Quantitative NMR Estimation of Primary Metabolites in Turmeric Spice

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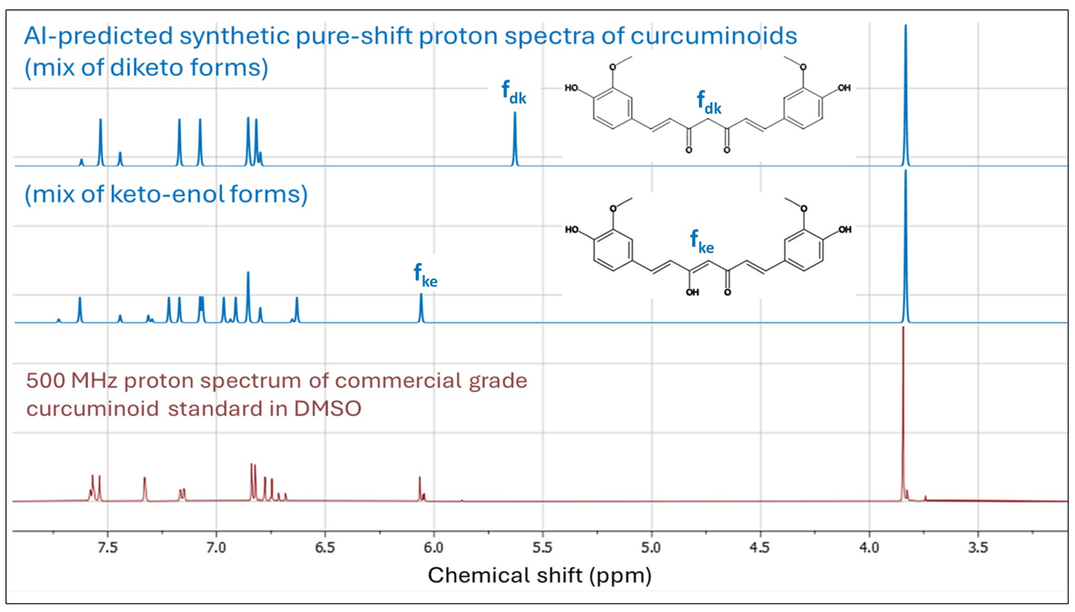
Why quantify turmeric metabolites?

Turmeric (Curcuma longa) has been cultivated for over 4,000 years, and the dried ground tubers are widely used as a culinary spice and dietary supplement. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin, collectively known as curcuminoids, give turmeric its vibrant yellow colour. Around 70 varieties are grown, that differ widely in their curcuminoid content; Lankadong is the most sought-after variety for its high curcumin content.

Accurate quantification of curcuminoids is important for detecting fraud, where lower-quality turmeric is mislabelled as premium or bulked with additives and recoloured with dyes. It is also crucial for manufacture of supplements, which alter the bioavailability of curcumin substantially such that its concentration needs to be controlled to avoid toxicity.

500 MHz ^{1}H- NMR spectrum of commercial curcumin

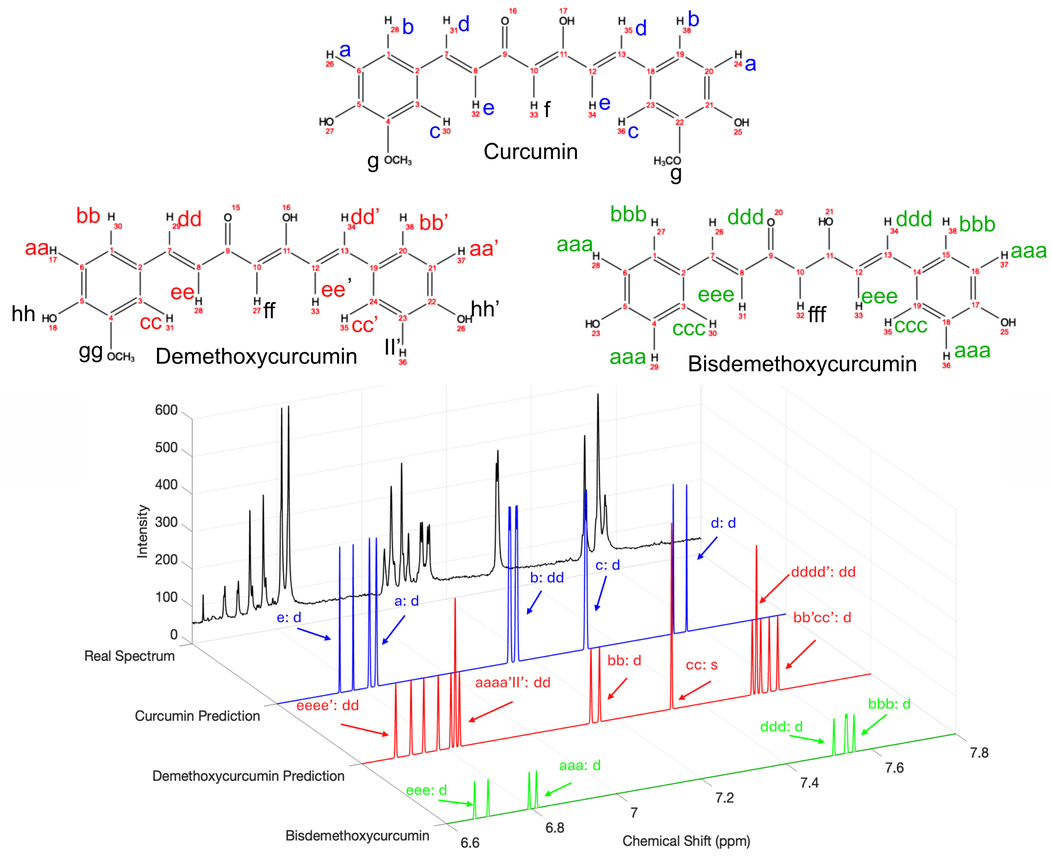
Commercial-grade 'curcumin' comprises a mix of curcumin (>65% w/w), demethoxycurcumin and bisdemethoxycurcumin. Its experimentally acquired 500 MHz ^{1}H-NMR spectrum is shown in the figure. In solution, curcuminoids can exist as various tautomers dependent on the solvent. Also shown are AI-synthesized pure-shift ^{1}H spectra of the diketo and keto-enol forms of 75:20:5 mixtures of the three curcuminoids. This confirms that in DMSO-d6, curcuminoids predominantly adopt the keto-enol tautomer. This is due to the stabilization of the intramolecular hydrogen bond between the enol proton and the adjacent carbonyl group.



