SUPPORTING INFORMATION

Associated with the paper

HPLC-DAD Quantification of Usnic Acid and Atranorin and Evaluation of Antibacterial and Antifungal Activities in Eight Peruvian Lichens

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1S. Method Validation

The validation of the analytical methods used to quantify UA and ATR in lichen extracts was performed according to the parameters recommended by the USP–NF (United States Pharmacopeia) [1], the Lab Guide to Method Validation by EURAChem [2], and the Harmonised Tripartite Guideline (ICH, 2005) [3]. Each method was independently evaluated, considering parameters such as linearity, precision, accuracy, limit of detection, and limit of quantification.

Linearity

Five calibration levels were prepared in the range of 12–202 μg/mL for UA and 45–683 μg/mL for ATR, dissolving the standards in acetonitrile. Each concentration level was analyzed in triplicate. The calibration curve was fitted using the least squares method, and the regression analysis of variance was performed. All were linear as their correlation coefficient (r) was greater than 0.999.

LOD and LOQ

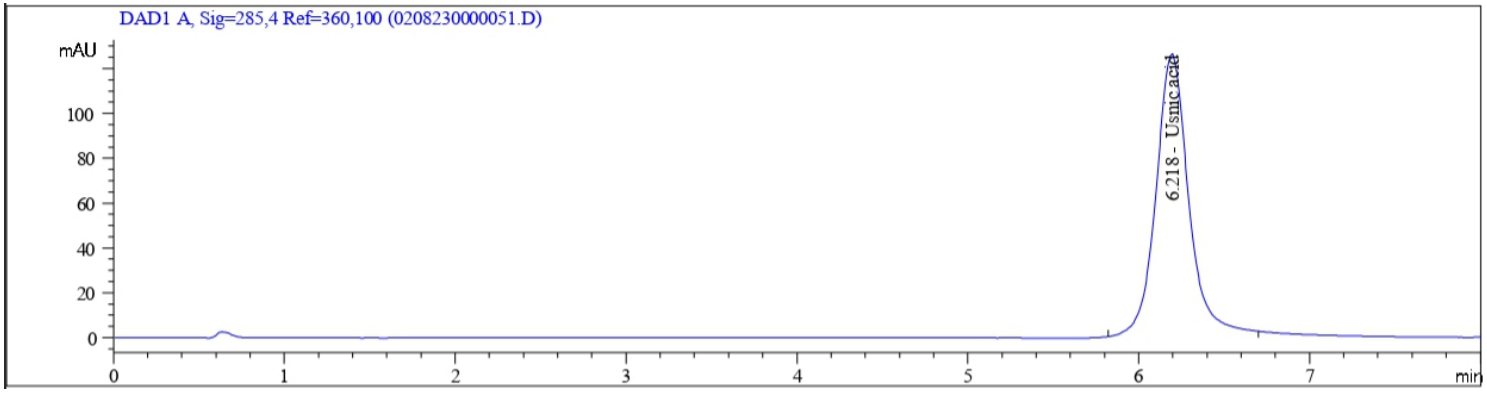
The LOD and LOQ were determined by preparing low-concentration standard solutions, measuring each level in triplicate to estimate background noise, constructing the calibration curve and calculating its slope; the values were then obtained using the formulas LOD = 3.3*σ/slope and LOQ = 10*σ/slope, and experimentally verified to ensure detectability at the LOD and reliable quantification at the LOQ [2, 3].

