OPTIMIZATION OF qNMR PARAMETERS FOR THE QUANTIFICATION OF BARBINERVIC ACID IN *Eugenia punicifolia* CRUDE EXTRACTS

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Introduction

Nuclear Magnetic Resonance (NMR) is a fast, non-destructive technique that is very efficient in structural characterization and qualitative and quantitative analysis. Therefore, this technique has been applied in several areas and was included in Brazilian Pharmacopeia 5th edition (2017).

NMR is inherently quantitative whereas the area of the response spectral signal is directly proportional to the number of nuclei that absorb radiofrequency energy for this resonance signal. So that NMR could be used quantitatively, is necessary to ensuring that all hydrogens relax completely before applying a new pulse, that is, the excited nuclear spins completely return to the fundamental state. For this, the interval between pulses (delay – d_1) must be at least equal to the relaxation time multiplied by 5. The great advantage of quantitative NMR (qNMR) over other techniques is that it eliminates the need to use internal standards with similar chemical structures and the construction of analytical curves. Thus, the qNMR technique can be considered a primary standardization method and allows to reach a precision greater than 99% when the signal-to-noise (S/N) ration is 200:1[2].

The aim of the present study was to optimize the acquisition parameters of the ^{1}H NMR spectra to enable its application in the calculation of barbinervic acid contents in crude extracts of *Eugenia punicifolia* leaves.

Material and Methods

Quantitative ^{1}H NMR spectra were acquired in a Varian VNMRS 500 MHz spectrometer from crude extracts solubilized in 600 µL of deuterated chloroform with tetrametylsilane as an internal standard at 27°C. From ^{1}H NMR spectrum of the barbinervic acid standard (BA) and *Eugenia punicifolia* crude extract (EPCE), the following parameters for the qHNMR were optimized: spectral window (sw), pulse width at 90° (pw90), longitudinal relaxation time (T_1), signal-to-noise ratio (S/N) and number of transients (nt). The experiment for each parameter was performed separately with the acquisition of scanning spectra. From the analysis of the spectra obtained, the value of each parameter was established and inserted in the acquisition of the qHNMR spectra.

Results and Discussion

A pw array was carried out by scanning 40 spectra with 1 µs increments pulse time variation. In this scan, the signal amplitude goes through a maximum corresponding to the 90° pulse and decays to zero, corresponding to 180° pulse (Figure 1A)[3]. The maximum value observed corresponds to the pw90 value to be used. In our experiment, the value of pw90 was equal to 9 µs.

To comply with the parameters validation conditions for quantitative NMR use, the T_1 value must correspond to the longer T_1 time. The inversion-recovery experiment (T_1 measurement) was carried out entering pw90 value previously obtained (Figure 1B). As a result of the calculation, we have an output table with relaxation times values of all signals. Excluding the solvent signals, we obtained a value of 6.88 s for the longer T_1 time of EPCE at 2.18 ppm. Thus, the value was rounded to 7 and multiplied by 5, obtaining the value of d1=35 s (d1=5*T_1) [3].

Considering that the S/N ratio is proportional to the square root of the number of scans, the influence of the number of transients (nt) on the values of the S/N ratio was analyzed using the VnmrJ 4.2 (Agilent)

software. For the acquisition of quantitative spectra, the S/N ratio must be ≥ 250:1. This parameter is essential to ensure that the signal area corresponds to the number of nuclei and, consequently, the precision of the method[4]. Observing the spectra obtained in nt scan (Figure 1C), we have an improvement in the resolution for nt=64 and S/N ratio value of 650:1. Thus, for the acquisition of quantitative spectra, 64 transients were used, satisfying specification (S/N≥ 250:1) and ensuring the precision of the method above of 99%.

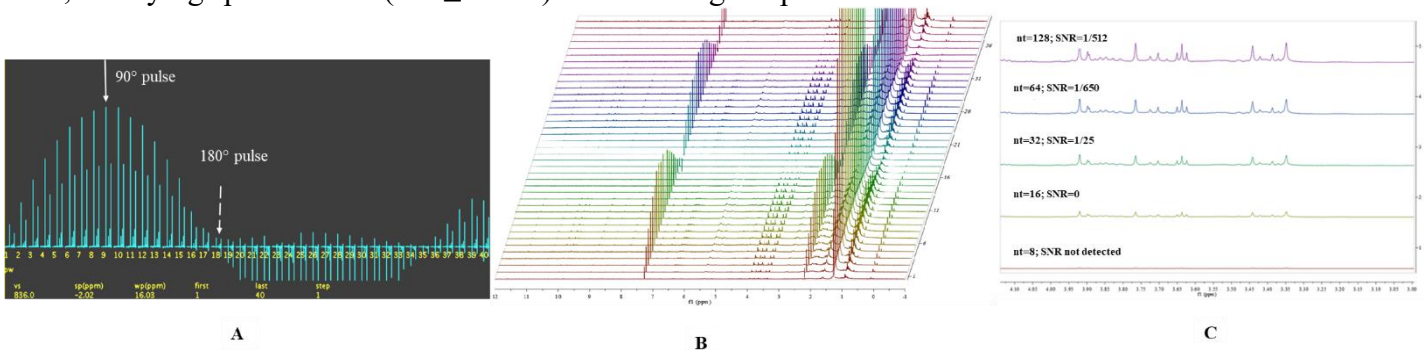

Figura 1 – A: A*rray* pw90 experiment from barbinervic acid standard solubilized in $CDCl_3$; B: Inversion-recovery experiment (T_1 measurement) from EPCE crude extract solubilized in $CDCl_3$; C: nt array experiment from EPCE crude extract solubilized in $CDCl_3$.

The values of the parameters found referring to the standard of barbinervic acid and *E. punicifolia* crude extract were the same and allowed the acquisition of spectra with optimum precision and resolution. Optimized parameters were inserted in the acquisition of the qHNMR spectra (pw90=9 µs, d1=35 e nt=64). In the spectrum of EPCE, the selected signal referring to BA was identified (3,64 ppm, triplet) and the area of this signal (2093.43) was used to calculate the content. The content of BA found in EPCE was 0,005%.

Conclusion

With the optimization experiments of the acquisition parameters of the ^{1}H NMR spectra it was possible obtaining valid signals for the calculation of barbinervic acid contents in crude extracts of *Eugenia punicifolia* leaves. These optimized parameters guarantee that all nuclear spins have returned to the fundamental state and, consequently, the signal area corresponds to the number of nuclei referring to the signal, resulting in spectra with optimal accuracy and resolution.

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