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Research Article

QUALITY ASSESSMENT AND QUANTIFICATION OF GENISTEIN IN DIETARY SUPPLEMENTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY, QUANTITATIVE NUCLEAR MAGNETIC RESONANCE, AND TWO-DIMENSIONAL DIFFUSION ORDERED SPECTROSCOPY ^{1}H

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ABSTRACT

Objective: The aim of the present study is to analyze four herbal dietary supplements containing genistein by liquid chromatography, quantitative nuclear magnetic resonance (qNMR), and diffusion ordered spectroscopy (DOSY) ^{1}H NMR.

Methods: Quantification of the active ingredient, genistein, is carried out by high-performance liquid chromatography (HPLC) and qNMR. Two-dimensional (2D) DOSY NMR also allows the qualitative analysis of the samples with the detection of active ingredient and excipients present in the formulations.

Results: The validated HPLC and qNMR methods showed that all four supplements contain genistein in different amounts, and 2D DOSY NMR provides a clear image of all ingredients in the formulations.

Conclusion: The use of the three techniques provides detailed information on each product and its contents, and all of them are currently used for the quality control of natural supplements by our laboratory.

Keywords: Genistein, Quantification, High-performance liquid chromatography, Quantitative nuclear magnetic resonance, Two-dimensional diffusion ordered spectroscopy.

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INTRODUCTION

Polyphenols are a group of mainly natural, but also synthetic or semisynthetic, organic chemicals [1,2], which have been investigated for their protective effects on human health over the past two decades [3-6]. Studies have shown that, when incorporated into the diet, they limit the development of cancers, cardiovascular and neurodegenerative diseases, and others [7-13]. Polyphenols are subdivided into two large groups: Flavonoids and non-flavonoids [14]. Isoflavones are flavonoids in which the B ring is linked to the heterocyclic ring at the C3 position [15,16]. The most important isoflavones are genistein (Fig. 1), daidzein, and glycitein, which can occur in foods both in free and esterified forms [17].

Dietary supplements containing genistein have been investigated for their potential health effects in areas including prevention of breast, colon, and prostate cancers, cardiovascular disease, and postmenopausal ailments [18-22].

High-performance liquid chromatography (HPLC) has been used for decades for the quantification of chemical compounds as it offers a precise, accurate, reproducible, highly sensitive, quick, and automated analysis compared to other quantification methods (ultraviolet [UV], titration, etc.) [23]. Quantitative nuclear magnetic resonance (qNMR) has gained more attention recently as a structural tool and quantitative analytical technique because it provides several advantages, such as simple method development and quick and easy sample preparation [24,25]. Two-dimensional (2D) diffusion ordered spectroscopy (DOSY) ^{1}H NMR is used for the accurate identification of the composition of drugs, dietary supplements, etc. The main advantage of DOSY NMR is to provide global information; it allows the fingerprinting of formulations and can be used to determine similarities or differences between samples [26]. The combination of these three techniques can give detailed information on the components of a complex sample.

The aim of this study was to analyze the genistein composition of commercially available capsules and our formulations of dietary supplements using simple, specific, accurate and precise methods, reversed-phase HPLC, and qNMR. The methods were validated with respect to specificity, linearity, precision, robustness, accuracy, limit of detection (LOD), and limit of quantification (LOQ) according to the International Conference of Harmonization guidelines [27]. 2D DOSY ^{1}H NMR was also used for the quality assessment of the different formulations, especially on their excipient composition. To the best of our knowledge, it is the 1st time, the combination of all these techniques is used for the qualification and quantification of genistein in dietary supplements.

MATERIALS AND METHODS

Materials

Genistein capsules were purchased on the internet or provided as free samples and produced in our pharmaceutical technology laboratory. A list of the analyzed capsules is given in Table 1. All samples were stored in the dark at ambient temperature and analyzed before expiry dates. The genistein analytical standard ≥97% (standard for HPLC) and the benzoic acid (standard for qNMR) were purchased from Sigma-Aldrich (Louis, MO, USA). Deuterated solvent dimethyl sulfoxide (DMSO-d_6, >99.96%), acetonitrile, and water for HPLC were also purchased from Sigma-Aldrich (Taufkirchen, Germany). Glacial acetic acid was purchased from Merck KGaA (Darmstadt, Germany).

Sample preparation and calculations

Chromatographic analysis

Both commercial and in-house supplements were analytically examined regarding their content. For HPLC analysis, the capsule content was weighed and dissolved in methanol resulting in genistein extraction (10.5 ppm). The volume of the sonicated extract was filtered through a 0.45 μm filter and analyzed further for the assay.

The purity of genistein was calculated using equation (1):

$$P_s = \frac{A_s}{A_{std}} \frac{m_{std}}{m_s} \times P_{std} \times 100\% \quad (1)$$

Where A_s and A_{std} are the peak response from the sample and standard solutions; m_{std} and m_s are the weighted mass of genistein standard and genistein substance, respectively, and P_{std} is the purity of genistein standard.

The amount of genistein per unit dose was calculated using equation (2):

$$\frac{mg}{unit} = \frac{A_s}{A_{std}} \times \frac{C_{std} \times P_{std}}{W \times f_{dil}} \times MW \quad (2)$$

Where A_s and A_{std} are the peak response from the sample and standard solutions; C_{std} the standard solution concentration; P_{std} the purity of genistein standard; MW the molecular weight of genistein; W the average capsule weight; and f_{dil} the dilution factor of the standard solution.

NMR analysis

For the calculation of the assay of the supplements, as well as for DOSY experiments, 20 capsules were weighed and finely powdered. A portion of the powder equivalent to 5 mg and 5 mg of the internal standard were accurately weighed and transferred into a vial. The mixture was dissolved in 0.5 ml of DMSO-d_6 using a vortex mixer. After centrifugation, the supernatant was transferred into an analytical NMR tube and the spectrum was obtained. The % purity was calculated using the following equation [28] (3):

$$P_{gen} = \frac{I_{gen}}{I_{ben}} \times \frac{N_{ben}}{N_{gen}} \times \frac{MW_{gen}}{MW_{ben}} \times \frac{m_{ben}}{m_{gen}} \times P_{ben} \times 100\% \quad (3)$$

Where I_{gen} and I_{ben} are the integral values of the signal that belongs to genistein and benzoic acid, respectively, N_{gen} and N_{ben} the number of spins of genistein and benzoic acid; MW_{gen} the molecular weight of genistein (270.24 g/mol); MW_{ben} the molecular weight of benzoic acid (122.12 g/mol); m_{gen} and m_{ben} weighed mass of genistein and benzoic acid; and P_{ben} the purity of benzoic acid (99.97%).

The amount of genistein per unit dose was calculated using equation (Pauli *et al*.) (4):

$$m_{gen} = \frac{I_{gen}}{I_{ben}} \times \frac{N_{ben}}{N_{gen}} \times \frac{MW_{gen}}{MW_{ben}} \times \frac{m_{ben}}{m_{powder}} \times P_{ben} \times T \quad (4)$$

Where m_{powder} is the weighed mass of capsule powder sample taken for the assay and T is the average capsule weight. The significance of the other parameters is identical to the ones mentioned in equation (3).

Instrumentation

Chromatographic system and conditions

HPLC analysis was performed using an Agilent 1260 infinity series equipped with a multiple wavelength detector (Agilent Technologies Inc., Richardson, TX, USA). Chromatograms were integrated and analyzed using OpenLAB Chemstation (version M8301AA, Revision C.01.07 Agilent Technologies Inc., Richardson, TX, USA).