Precision

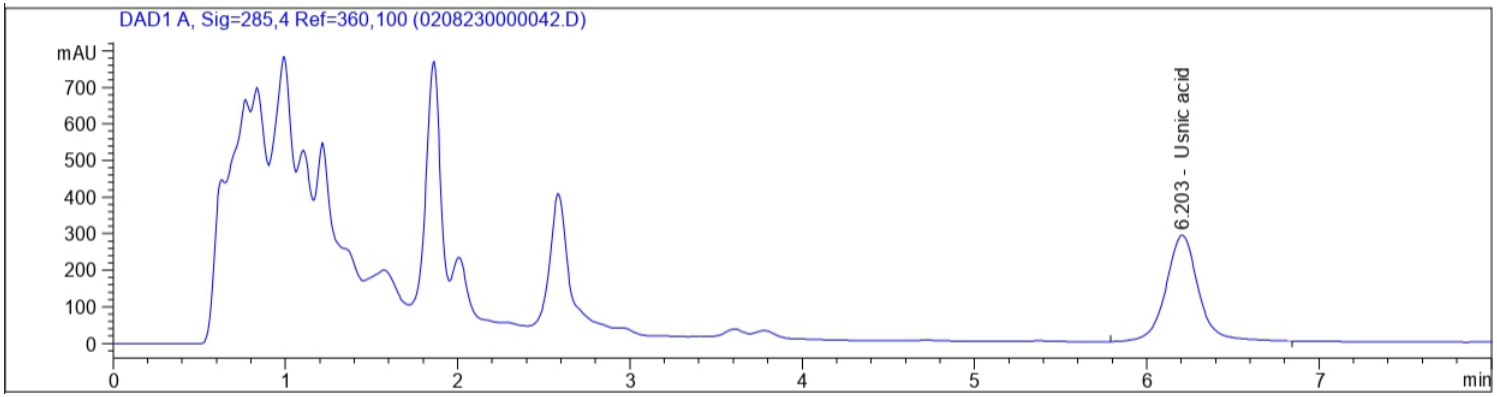
Repeatability was assessed by analyzing samples at three concentration levels: 18, 50, and 100 μg/mL for UA, and 50, 200, and 400 μg/mL for ATR. Experiments were conducted on three separate days, with ten replicates (n = 10) at each concentration level per day. Results were evaluated based on the relative standard deviation (%RSD < 2) and recovery percentage, calculated according to the Horwitz equation [4].

Accuracy

The accuracy of the method was evaluated by preparing standard solutions at three concentration levels (18, 50, and 100 μg/mL), each analyzed in independent replicates under identical conditions; the obtained results were compared with the theoretical values, and both the recovery percentage and % RSD were calculated to assess dispersion; recovery values were considered acceptable within the 95–105 % range [2, 4].

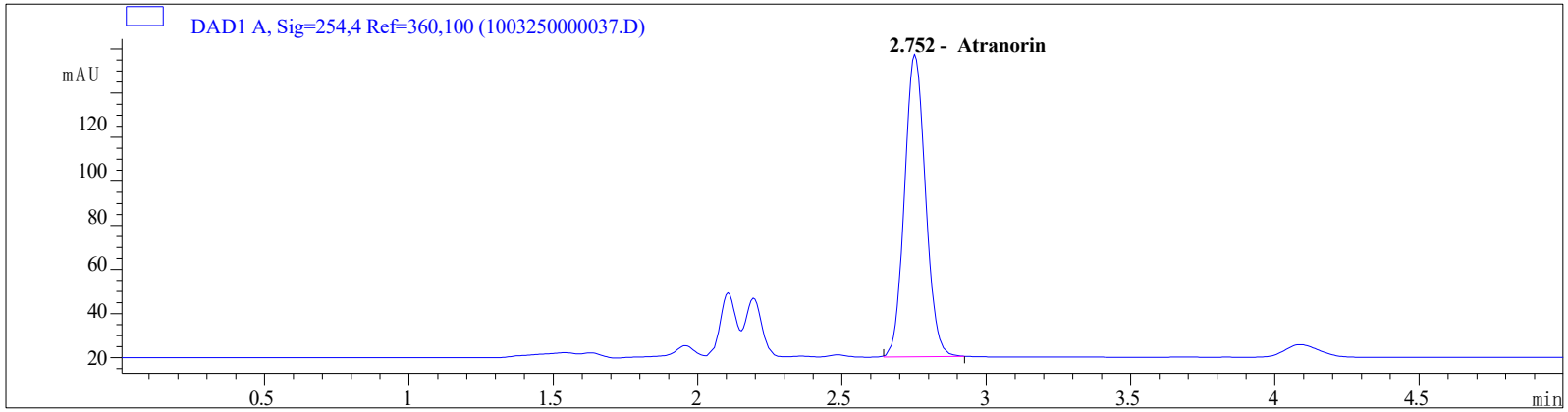


(a)

