Quantitative Nuclear Magnetic Resonance for the determination of Genistein in capsules

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Introduction

Genistein is a natural flavonoid in which the B ring is linked to the heterocyclic ring at the C3 and can occur in foods both in free and esterified form. Dietary supplements containing genistein have positive health effects in areas including prevention of breast, colon and prostate cancers, cardiovascular disease and post-menopausal ailments. Genistein (Figure 1) acts as a chemotherapeutic agent against different types of cancer, by altering apoptosis, the cell cycle, angiogenesis and inhibiting metastasis and many in vitro and in vivo studies support that genistein can be considered a promising chemo preventive agent for the treatment of different types of cancer.

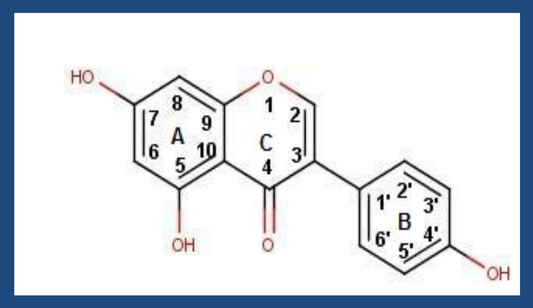

Figure 1: Chemical structure of Genistein

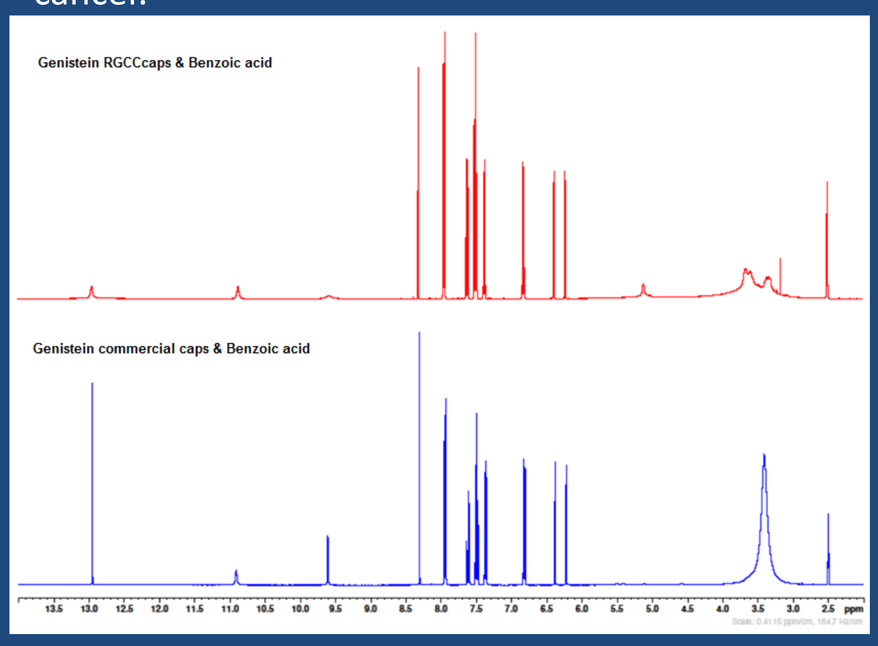

Figure 2: ^{1}H-NMR of Genistein capsules with Internal Standard

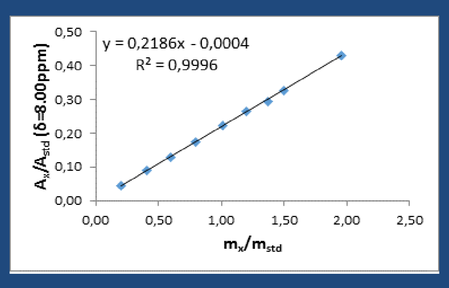

Figure 4: Linearity curve for signal 8.00ppm

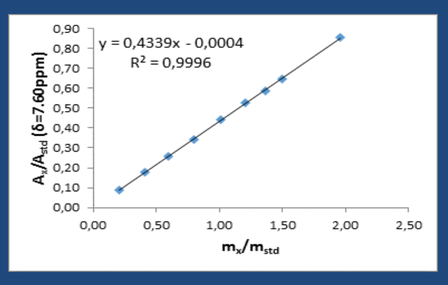

Figure 5: Linearity curve for signal 7.60ppm

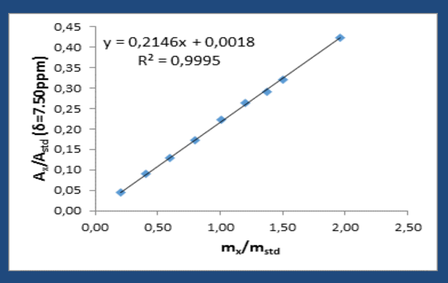

Figure 6: Linearity curve for signal 7.50ppm

Quantitative NMR is specific and selective and therefore, a good analytical technique for quantitative estimation. Through optimization of its acquisition, processing parameters and referencing techniques, qNMR can achieve a high degree of accuracy and precision.
The aim of this study was to develop a qNMR method and analyze genistein composition of commercial capsules and our in-house formulations of dietary supplements (Figure 2). The method was validated according to ICH guidelines and this led us to incorporate this method to our routine analysis of genistein capsules.

