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QUALITY IN ANALYTICAL MEASUREMENTS

Validation of of the Quantification of Mitragynine in Kratom by HPLC-DAD

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INTRODUCTION

Mitragynine is the most abundant indole alkaloid present in *Mitragyna speciosa* Korth (Rubiaceae family), an endemic plant from Southeast Asia marketed in Europe as Kratom (dried leaves or as an extract). This alkaloid, besides being a chemical marker for *M. speciosa*, showed a strong antinociceptive effect and yet, acts like a psychostimulant. The consumption of these vegetable products is dangerous due to the psychotropic effects of their alkaloids. The content of these natural products varies according to geographical region and season. The vegetal material processing can also play a relevant role in composition variability. This work presents the validation of the quantification of mitragynine in kratom by HPLC-DAD, after a solid-liquid extraction, to allow studying the contents of this alkaloid in commercialised products. This variability is relevant to evaluate the consumption risk.

METHODOLOGY

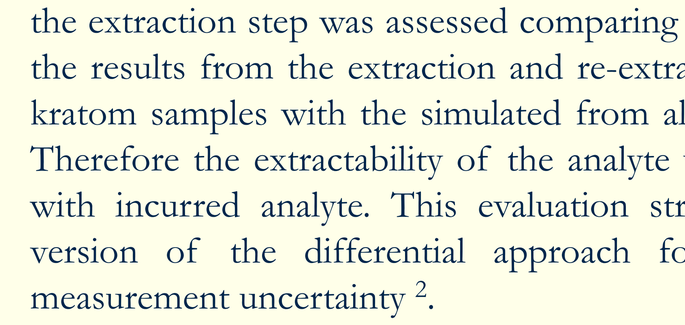
The validation involved studying the performance of the analytical steps separately and combining their uncertainty using Monte Carlo simulations.

The uncertainty associated with gravimetric and volumetric steps was assessed through Monte Carlo simulations of components described in the Eurachem/CITAC guide [1]. The instrumental quantification was assessed through Monte Carlo simulations of signals taking into account models of the variation of signal precision with the mass concentration of the analyte and the correlation of operations and effects involved in calibrators preparations, and using the non-parametric Theil's regression method. The uncertainty associated with the extraction step was assessed comparing the observed dispersion of the results from the extraction and re-extraction of mitragynine from kratom samples with the simulated from all the other analytical steps. Therefore the extractability of the analyte was assessed from samples with incurred analyte. This evaluation strategy is the Monte Carlo version of the differential approach for the evaluation of the measurement uncertainty [2].

The defined relative target standard uncertainty, u^{tg} , is 2.4% since it was intended to be discriminated differences of mitragynine mass fraction in samples larger than 10% (u^{tg} =10%/4.24)[3].

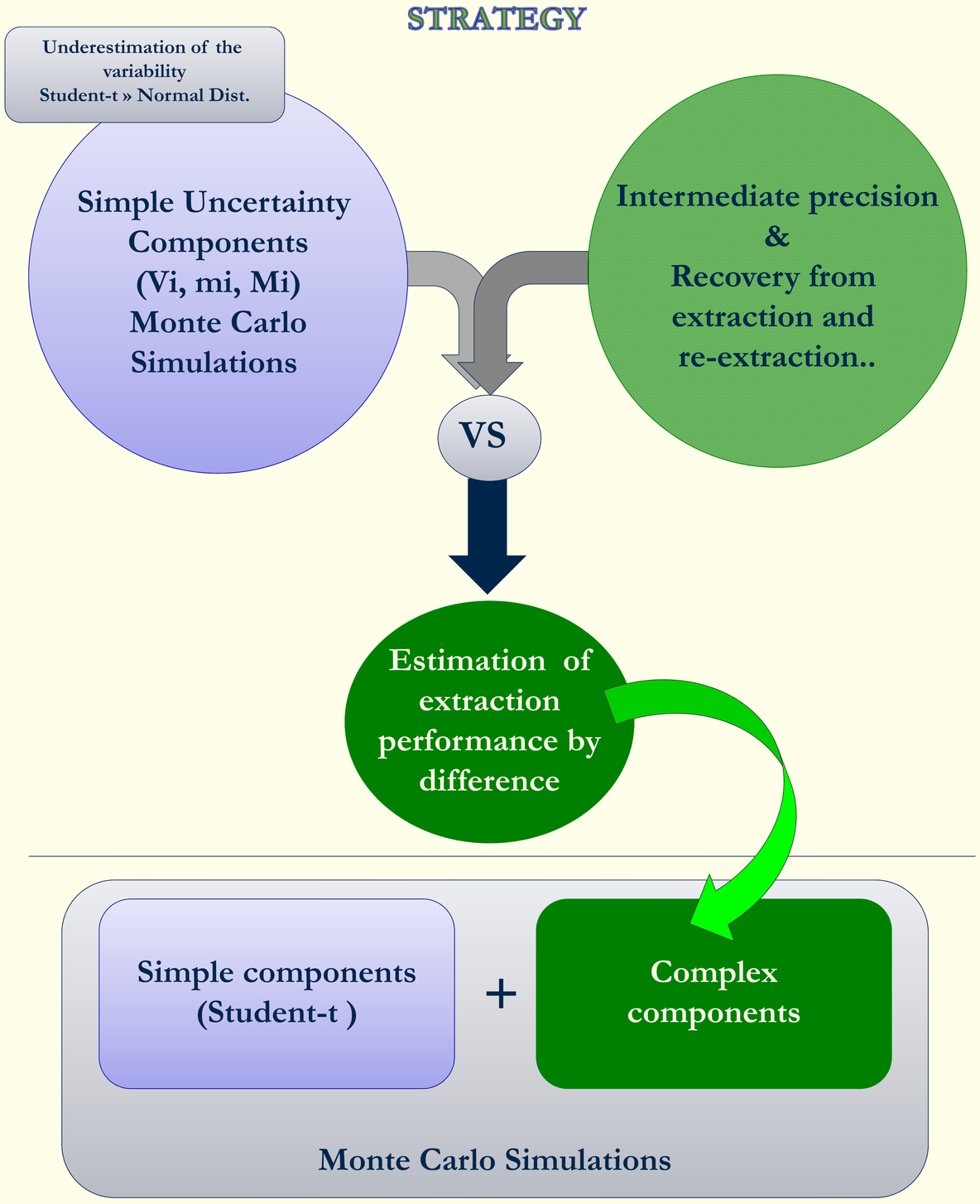
Mitragynine measurements are fit for the intended use in the studied mass fraction range, 0.08-5 % (w/w), since presented relative standard uncertainties between 1.6-2.4%. The analysed kratom products present mitragynine mass fraction between 1.2-2.7% (w/w).

RESULTS



Sample	Mitragynine %(m/m)
Kr01	1.551 ± 0.057
Kr02	2.67 ± 0.10
Kr03	1.608 ± 0.059
Kr04	1.036 ± 0.041
Kr05	1.601 ± 0.059
Kr06	1.436 ± 0.054
Kr07	1.189 ± 0.045

STRATEGY



Conclusion: The developed strategy was successfully applied to the determination of Mitragynine in Kratiom

References: (1) A. Williams, S. L. R. Ellison (Eds), Quantifying Uncertainty in Analytical Measurement, 3rd Ed., Eurachem/CITAC, 2012; **(2)** R. J. N. Bettencourt da Silva, M. J. Lino, J. R. Santos, M. F. G. F. C. Camões, Analyst 125 (2000) 1459-1464; **(3)** R. J. N. Bettencourt da Silva, Water 5 (2013) 1279-1302.

Acknowledgements

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