ISOLATION, CHARACTERIZATION AND QUANTITATION OF CURCUMINOIDS: NMR studies in turmeric market products and pharmaceutical supplements

M.K. Daskalopoulou[1,2], D. Matiadis[2], M. Sagnou[2], M. Pelecanou[2], T. Mavromoustakos[1], A. Panagiotopoulou[2,*]

[1]Department of Chemistry, University of Athens, Athens, Greece

[2]Institute of Biosciences & Applications, NCSR "Demokritos", 15310, Athens, Greece

*(*apanagio@bio.demokritos.gr)*

ABSTRACT

Turmeric is the common name for the rhizomatous herb *Curcuma longa L.* (Family: Zingiberaceae), the dried roots of which have been widely used as spice, food coloring (E100) and taste enhancer, as well as in traditional medicine for a great variety of therapeutic purposes. Investigation on turmeric extracts (ethanol, methanol, water, and ethyl acetate) revealed the group of active ingredients, the "curcuminoids", which are responsible for the characteristic yellow color of the rhizome. More importantly, curcuminoids have been endorsed with pleiotropic pharmaceutical activity [1]. The three main curcuminoids accounting for more than 90% of the extract are: curcumin (70-80 %), demethoxycurcumin (15-25 %) and bisdemethoxycurcumin (2.5-6.5 %) [2]. In most of the earlier studies, it was this curcuminoid mixture that was shown to have numerous pharmacological properties, including antioxidant, neuroprotective, anticancer, antifungal, antimicrobial, antiviral, and anti-inflammatory activities, whereas more recent reports investigate and reveal the multifunctional potential of each individual compound of the mixture [3,4].

In the present study, we performed quantitative analysis of the curcumin present in the turmeric market products and pharmaceutical supplements. More specifically, we have quantified curcumin in the mixtures by rapid quantitative ^{1}H NMR (qNMR) using 3, 5 bis-trifluoromethyl benzoic acid as an internal standard. This qNMR analysis can in principle be used as a tool of quality control and standardization of natural products, pharmaceutical and dietary supplements.

INTRODUCTION

Turmeric, scientifically known as *Curcuma longa* is a member of the *Zingiberaceae* family. Turmeric's major bioactive curcuminoid substance is curcumin, with the IUPAC name (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione and chemical formula $C_{21}H_{20}O_6$. Curcumin is the major component (70-80 %) but also other substances of much related structure are present in the turmeric extract, the most significant being demethoxycurcumin (15-25%) and bisdemethoxycurcumin (2.5-6.5 %) [2]. Much uncertainty exists in the scientific literature and also in the herbal market on the exact composition of turmeric, turmeric extract, curcuminoid-enriched turmeric extract, or curcumin as a single-chemical entity.

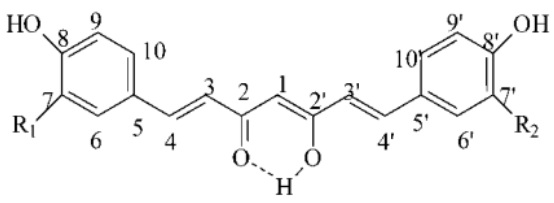

Figure 1 *Chemical structure of curcuminoids* [7]

Structure	R_1	R_2
curcumin	OMe	OMe
demethoxycurcumin	H	OMe
bisdemethoxycurcumin	H	H

The medicinal properties of turmeric have been known for thousands of years, as it is proved to have strong antioxidant, anti-inflammatory, antimicrobial and anti-tumor properties. [5, 6] However, in recent times, ascertaining the chemical composition of a sample with biological activity is a fundamental requirement for assessing its potential medicinal value, especially regarding products of natural origin. Simply reporting the content (often termed "purity") of the major component ignores potential contributions of other constituents ("impurities") and their in vivo metabolites to the observed biological activity. In the case of turmeric, the investigation of the biological activity of each of the curcuminoid components has revealed differentiation in their activity and spurred interest in the separate evaluation of the activity of curcuminoids for their potential applications not as "mixtures" but as individual bioactive entities. [7]

Within this framework, this study focused on isolation and characterization of curcuminoids by column chromatography, followed by their detailed characterization by one and two-dimensional NMR. The NMR spectral data were utilized for the quantitation of curcumin in commercial curcumin products through the application of rapid quantitative ^{1}H NMR (qNMR) using 3,5 trifluoromethyl benzoic acid, as a validated reference internal standard.

MATERIALS

For isolation

- 10 g curcumin mixture: "Alfa Aesar" curcumin 95% (concentration in total curcuminoids), from turmeric rhizome $C_{21}H_{20}O_6$ (B21573, LOT: 10165835, CAS : 458-37-7, FW: 368.39 , EINECS : 207-280-5, MP : 170-175˚C)
- Dichloromethane
- Methanol

For characterization and quantitation

- Curcumin mixture Alfa Aesar 95% (concentration in total curcuminoids)
- Pharmaceutical curcumin complex 93% (concentration in total curcuminoids)
- Commercial turmeric
- Organic, commercial turmeric
- Solvent: d_6-DMSO
- Internal standard: 3,5 bis trifluoromethyl benzoic acid [3,5-BTMFBA, 99.96±0.06% (w/w)]