Materials and Methods

qNMR analysis was carried out by a Bruker Avance Spectrometer at 400 MHz proton frequency and TOPSPIN software (Table 1). All chemical shifts were reported in parts per million (ppm) relative to deuterated dimethyl sulfoxide (DMSO-d_6) at 2.50ppm and benzoic acid CRS was used as an internal standard (Figure 3). Each measurement was repeated six times.
T1 relaxation time values for targeted protons were: for benzoic acid, δ 8.00 ppm (H-2 & H-6), T1 = 2.041s; δ 7.60 ppm (H-4), T1 = 3.071s; δ 7.50 ppm (H-3 & H-5), T1 = 2.255s; for genistein, δ 8.3 ppm (H-2), T1 = 2.217s.
The amount per unit dose and assay of genistein was calculated by using Equations 1 & 2: $Px = \frac{Ax}{Astd}\frac{Nstd}{Nx}\frac{Mx}{Mstd} \times Pstd \times 100\%\ (1)$ $mx = \frac{Ax}{Astd}\frac{Nstd}{Nx}\frac{Mx}{Mstd}\frac{mstd}{m_{powder}} \times Pstd \times T (2)$

Table 1: Analysis parameters

Optimized Parameter	Value
Pulse Angle	30°
Pulse Width	41.6μs
Datapoints	96152
Number of Scans	64
Acquisition Time	3.999s
Spectral Width	12019.23Hz
Line-broadening Factor	0.1Hz
Repetition Delay	60s

Validation Parameter		STDV (<2%)			% RSD (<2%)		
Analytical Signals		δ*8.00	δ*7.60	δ*7.50	δ*8.00	δ*7.60	δ*7.50
Precision (n=3)		0.57	0.49	0.57	0.59	0.52	0.60
Repeatability		0.71	0.66	0.68	0.74	0.69	0.71
Stability (0-24 h)		0.75	0.57	0.95	0.77	0.59	0.99
Robustness	Number of Scans	0.77	0.69	0.76	0.80	0.72	0.79
	Acquisition Time	0.44	0.70	0.37	0.46	0.73	0.39
	Relaxation Delay	0.61	0.52	0.61	0.63	0.54	0.63
	Analyte Protons	0	0	0	0	0	0
Recovery		1.72	1.79	1.62	1.75	1.83	1.66

