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Research Article

Determination of myo-inositol and D-chiro-inositol in dietary supplements by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

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Abstract

Myo-inositol and D-*chiro*-inositol are inositol isomers involved in key cellular functions. They are widely used in dietary supplements for their benefits in improving insulin sensitivity, hormonal balance, and fertility in polycystic ovary syndrome PCOS, as well as managing adiposity, hyperglycemia, and insulin resistance. Therefore, determining *myo*-inositol and D-*chiro*-inositol is essential to assess health supplement quality and efficacy. In this study, a simple and reliable analytical method to analyze *myo*-inositol and D-*chiro*-inositol in food supplements was developed and validated using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The dietary supplements were ultrasonicated, centrifuged, and filtered before quantification. Myo-inositol and D-chiro-inositol were completely separated on a DionexTM CarboPacTM MA1 IC column (250 mm × 4 mm × 7.5 µm) with a gradient elusion using a 50 mM and 1M NaOH mixture at a flow rate of 0.4 mL/min. The method has good specificity, linearity ($R^2 > 0.999$), and accuracy with repeatability and recovery satisfying the AOAC requirements. The limit of detection (LOD) and the limit of quantification (LOQ) of the two analytes were 0.5 mg/kg and 1.5 mg/kg, respectively. The method was applied to simultaneously determine *myo*-inositol and D-*chiro*-inositol in different dietary supplement products.

Keywords: *Myo-inositol, D-chiro-inositol, dietary supplements, HPAEC-PAD.*

1. INTRODUCTION

The escalating global demand for nutrition and preventive healthcare has catalyzed the rapid expansion of the dietary supplement industry. These products, available in various forms such as capsules, tablets, and liquids, are often formulated with essential micronutrients, including vitamins, minerals, amino acids, and botanical extracts. Among these bioactive compounds, inositol—particularly its stereoisomers *myo*-inositol (MI) and D-*chiro*-inositol (DCI)—has garnered significant attention due to its diverse biological functions and potential health benefits. Inositol is a small, polar cyclohexanehexol belonging to the sugar family, comprising nine stereoisomeric forms, of which MI and DCI are the most biologically relevant [1, 2]. Although structurally similar to glucose, inositol is not classified as an essential nutrient because it can be synthesized endogenously in both prokaryotic and eukaryotic organisms [1]. In nature, inositol is found in both free and phosphate-bound forms and is particularly abundant in legumes and fruits [3].

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Myo inositol is the predominant isomer and acts as a precursor to inositol phosphates and phosphoinositides involved in key cellular processes such as membrane signaling, cytoskeletal organization, gene expression, and vesicle trafficking [1, 2]. Functionally, MI and DCI complement each other in modulating insulin signaling and glucose metabolism. MI enhances estrogen synthesis and oocyte maturation by upregulating follicle-stimulating hormone (FSH) receptors and aromatase in granulosa cells, whereas DCI promotes insulin-stimulated androgen production in theca cells while downregulating aromatase expression [3, 4]. This divergence underlies their clinical application in managing polycystic ovary syndrome (PCOS), a common endocrine-metabolic disorder characterized by insulin resistance and hyperandrogenism. Numerous studies have shown that supplementation with MI and DCI in physiological ratios restores hormonal balance and improves metabolic and reproductive outcomes in PCOS patients [5].

Given their expanding use in metabolic and reproductive health supplements, accurate quantification of MI and DCI is essential for quality control and regulatory compliance. Currently, several studies worldwide have been conducted to determine the content of *myo*-inositol and D-*chiro*-inositol in various sample matrices such as milk, black rice bran, beans, nuts, and cereals using analytical techniques including capillary electrophoresis (CE) [6], gas chromatography (GC) [7], and high-performance liquid chromatography (HPLC) [8]. However, to the best of our knowledge, limited attention has been paid to the simultaneous determination of MI and DCI in dietary supplements using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Therefore, the objective of this study was to develop, validate, and apply an analytical method for the quantification of *myo*-inositol and D-*chiro*-inositol in dietary supplement products. The method employs high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) following an ultrasonic-assisted extraction procedure. The proposed approach offers a sensitive, selective, and reproducible solution for monitoring inositol content in commercial formulations, thereby supporting product quality assurance and consumer protection.

2. MATERIALS AND METHODS

2.1. Chemicals and materials

Myo-inositol and *D*-chiro-inositol standards purity ≥ 99% were obtained from Sigma-Aldrich (USA). Deionized water used in this study was purified by a WaterPro RO System (Labconco Cooperation, USA). Other chemicals including sodium hydroxide 50% were purchased from Merck (Germany).