METHODOLOGY

The curcuminoids were isolated from the curcumin mixture using a silica gel flash column chromatography (eluting solvent system, chloroform: methanol 99:1), and curcumin, demethoxycurcumin and bis-demethoxycurcumin were separated based on their different polarity. All fractions were collected and compound purity was assessed by TLC (chloroform: methanol 98:2). One and two-dimensional NMR studies performed on a Bruker Avance DRX, 500 MHz NMR spectrometer with a ^{1}H-^{13}C inverse BBI probe, confirmed the molecular structures of the three curcuminoids and their presence in each product under study. For the qNMR study, the analyte and the internal standard were weighed to the nearest 0.1 mg on an analytical balance (Mettler-Toledo ME204T) in such amounts that the ratio of the proton signals to be determined being 1:1. The solids were dissolved in 0.75 mL of deuterated solvent, DMSO-d_6. Their NMR spectra were integrated in order to determine the % composition of the curcumin in each product. The spectra were obtained at 25 °C according to the following protocol:

Number of scans: ns=32

Spectral width: SW=14 ppm centered at 7.9 ppm (O1p=7.9 ppm)

Relaxation delay: d1=20 sec

Pulse width, P1 calibrated at 90°: p1=8.5 μs

Exponential line- broadening window function: lb=0.3 Hz
A total of 32 scans were acquired, collecting 64 K data points of time domain data, using a 90° pulse duration of 8.5 μs. To increase sensitivity, FIDs were multiplied by a suitable exponential weighting function corresponding to 0.3 Hz.
The quantification of the purity of the products are based on the so-called internal standard method where the analyte signal is directly compared with an internal reference signal. The following equation shows the relevant parameters for the calculation of the purity of the sample (p_s), which is finally expressed as percent (%) mass fraction.

$$p_s = \frac{I_s}{I_{is}} \frac{N_{is}}{N_s} \frac{mw_s}{mw_{is}} \frac{m_{is}}{m_s} p_{is} \qquad (1)$$

In equation 1, *w* is mass fraction (mg/g), *I* is signal integration, *N* is the number of nuclei ^{1}H corresponding to each signal, *MW*, is molecular mass, *m* is weighed mass. The indices *s* and *is* declare the sample and the internal standard, respectively. [8]
Two-dimensional spectra were obtained in all products to identify the presence of the compound curcumin. As internal standard (IS) the 3, 5-bis (trifluoromethyl) benzoic acid was used, because its ^{1}H peaks do not overlap with any curcumin studied. The quantification was based on the ratio of the integrals of the 3, 5-bis (trifluoromethyl) benzoic acid peaks at 8.44 ppm and 8.40 ppm (total three protons, 3H) to those of curcumin at 7.32 ppm corresponding to position-6 (Figure 1) of curcumin (total two protons, 2H). The same method was applied to all samples. Each sample was counted twice and the average of the two resulting values was calculated.

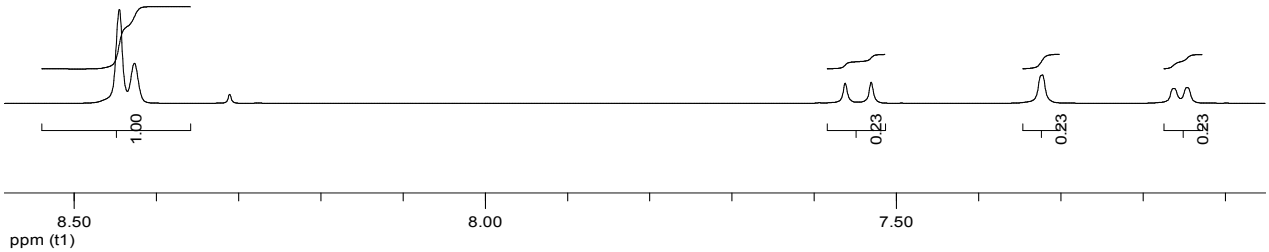

Figure 2 *Curcumin in DMSO-d_6 with 3, 5-bis (trifluoromethyl) benzoic acid for qNMR*

RESULTS

Post-acquisition processing with MestReNova-12.0.0-20080 (Mestrelab Research, Santiago de Compostela, Spain) software produced a table showing the integration ratio and the weighing values for each sample separately. By applying equation 1 the following results were obtained:

Table 1 *Content of market products and pharmaceutical supplements in curcumin*

SUBSTANCE	PURITY / CURCUMIN CONTENT
CURCUMIN	98,43%
Alfa Aesar	84,82%
Solgar Turmeric	72,75%
Macrolife Curcumin	32,11%
Bio Turmeric	30,19%
ANATOLI Turmeric	30,18%
CAPTAINS Turmeric	30,21%

DISCUSSION

The quantitative ^{1}H NMR (qNMR) spectroscopy analysis revealed approximately 30% curcumin in market products and 32-98% in pharmaceutical and dietary supplements. It was also proved that

bio turmeric does not have higher amount of active curcumin compared to regular market products. This method is suitable for quantitation of components within a mixture, because there is no necessity for isolation and purification of the analyte from its mixture and additionally it is simple, rapid and convenient for quantitation of multiple components using single reference compound.

CONCLUSION

NMR spectroscopy is the most suitable technique for structural identification and quantitative analysis of mixtures and qNMR can do that with very high precision. In the present case, the quantification of curcumin, and eventually of each curcuminoid component, in commercial turmeric and curcumin products will help correlate biological activity and expected health benefits with curcuminoid content.

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