Table 2: Validation parameters

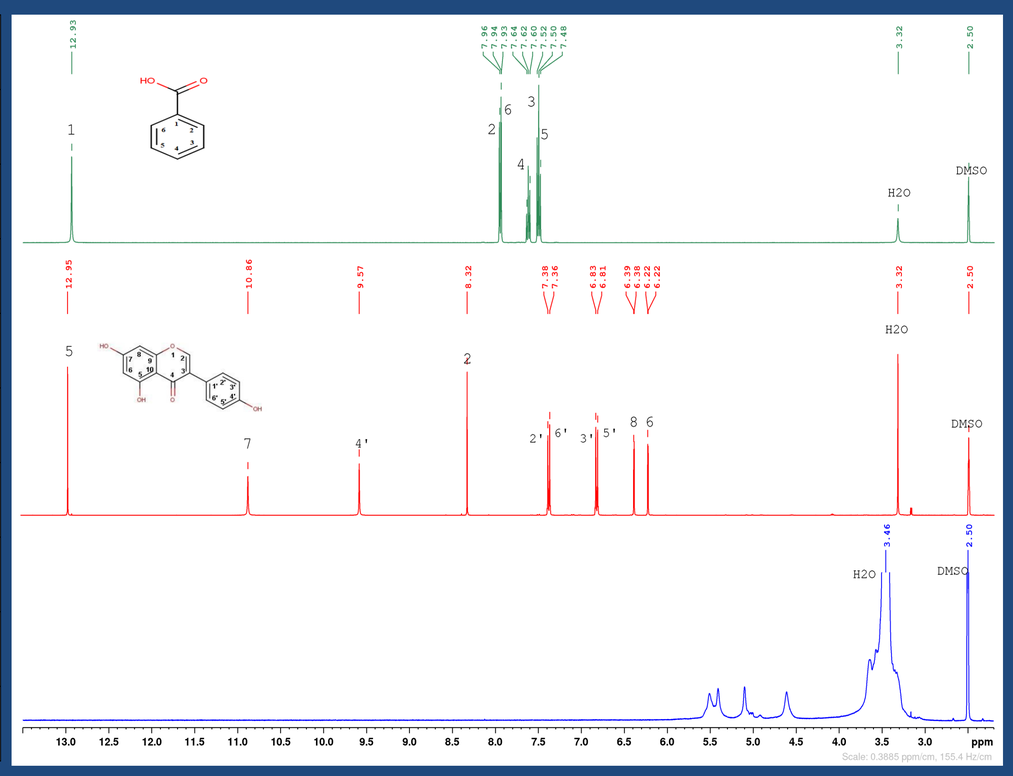

Figure 3: ^{1}H-NMR of Benzoic acid, Genistein & Excipients

Results-Discussion

The qNMR method was validated with respect to specificity, linearity, precision, repeatability, stability, robustness and accuracy according to ICH guidelines (Figure 3, Figures 4-6, Table 2).
Genistein capsules manufactured in our laboratory and commercial capsules were analyzed and their % Purity was calculated. Our formulations presented higher purity (Tables 3 & 4). Finally, this qNMR method is a useful and practical tool for the quantification of genistein in capsules and it is already used for the determination of genistein content in our laboratory.

Purity Determination: $Px = \frac{Ax}{Astd}\frac{Nstd}{Nx}\frac{Mx}{Mstd}\frac{mstd}{mx} \times Pstd \times 100\%$	
m_x = 10.00 mg	m_{std} = 5.30 mg P_{std} = 99.97%
Ax = 0.50	**Nx** = 1
Astd = 1.23	**Nstd**= 1
Mx = 270.24 g/mol	**Mstd** = 122.12 g/mol
P [%] = 48%	

Table 3: Purity of Genistein RGCC capsules

Purity Determination: $Px = \frac{Ax}{Astd}\frac{Nstd}{Nx}\frac{Mx}{Mstd}\frac{mstd}{mx} \times Pstd \times 100\%$	
m_x = 10.00 mg	m_{std} = 5.00 mg P_{std} = 99.97%
Ax = 0.63	**Nx**= 1
Astd = 1.18	**Nstd** = 1
Mx = 270.24 g/mol	**Mstd** = 122.12 g/mol
P [%] = 47%	

Table 4: Purity of Genistein commercial capsules

Selected References
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