The analysis of genistein was performed using a column (Zorbax Eclipse RP C18 reversed-phase, 250 mm×4.6 mm I.D., 5 µm, Agilent, Santa Clara, CA, USA) maintained at 40°C. Mobile Phase A was water, mobile Phase B was acetonitrile, and mobile Phase C was glacial acetic acid. The mobile Phases A, B, and C were mixed at a ratio of 67.5:25.0:7.5 (v/v). The flow rate was kept at 1.5 ml/min, injection volume was 10 µL and UV detection was carried out at 260 nm. The retention time of the genistein was 7.30 min.

^{1}H and DOSY ^{1}H NMR

^{1}H-NMR and DOSY spectra were obtained using a Bruker Avance spectrometer at 400 MHz proton frequency (AV-III-HD, 400, Rheinstetten, Germany). The qNMR experiments were performed with the following optimized parameters: Pulse angle, 30°; pulse width, 41.6 µs; data points 96152; and number of scans, 64; acquisition time (AQ), 3.999 s; spectral width, 12019.23 Hz. A line-broadening factor of 0.1 Hz was applied to FIDs before Fourier transformation, and the repetition delay was 60 s. All chemical shifts were reported in parts per million (ppm) relative to deuterated DMSO-d6 at 2.50 ppm. All spectra were automatically corrected for phase and baseline distortions using TOPSPIN (version 3.5pl5, Bruker Biospin, Spring, TX, USA). For statistical reasons, each measurement was repeated 6 times. The

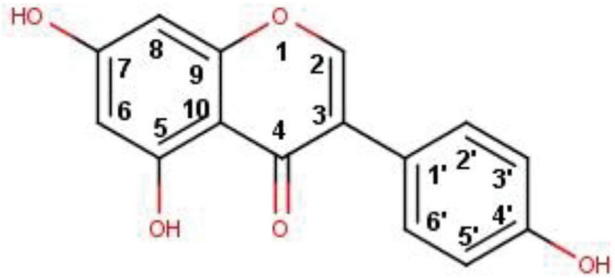

Fig. 1: Numbered chemical structure of genistein

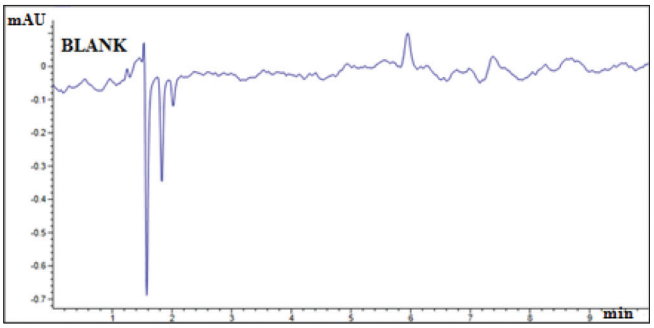

Fig. 2: High-performance liquid chromatography chromatogram of the blank sample. Chromatogram obtained by Zorbax Eclipse RP C18 reversed-phase column (250 mm×4.6 mm, 5 µm), mobile phase of water: acetonitrile: glacial acetic acid (67.5:25.0:7.5) and flow rate of 1.5 ml/min

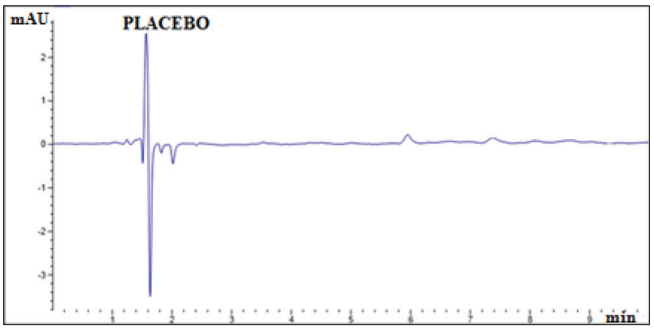

Fig. 3: High-performance liquid chromatography chromatogram of the placebo sample (sample concentration=10.5 ppm). Chromatogram obtained by Zorbax Eclipse RP C18 reversed-phase column (250 mm×4.6 mm, 5 µm), mobile phase of water: acetonitrile:glacial acetic acid (67.5:25.0:7.5) and flow rate of 1.5 ml/min

Table 1: Dietary supplements analyzed in this study

S. No	Formulation name	Batch number	Product form
1	RGCC	EXP2.2	Capsule
2	Swanson	B225947	Capsule
3	Now	1937090	Capsule
4	Vital	N/A	Capsule

N/A: Not available, RGCC: Research Genetic Cancer Center

Table 2: Validation parameters for the determination of genistein by high-performance liquid chromatography

HPLC parameters	Results		
System suitability			
Theoretical plates	11086		
Symmetry factor	0.69		
%RSD	0.20		
Linearity			
Concentration range (ppm)	5.25–15.75		
HPLC parameters	**RT**	**Plate count**	**Peak symmetry**
Robustness			
Column temperature (42 and 38°C)	7.09	11347	0.682
	7.58	11692	0.701
Column batch (B15043 and B16021)	7.33	11377	0.688
	7.33	11527	0.747
Flow rate (1.53 and 1.47 ml/min)	7.30	11619	0.677
	7.31	11692	0.682
HPLC parameters	**Intraday**	**Interday**	
Precision			
Mean peak area	497.9	489.14	
%RSD	0.19	0.19	
Accuracy			
Number of solutions analyzed	18		
Concentration range (ppm)	5.25–15.75		
Mean recovery±%RSD	100.1±0.05		
LOD (ppm)	0.225		
LOQ (ppm)	0.675		

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation, HPLC: High-performance liquid chromatography

values of T1 relaxation time for targeted protons are listed below: For benzoic acid, δ 8.00 ppm (H-2 and H-6), T1=2.041 s; δ 7.60 ppm (H-4), T1=3.071 s; δ 7.50 ppm (H-3 & H-5), T1=2.255 s; and for genistein, δ 8.3 ppm (H-2), T1=2.217 s. For DOSY ^{1}H NMR, stimulated echo bipolar gradient pulse experiments were used with a pulse delay of 5 ms after each gradient, a pulse-field gradient length of 1 ms and a diffusion delay of 100 ms. Sequence parameters were adapted to have the intensity of the H2 NMR signal of genistein strongly decreased at 95% of the full gradient length. All data were processed with TOPSPIN (version 3.5pl5, Bruker Biospin, Spring, TX, USA) software using the maximum entropy algorithm (MaxEnt). The processing parameters were 1024 points along the Laplace spectrum diffusion axis and 20000 MaxEnt iterations. DOSY spectra are presented with chemical shift on the horizontal axis and diffusion coefficients expressed in $\mu m^2 s^{-1}$ on the vertical axis.

RESULTS

Quantification of genistein by HPLC

Method validation

System suitability

The HPLC system was allowed to equilibrate for 30 min. A blank and a standard sample (10 replicates) were injected, and the chromatograms were recorded to evaluate the system suitability parameters such as % relative standard deviation (RSD) between replicate injections (<2%), theoretical plates (NLT 3000), and symmetry factor (0.8–1.5).

Specificity and selectivity

The specificity of the method was demonstrated by studying the effect of the excipients present in genistein formulations under optimum conditions. The blank, placebo, and genistein capsule samples were analyzed to examine the interference of blank and placebo with genistein peaks. No interference was observed. Furthermore, the well-shaped peaks indicate the specificity of the method. The chromatograms of the blank, placebo, and genistein samples are presented in Figs. 2-4:

Linearity

The linearity of the method was determined by preparing standard solutions at different concentrations from 50% to 150% of the test concentration. The calibration curve is shown in Fig. 5. The least-square analysis method was applied for the calculation of the slope, intercept, and correlation coefficient.