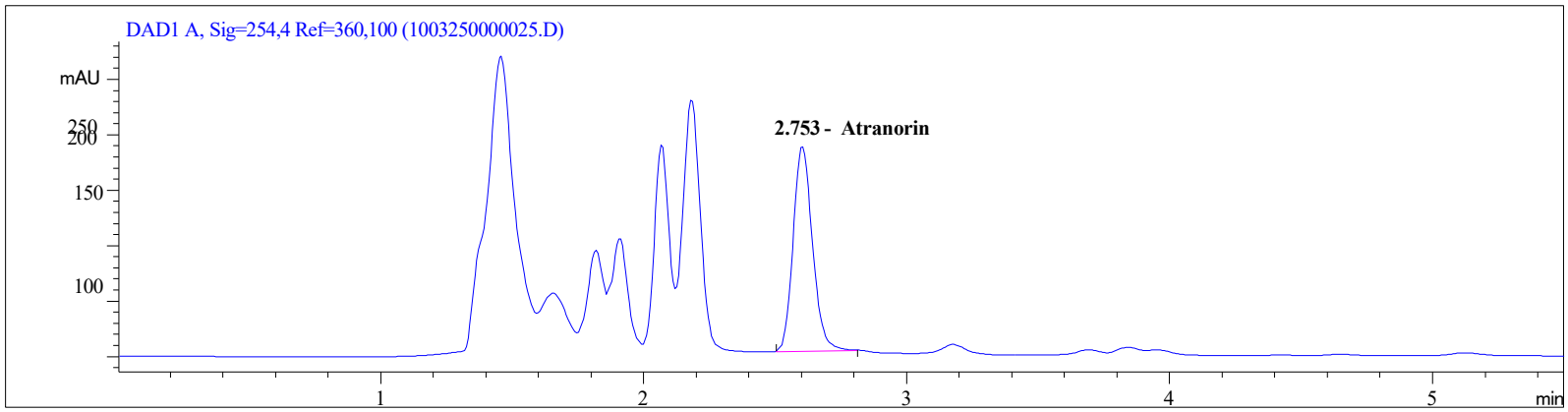


(b)

Figure S1. Comparison of chromatograms for (a) UA reference compound and (b) lichen extract obtained at 285.4 nm



(a)



(b)

Figure S2. Comparison of chromatograms for (a) ATR reference compound and (b) lichen extract obtained at 254.4 nm

