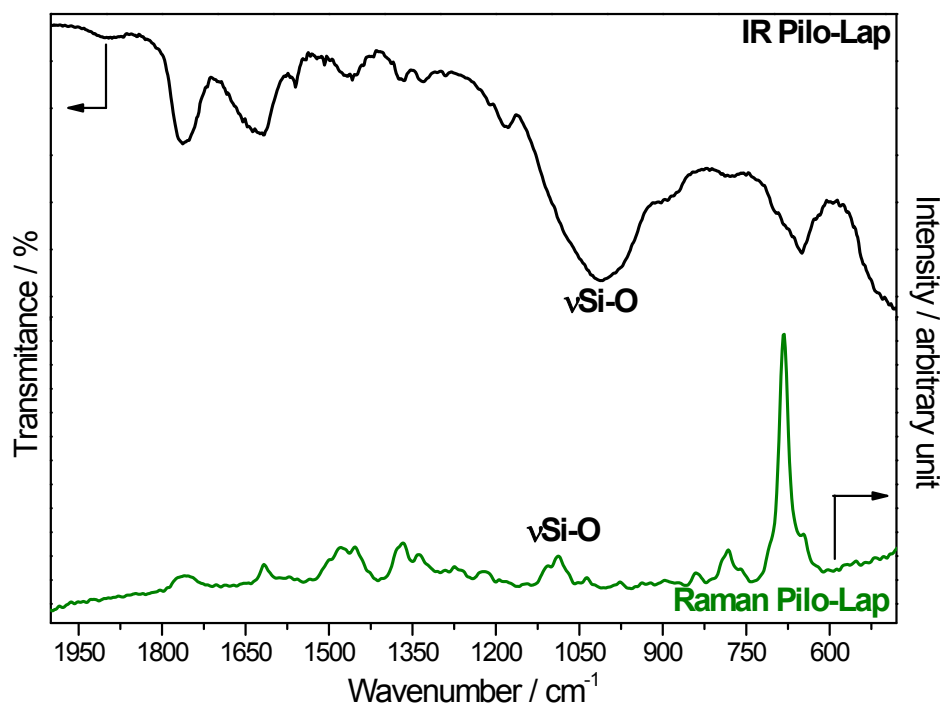


Figure S6: FTIR and Raman spectra of the Pilo-Lap sample.

Figure S7 shows the linear correlation between the two theoretical conformers and the experimental results.

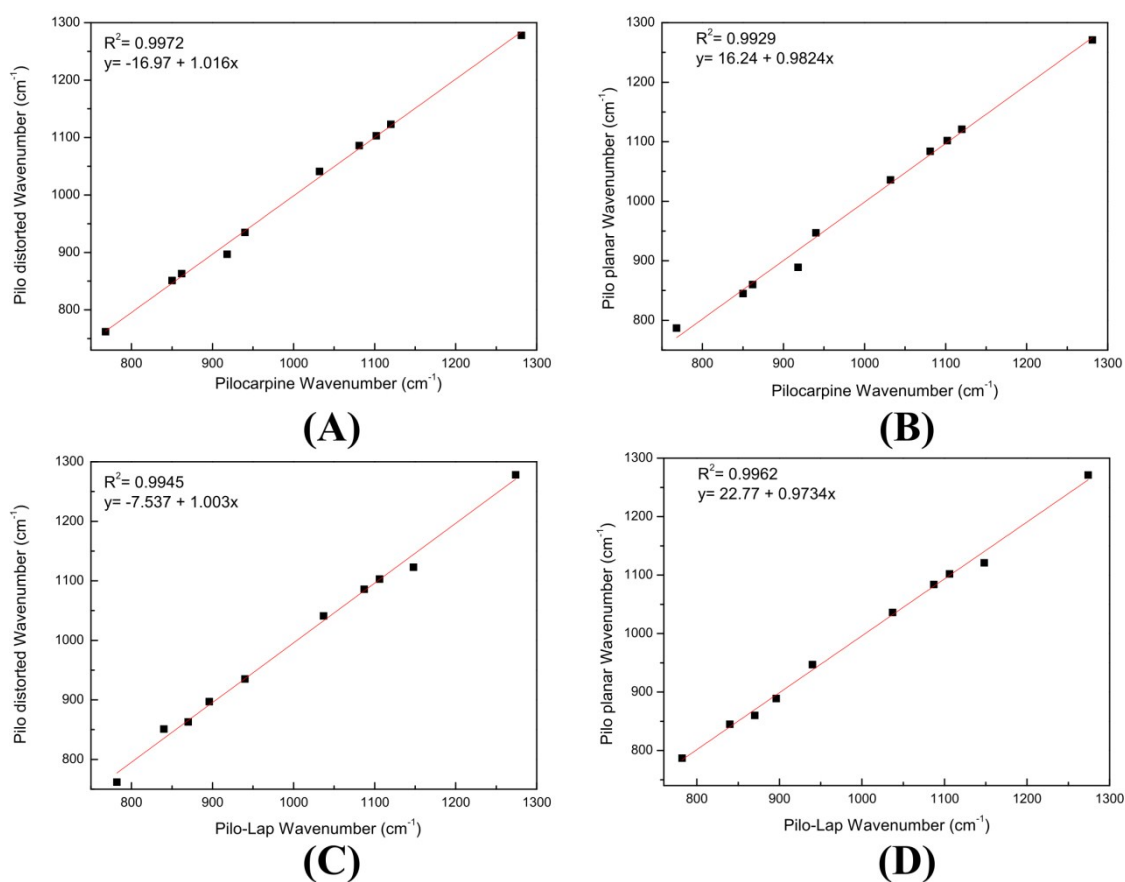


Figure S7: Raman wavenumber linear correlation: **(A)** PiloHCl and Pilo⁺ distorted; **(B)** PiloHCl and Pilo⁺ planar; **(C)** Pilo-Lap and Pilo⁺ distorted; **(D)** Pilo-Lap and Pilo⁺ planar.

References

- (1) L. M. Veras, M. A. Guimaraes, Y. D. Campelo, M. M. Vieira, C. Nascimento, D. F. Lima, L. Vasconcelos, E. Nakano, S. S. Kuckelhaus, M. C. Batista, J. R. Leite and J. Moraes, *Curr. Med. Chem.*, 2012, **19**, 2051–2058.