Table 3: Validation parameters for the determination of genistein by quantitative nuclear magnetic resonance

qNMR parameters	Results											
Linearity	δ*8.00				δ*7.60				δ*7.50			
Equation	y=0.2186x−0.0004				y=0.4339x−0.0004				y=0.4339x−0.0004			
R^2	0.9996				0.9996				0.9996			
Concentration range (w/w)	0.20–1.96											
Precision												
Mean P_{gen} (%)	96.11				95.67				95.68			
%RSD	0.59				0.52				0.60			
Repeatability												
Mean P_{gen} (%)	96.39				96.13				96.13			
%RSD	0.74				0.69				0.71			
Accuracy												
%Recovery	98.13				97.86				97.90			
%RSD	1.75				1.83				1.66			
qNMR parameters	**Number of scans**			**AQ (s)**			**D1 (s)**			**Protons (ppm)**		
Robustness												
Mean P_{gen} (%)	96.07	95.74	95.58	96.34	95.97	96.01	96.18	96.00	95.90	97.42	96.37	96.89
%RSD	0.80	0.72	0.79	0.46	0.73	0.39	0.63	0.54	0.63	0	0	0
LOD (ppm)	0.175											
LOQ (ppm)	0.526											

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation, qNMR: Quantitative nuclear magnetic resonance, AQ: Acquisition time

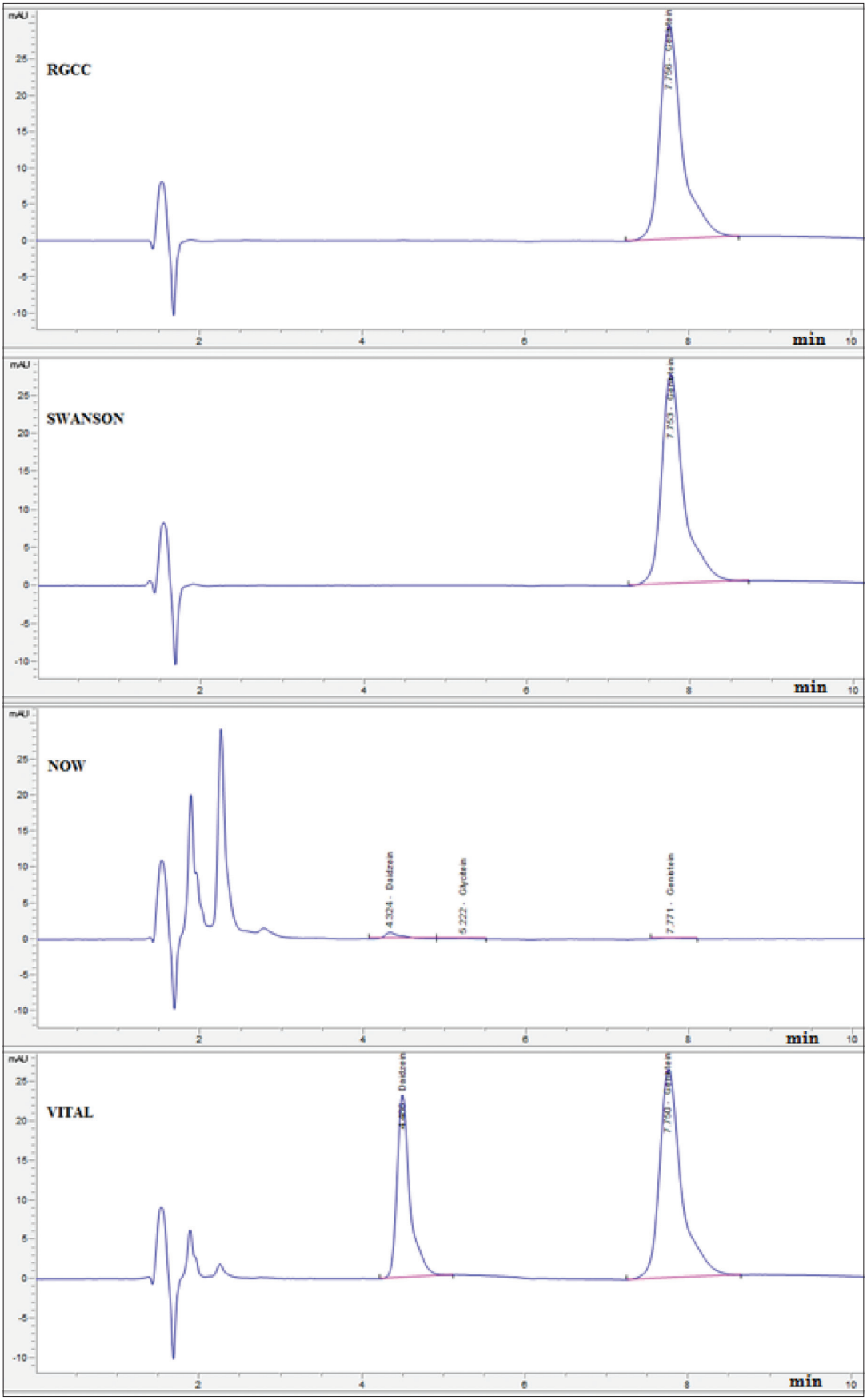

Fig. 4: High-performance liquid chromatography chromatographs of Research Genetic Cancer Centre dietary supplement, Swanson dietary supplement, Now dietary supplement and Vital dietary supplement (sample concentration=10.5 ppm). All chromatograms obtained by Zorbax Eclipse RP C18 reversed-phase column (250 mm×4.6 mm, 5 µm), mobile phase of water: acetonitrile: glacial acetic acid (67.5:25.0:7.5) and flow rate of 1.5 ml/min

Table 4: Genistein content (mg) in commercial capsules determined by high-performance liquid chromatography and quantitative nuclear magnetic resonance

Brand	Specifications (mg)	HPLC (mg/unit)	qNMR (mg/unit)	%RSD
RGCC	125	123.8	122.0	1.3
Swanson	125	122.2	119.9	1.6
Vital	250	248.1	246	1.5
Now	60	1.4	1.2	0.1

qNMR: Quantitative nuclear magnetic resonance, RSD: Relative standard deviation, HPLC: High-performance liquid chromatography, RGCC: Research Genetic Cancer Center

Table 5: Formulation ingredients

Ingredients	RGCC	Swanson	Vital	Now
Genistein	+	+	+	+
Daidzein	−	−	+	+
Glycitein	−	−	−	+
L-leucine	−	+	−	−
Calcium carbonate	−	−	+	−
Ascorbyl palmitate	−	−	+	−
Magnesium stearate	−	−	−	+
Rice flour	−	+	+	+
Corn starch	+	−	−	−
StarCap 1500	+	−	−	−
Nuflow	+	−	−	−

RGCC: Research Genetic Cancer Center

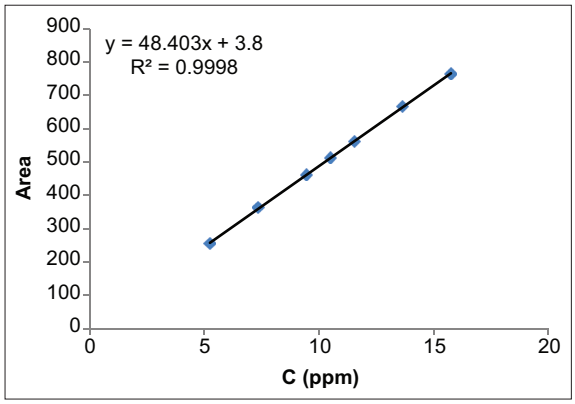

Fig. 5: The relationship between the concentration of genistein (X-axis) and peak area (Y-axis)

Robustness

Robustness was established by analyzing genistein under different experimental conditions, which entails making changes in parameters such as column temperature, column batch from the same manufacturer, and flow rate. All results were within the acceptance criteria (peak symmetry <1.5, plate count >3000) and no significant change in retention time.