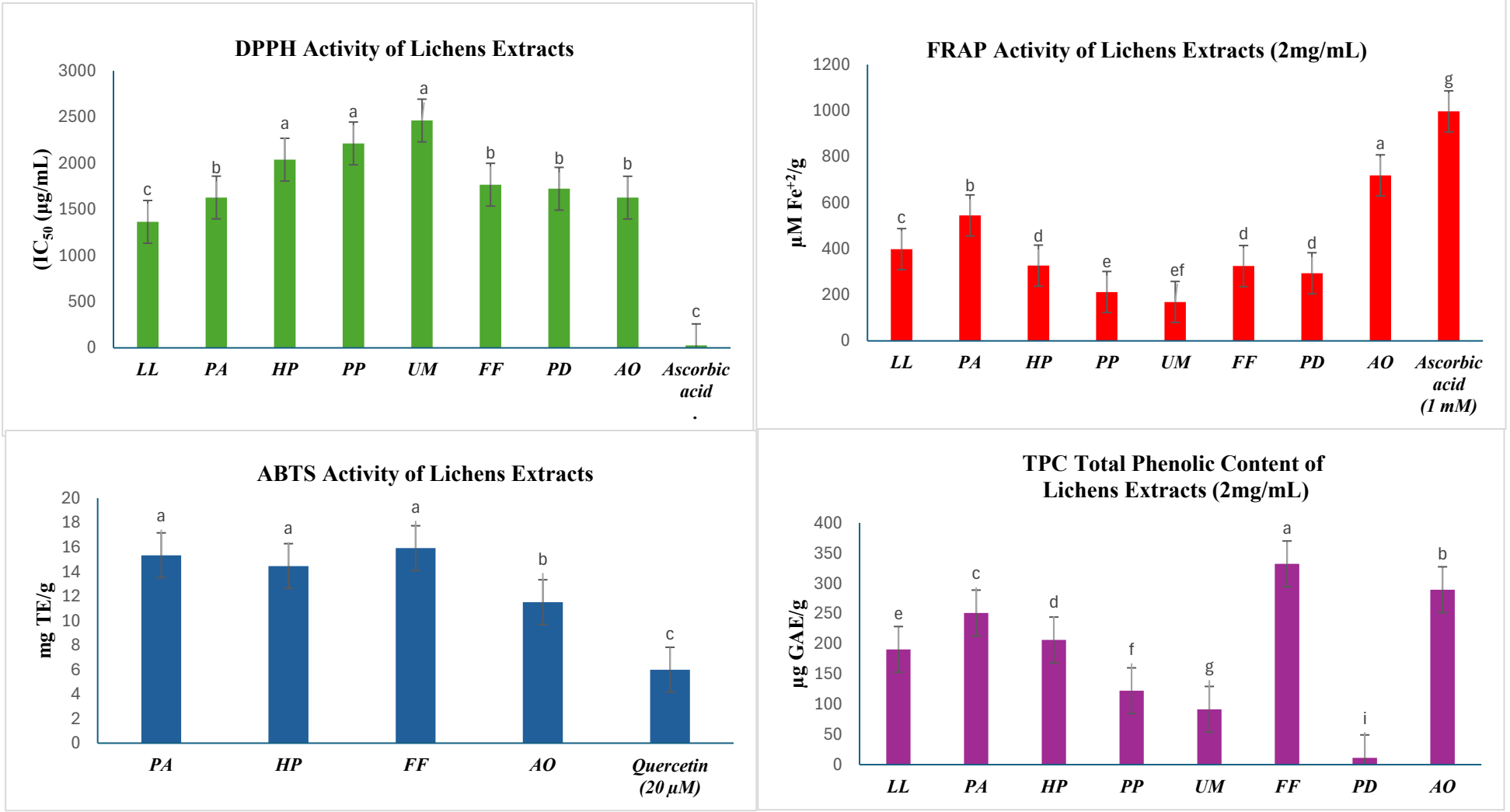


Figure S3. Evaluation of DPPH, FRAP, ABTS, and TPC in Methanol:Acetone (1:1) Lichen extract

[1] United States Pharmacopeia–National Formulary (USP–NF). General chapter <1225> validation of compendial procedures. Rockville (MD): United States Pharmacopeial Convention; [USP vigente].

[2] Magnusson B, Örnemark U, editors. Eurachem guide: the fitness for purpose of analytical methods – a laboratory guide to method validation. 2nd ed. Teddington (UK): Eurachem; 2014.

[3] International Conference on Harmonisation (ICH). ICH harmonised tripartite guideline: validation of analytical procedures: text and methodology Q2(R1). Geneva: ICH; 2005.

[4] AOAC International. Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals. Gaithersburg (MD): AOAC International; 2002. Available from: https://s27415.pcdn.co/wp-content/uploads/2020/01/64ER20-7/Validation_Methods/d-AOAC_Guidelines_For_Single_Laboratory_Validation_Dietary_Supplements_and_Botanicals.pdf