AI-synthesised pure-shift proton spectra are shown for the diketo and keto-enol forms of 75:20:5 mixtures of the main curcuminoids, along with the experimentally acquired spectrum of the commercial-grade curcumin (≥65% w/w).

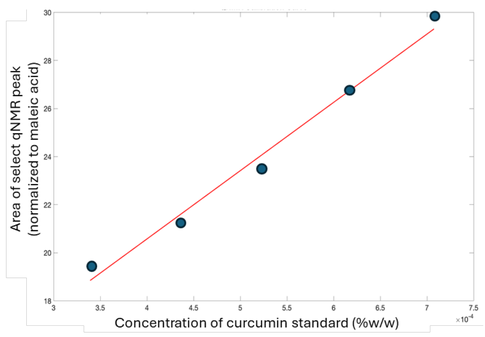
qNMR to quantify individual curcuminoids

The main 500 MHz ^{1}H-NMR signals with specificity for individual curcuminoids are in the aromatic region between 6 and 8 ppm.



Experimental NMR spectrum of extract of turmeric powder from a trusted source (black line) stacked with synthetic spectra of individual curcuminoids. These were constructed by peak simulation using chemical shifts, coupling constants and multiplicities obtained from the literature. Proton assignments are marked with reference to the structure diagrams above.

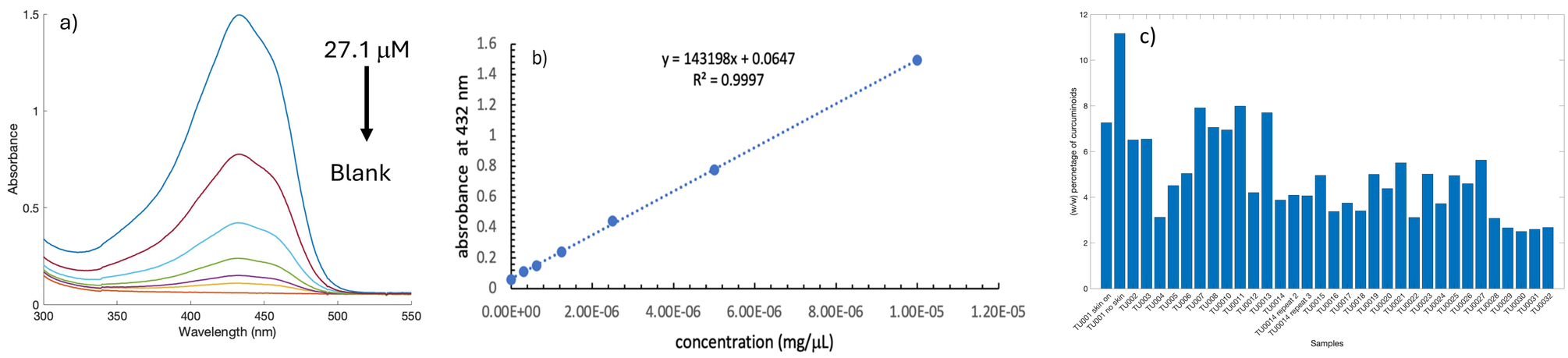
Using maleic acid as an internal standard, qNMR was used to accurately quantify curcuminoid concentration in spiked turmeric extracts. The qNMR calculations require knowledge of the number of protons in each peak, along with careful choice of the peaks to use, because in the spectra of real samples, there is some overlap of the curcuminoids with peaks from other minor metabolites. An iterative machine learning approach was implemented to make this choice, yielding the calibration curve below. By extrapolation, the curcuminoid content for this turmeric sample is estimated to be 5.90 %w/w, comparable to the value by UV-Vis of 6.51%w/w.



Iteratively fitted qNMR calibration curve of turmeric powder spiked with commercial grade 'curcumin'.

The UV-Vis approach

As brightly coloured extracts, curcuminoids are amenable to analysis by Ultraviolet-Visible (UV-Vis) spectroscopy, a well-established approach for measuring concentrations of solutions. A calibration curve can be used to estimate the curcuminoid content of commercially sold turmeric samples; the average is 4.80 ± 1.7 %w/w. While this method is quick and cost-effective, a main disadvantage is that it cannot accurately differentiate between the three curcuminoids. This limits its utility, as the individual compounds have different biological effects and are ideally quantified individually..



(a) UV-Vis data of curcumin for calibration curve: concentrations: 27.1, 13.6, 6.8, 3.4, 1.7, 0.85 µM and a blank. (b): Calibration curve of curcumin constructed from peak absorbance values at 432 nm. (c): (w/w) percentage of curcuminoids for all turmeric samples