Precision

Intraday and interday precision of the method was determined by analyzing ten test solutions at the same concentration (10.5 ppm) of genistein during the same day, under the same experimental conditions and on a different day, respectively. The RSD was calculated and is within the acceptable criteria (%RSD <2.0).

Accuracy and recovery tests

The accuracy was evaluated at three different concentrations equivalent to 50%, 100%, and 150% of the active ingredient, by adding a known amount of genistein standard to a sample with a pre-determined amount of genistein. Each concentration was evaluated in six replicates. The recovered amount of genistein, % recovery of each concentration, and %RSD of recovery were calculated to determine the accuracy.

LOD and LOQ

LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve. This procedure is approved by the German Institute for Standardization and is regarded as a method, from which reliable and practically meaningful values can be obtained. The following formulas were used: LOD=$3.3S_{\beta}/\alpha$ and LOQ=3LOD, where S_{β}=standard deviation of response and α=slope of the calibration curve.

All the HPLC validation parameters are shown in Table 2:

Quantification of genistein by ^{1}H NMR

Method validation

Specificity and selectivity

NMR spectroscopy provides very detailed structural information and, therefore, it possesses in principle, high specificity. qNMR is considered to be specific and selective the analyzed signal does not overlap with other components in the sample solution, including solvent and excipients in the formulation. Figs. 6 and 7 show ^{1}H NMR spectra of internal calibrant solution, standard solution, placebo solution, and sample solution individually. It is evident that the signals at 8.32 for genistein and at 8.00, 7.60, and 7.50 ppm for benzoic acid are not overlapped by the solvent and excipients present. In addition, these signals are well separated from each other in standard and sample preparations.

Linearity

To check the linearity of the method, linearity tests were performed over a concentration range (w/w) for genistein per mg of benzoic acid. The results showed that the method had excellent linearity over these concentration ranges.

Precision and repeatability

The precision of the method was determined by measuring within a short time a 5 mg sample of genistein. Six samples were prepared and each sample was measured in triplicates. Repeatability was determined by measuring the 5 mg sample of genistein 6 times.

Robustness

The robustness of the method was estimated by varying four parameters independently: (1) Number of scans (32, 48, and 80), (2) AQ (3, 5, and 6 s), (3) relaxation delay (30, 40, and 50 s), and (4) different analyte protons (6.39, and 6.23 ppm) chosen.

Accuracy and recovery tests

The accuracy of the method was estimated by analyzing nine mixtures with the known composition of genistein, genistein capsule powder, and benzoic acid and by measuring the mean recovery and RSD. Quantitative genistein was added to the capsules to investigate the recovery of genistein from capsules. The results indicate that the relative proportions of genistein and internal standard have no influence on the methods' accuracy.

LOD and LOQ

LOD and LOQ were calculated in the same manner as in HPLC, using the same formulas.

All qNMR validation parameters are shown in Table 3.

The samples were analyzed in triplicates by HPLC and qNMR method, and the results are presented in Table 4. It is worth mentioning that two other isoflavones, glycitein and daidzein, were able to be identified and quantified, in comparison with their respective reference standards, in two supplements by HPLC. In particular, Now brand was found to contain 3.6 mg of daidzein and 2.6 mg glycitein and Vital 187.9 mg of daidzein. The chromatographs of all brands are shown in Figs. 8-12.

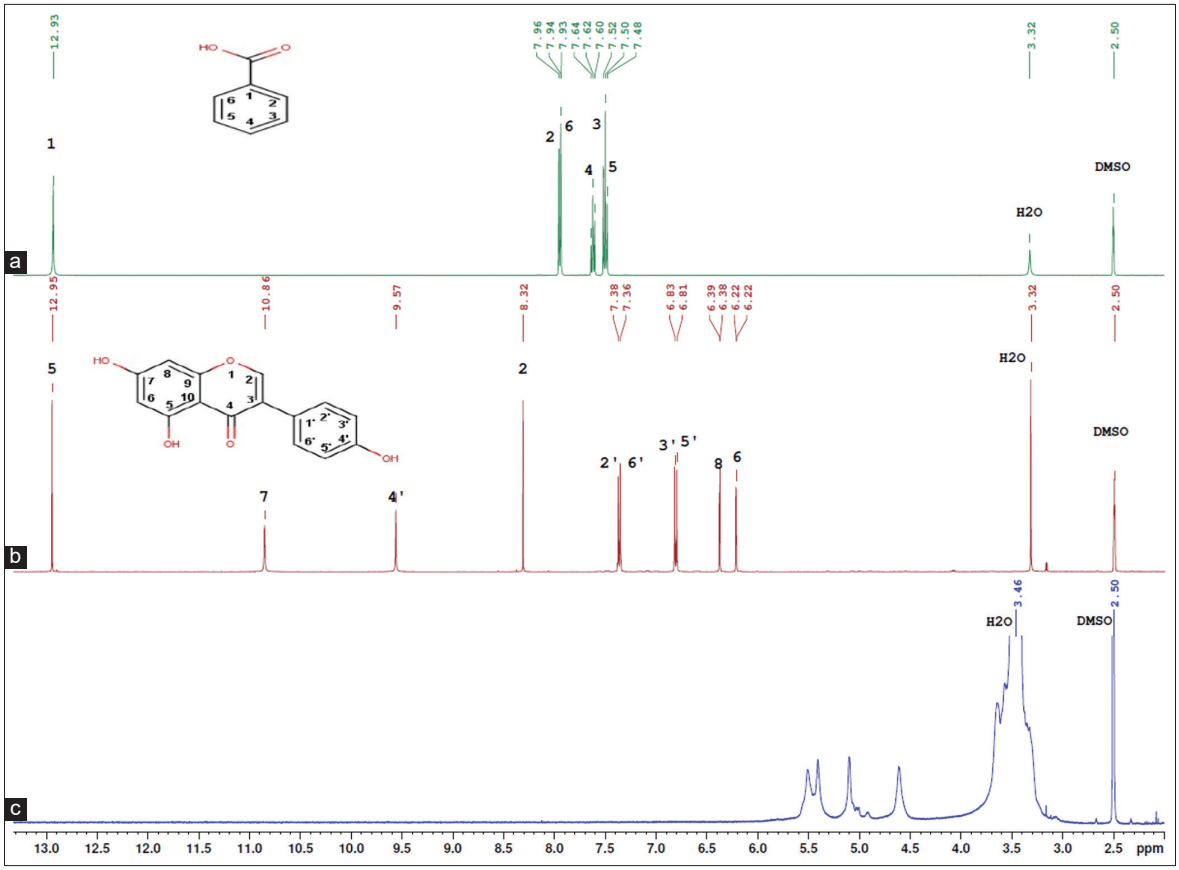

Fig. 6: (a) ^{1}H nuclear magnetic resonance (NMR) spectrum of 5 mg benzoic acid in 0.5 ml dimethyl sulfoxide (DMSO-d_6), (b) ^{1}H NMR spectrum of 5 mg genistein in 0.5 ml DMSO-d_6, (c) ^{1}H-NMR spectrum of 5 mg excipients in 0.5 ml DMSO-d_6

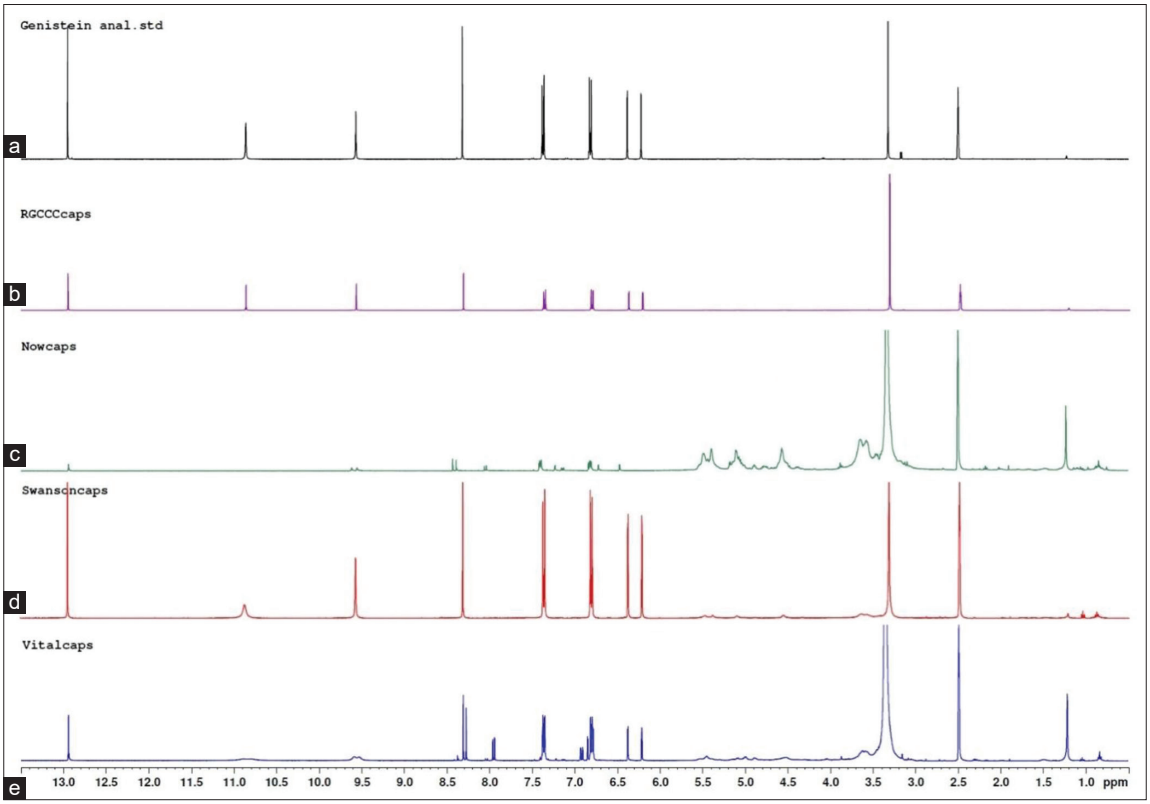

Fig. 7: (a) ^{1}H nuclear magnetic resonance (NMR) spectrum of 5 mg genistein in 0.5 ml dimethyl sulfoxide (DMSO-d_6). (b) ^{1}H NMR spectrum of 5 mg Research Genetic Cancer Center capsules in 0.5 ml DMSO-d_6. (c) ^{1}H NMR spectrum of 5 mg Now capsules in 0.5 ml DMSO-d_6. (d) ^{1}H-NMR spectrum of 5 mg Swanson capsules in 0.5 ml DMSO-d_6. (e) ^{1}H-NMR spectrum of 5 mg Vital capsules in 0.5 ml DMSO-d_6

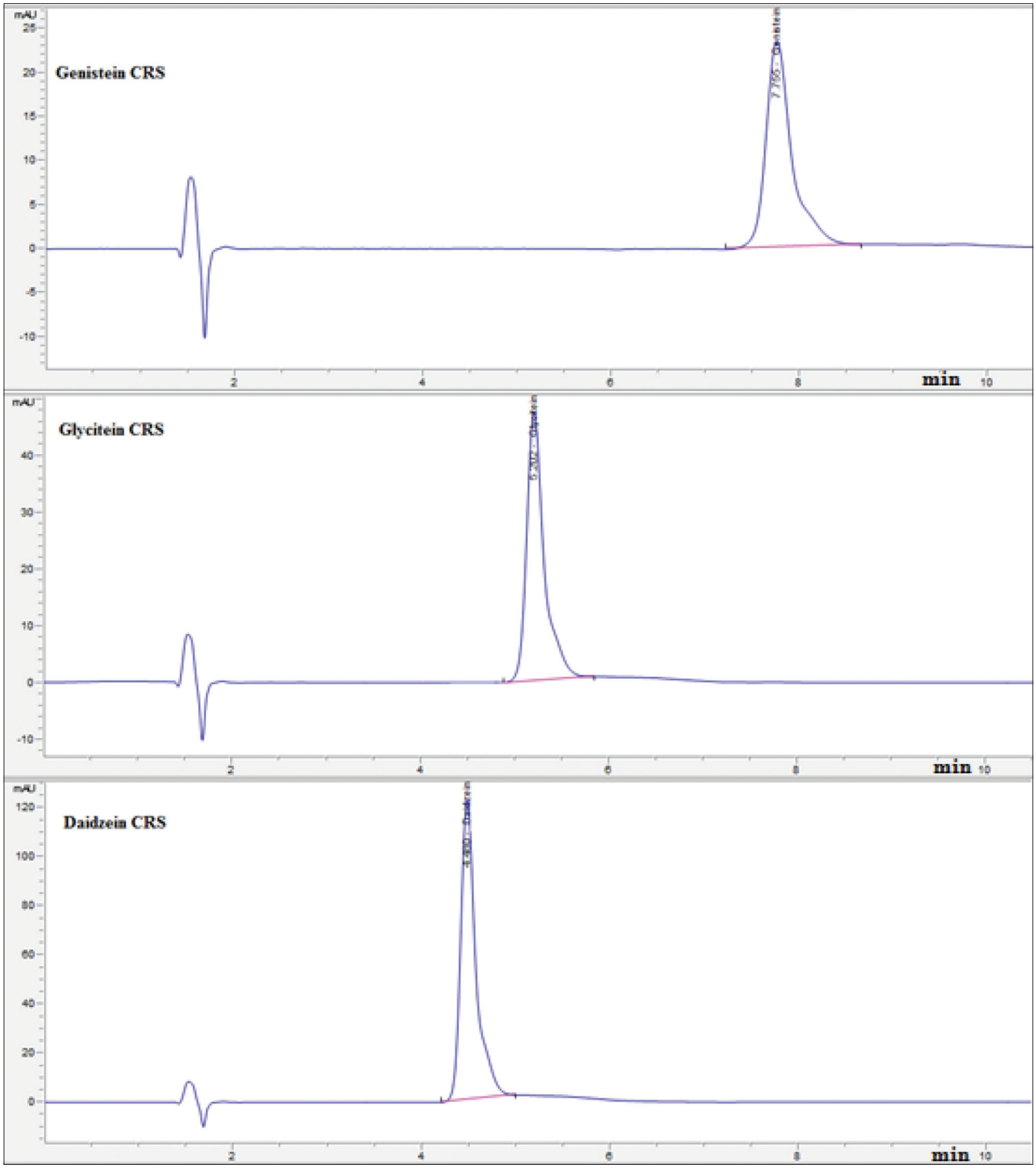

Fig. 8: High-performance liquid chromatography chromatographs of genistein, glyciten and daidzein reference standards (sample concentration=10.5 ppm). All chromatograms obtained by Zorbax Eclipse RP C18 reversed-phase column (250 mm×4.6 mm, 5 µm), mobile phase of water:acetonitrile:glacial acetic acid (67.5:25.0:7.5) and flow rate of 1.5 ml/min

Quality assessment by 2D DOSY ^{1}H NMR

Four formulations of genistein were analyzed with ^{1}H DOSY NMR, and their spectra along with their ^{1}H spectra are presented in Figs. 13-16. The ingredients of each formulation are displayed in Table 5. Peaks at 3.3 and 2.5 ppm correspond to the signals of water and DMSO, respectively. All the peaks of the isoflavones present (genistein, glycitein, and daidzein) are lined up in the Research Genetic Cancer Center formulation. A deviation of one peak from the line is observed in the remaining three formulations due to a hydrogen exchange between the hydroxyl group in 7' position and water. Although isoflavones glycitein and daidzein were identified and quantified in the Vital and Now supplements by HPLC, they could not be clearly observed in the DOSY spectra due to overlapping peaks with the peaks of genistein and similar molecular weights which result to similar diffusion

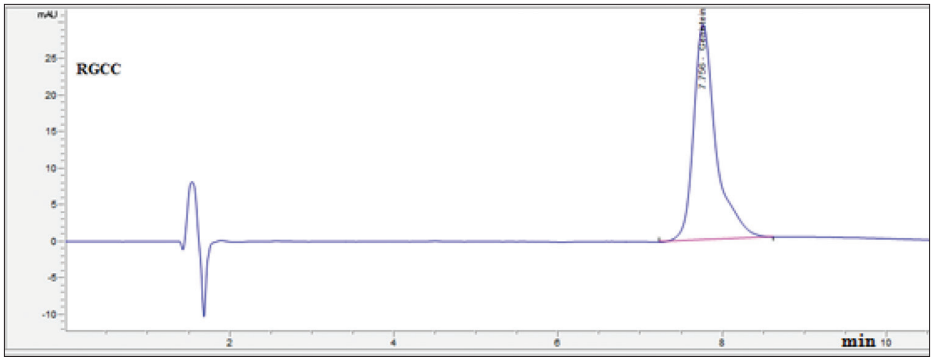

Fig. 9: High-performance liquid chromatography chromatograph of Research Genetic Cancer Centre dietary supplement (sample concentration=10.5 ppm). Chromatogram obtained by Zorbax Eclipse RP C18 reversed-phase column (250 mm×4.6 mm, 5 µm), mobile phase of water:acetonitrile:glacial acetic acid (67.5:25.0:7.5) and flow rate of 1.5 ml/min

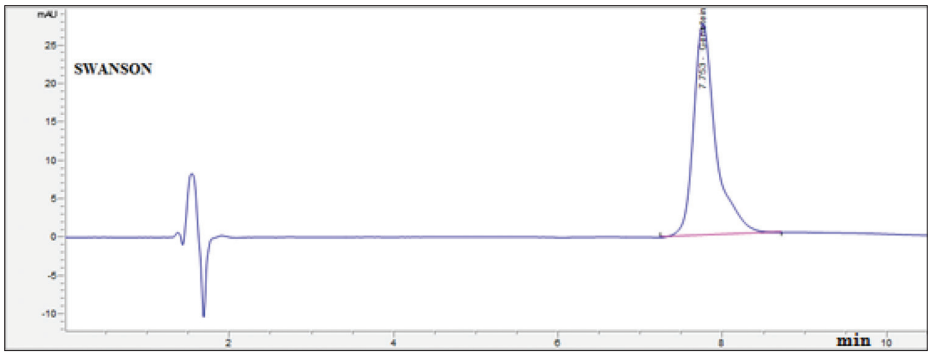

Fig. 10: High-performance liquid chromatography chromatograph of Swanson dietary supplement (sample concentration=10.5 ppm). Chromatogram obtained by Zorbax Eclipse RP C18 reversed-phase column (250 mm×4.6 mm, 5 µm), mobile phase of water: acetonitrile:glacial acetic acid (67.5:25.0:7.5) and flow rate of 1.5 ml/min

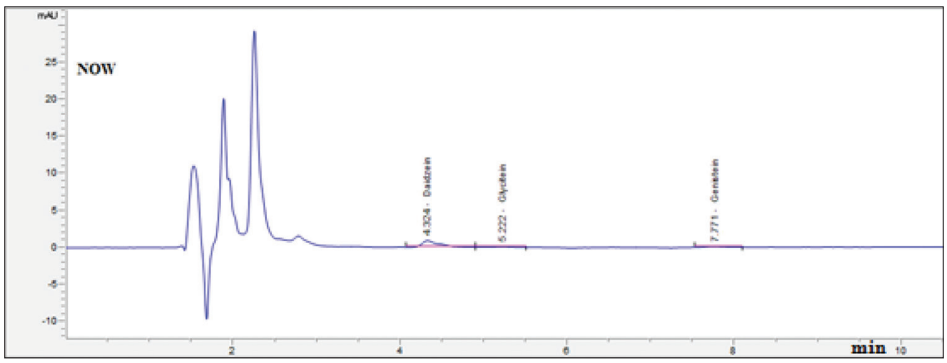

Fig. 11: High-performance liquid chromatography chromatograph of Now dietary supplement (sample concentration=10.5 ppm). Chromatogram obtained by Zorbax Eclipse RP C18 reversed-phase column (250 mm×4.6 mm, 5 µm), mobile phase of water: acetonitrile:glacial acetic acid (67.5:25.0:7.5) and flow rate of 1.5 ml/min

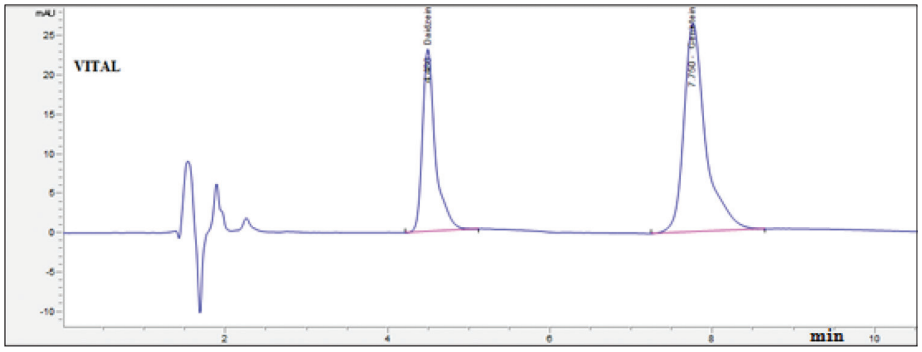

Fig. 12: High-performance liquid chromatography chromatograph of Vital dietary supplement (sample concentration=10.5 ppm). Chromatogram obtained by Zorbax Eclipse RP C18 reversed-phase column (250 mm×4.6 mm, 5 µm), mobile phase of water: acetonitrile:glacial acetic acid (67.5:25.0:7.5) and flow rate of 1.5 ml/min

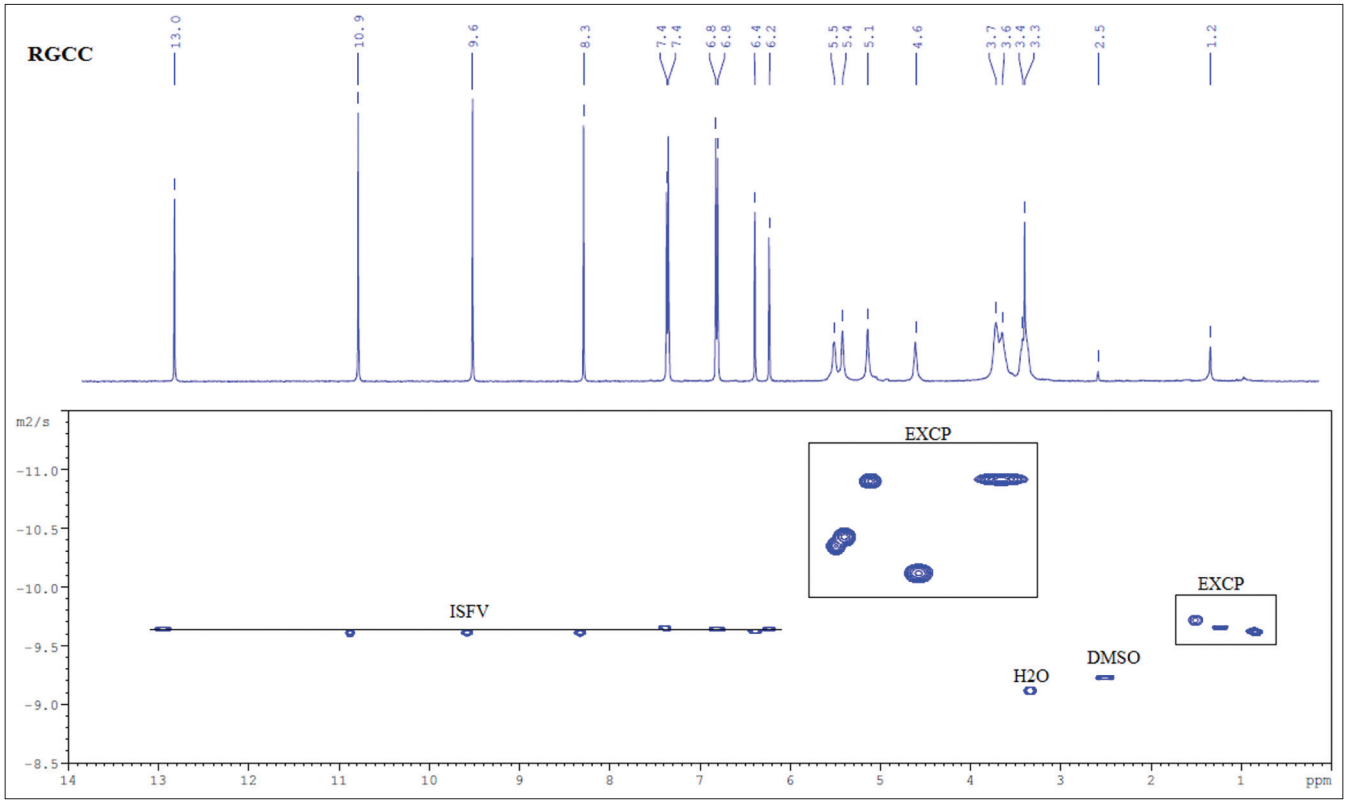

Fig. 13: ^{1}H diffusion ordered spectroscopy nuclear magnetic resonance spectra of 5 mg Research Genetic Cancer Center formulation in 0.5 ml of dimethyl sulfoxide (DMSO-d_6)

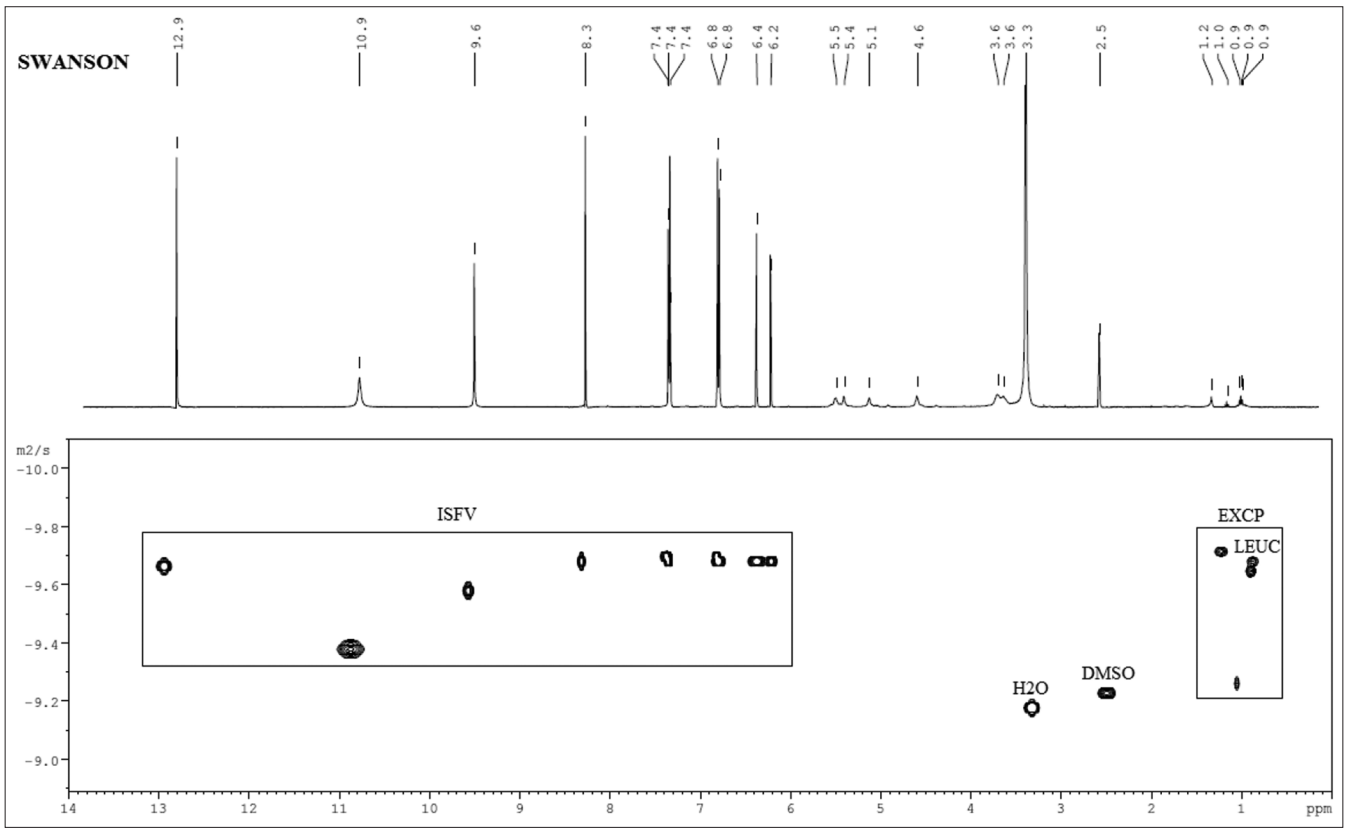

Fig. 14: ^{1}H diffusion ordered spectroscopy nuclear magnetic resonance spectra of 5 mg Swanson formulation in 0.5 ml of dimethyl sulfoxide (DMSO-d_6)

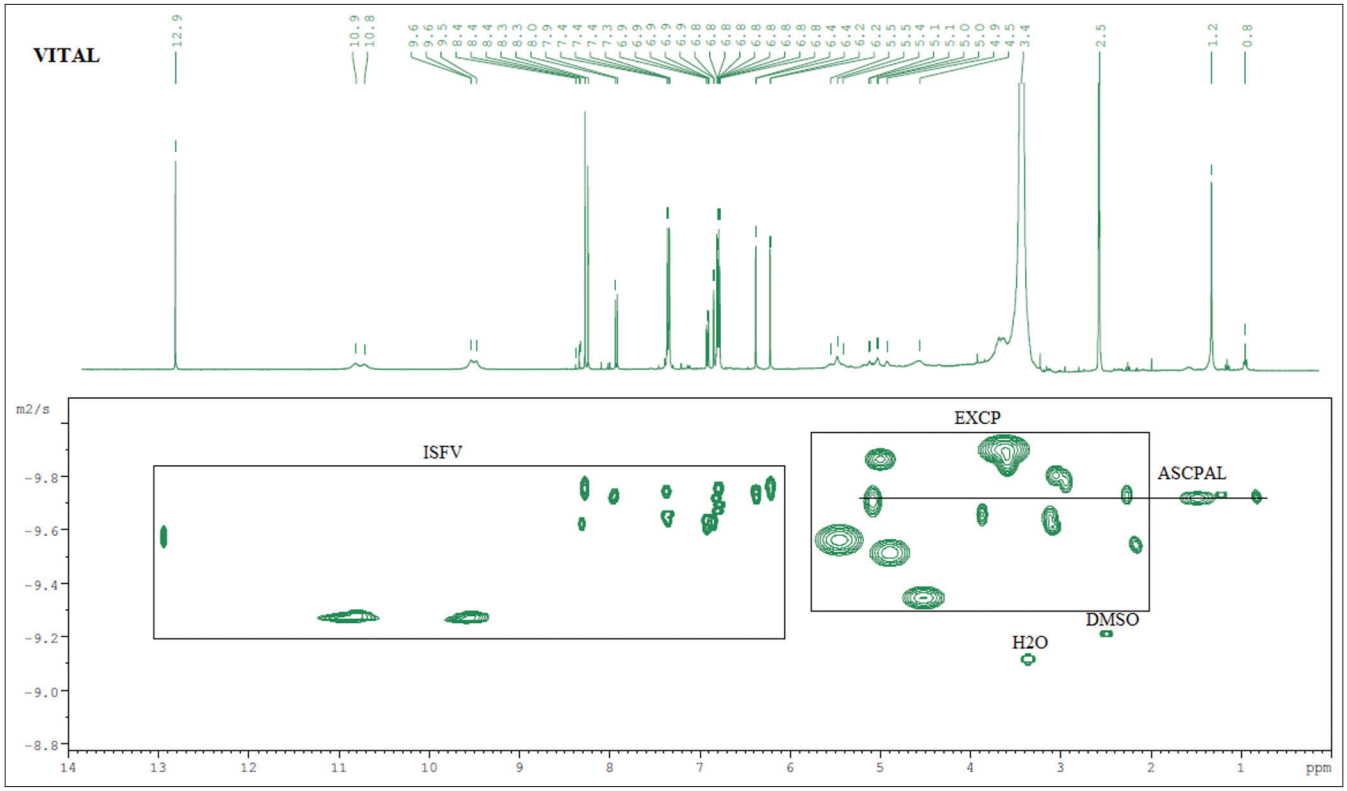

Fig. 15: ^{1}H diffusion ordered spectroscopy nuclear magnetic resonance spectra of 5 mg Vital formulation in 0.5 ml of dimethyl sulfoxide (DMSO-d_6)

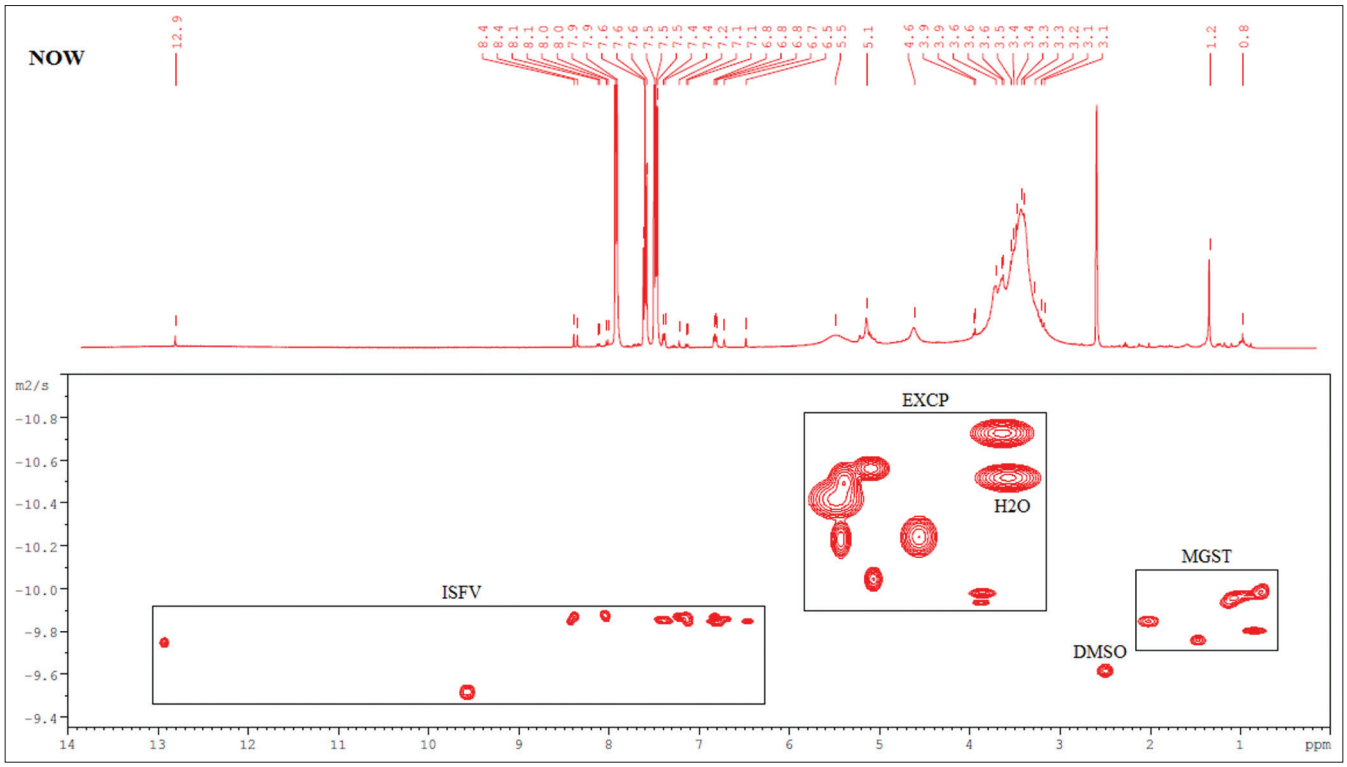

Fig. 16: ^{1}H diffusion ordered spectroscopy nuclear magnetic resonance spectra of 5 mg Now formulation in 0.5 ml of dimethyl sulfoxide (DMSO-d_6)

coefficients. Several excipients could be observed depending on the formulation. L-leucine peaks (LEUC) were observed in the Swanson formulation at 0.9, 1.0, and 1.2 ppm. Ascorbyl palmitate (ASCPAL) peaks were identified in the Vital formulation at 0.9, 1.1, 1.5, 2.3, and 5.1 ppm. Magnesium stearate peaks (MGST) were present in the Now formulation at 0.89, 1.3, 1.5, and 2.13 ppm. The remaining excipients (rice flour, cornstarch, StarCap 1500, Nuflow) gave broad signals in the 3–6 ppm region.

DISCUSSION

The isoflavone content of four dietary supplements has been determined by HPLC, qNMR, and 2D ^{1}H DOSY NMR. The quantification of these compounds is generally carried out by HPLC or qNMR [29-32]. However, the use of 2D ^{1}H DOSY NMR allowed the detection of various components of these formulations in a single run. The results allow some conclusions to be made: The quantities of all isoflavones were calculated in Now brand, even though they are mentioned only as a total amount on the label and Vital brand was found to also contain daidzein and not only genistein as it is stated on the supplements' label. Finally, all excipients and other ingredients were able to be identified by diffusion NMR.

CONCLUSION

Two methods (HPLC and qNMR) were developed and validated for genistein content determination in different supplements. The methods were proven accurate, precise, selective, and linear over the assessed concentration ranges. HPLC and quantitative NMR were used for the quantification of each formulation content in genistein. Diffusion NMR, which is considered as a strong analytical tool for the analysis of complex mixtures, was also used to assess the supplement quality. The combination of these techniques provided detailed information on each product and its contents and all of them are currently used for the quality control of natural supplements by our laboratory.

AUTHOR'S CONTRIBUTION

All authors have contributed equally to the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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