The analyzed samples included one blank dietary supplement sample (without *myo*-inositol and D-*chiro*-inositol) and ten dietary supplement products labeled to contain inositols. All samples were randomly collected from pharmacies across Hanoi in March 2025, with a total of 11 samples.

2.2. Equipments

High-performance anion-exchange chromatography (HPAEC) was performed using a Dionex ICS-5000 system (Thermo Scientific, USA) equipped with a pulsed amperometric detector (PAD). Separation was achieved using a Dionex™ CarboPac™ MA1 IC column (250 mm × 4 mm × 7.5 µm). Additional standard laboratory accessories were used as required.

2.3. Research methods

In this study, several research methods were employed to ensure the accurate determination and evaluation of *myo*-inositol and D-*chiro*-inositol in dietary supplements. The analytical method used was HPAEC-PAD. Sample preparation was performed using ultrasonic-assisted extraction to enhance the efficiency of analyte recovery. The method was validated through assessments of specificity, limit of detection (LOD), limit of quantification (LOQ), repeatability, and recovery. Finally, data analysis and calculations were carried out using Microsoft Excel to interpret chromatographic results and perform statistical evaluations.

2.3.1. Methods of analysis

The chromatographic separation of *myo*-inositol and D-*chiro*-inositol was performed using high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). HPAEC-PAD is a highly selective and sensitive method for determining polyhydroxy compounds such as inositol, owing to the use of a pulsed amperometric detector (PAD) that selectively detects oxidizable hydroxyl groups without the need for derivatization. The instrumental conditions were referred to previous studies with

slight modifications [9, 12, 13, 14]. The analysis was conducted at a flow rate of 0.4 mL/min with an injection volume of 25 µL. Two gradient programs were investigated, both using a mobile phase composed of 50 mM sodium hydroxide (eluent A) and 1 M sodium hydroxide (eluent B). In gradient program 1 (**Table 1**), the run started with 90% A and 10% B at −5.00 min, increased to 100% A (0% B) at 0.00 min, and was held isocratically until 10.00 min. The composition then shifted abruptly to 0% A (100% B) at 10.01 min and was maintained until 20.00 min, followed by a return to 100% A at 20.01 min, which was maintained until 30.00 min. The total run time was 35 min. The gradient program 2 (**Table 2**) started with 90% eluent A held isocratically for 5 min, followed by sequential decreases from 50% A to 35% A, then to 25% A, and finally to 0% A, each over 5-min intervals. The mobile phase was maintained at 0% A for 13 min before being returned to 50% A over the next 2 min. The total run time was 35 min. The gradient program providing adequate resolution, sensitivity, and reproducibility was selected for the simultaneous determination of the two inositol isomers.

The detailed compositions of the two gradient programs are shown in **Table 1** and **Table 2**.

Table 1. *Gradient program 1*

Time (min)	Flow rate (mL/min)	NaOH 50 mM (%)	NaOH 1 M (%)
-5.00	0.4	90	10
0.00	0.4	100	0
10.00	0.4	100	0
10.01	0.4	0	100
20.00	0.4	0	100
20.01	0.4	100	0
30.00	0.4	100	0

Table 2. *Gradient program 2*

Time (min)	Flow rate (mL/min)	NaOH 50 mM (%)	NaOH 1 M (%)
-5.00	0.4	90	10
0.00	0.4	50	50
5.00	0.4	35	65
10.00	0.4	25	75
15.00	0.4	0	100
28.00	0.4	0	100
30.00	0.4	50	50

2.3.2. Sample preparation

To efficiently extract *myo*-inositol and D-*chiro*-inositol from dietary supplement matrices, ultrasonic-assisted extraction (UAE) was employed as the sample preparation method in this study. The procedure was based on previous studies with several modifications to optimize extraction performance [15]. A 0.5 g portion of homogenized dietary supplement powder was accurately weighed and transferred to a 50 mL centrifuge tube. Deionized water was added to reach a final volume of 50 mL. The suspension was then subjected to ultrasonic-assisted extraction using an ultrasonic bath. In this study, extraction temperature and extraction time were selected as the two conditions for investigation. Specifically, extraction was performed under various temperatures (room temperature, 40°C, 50°C, and 60°C) and extraction times (5, 10, 20, 30, and 40 minutes), following the experimental design to determine optimal conditions. Other parameters were kept constant, including sample mass, solvent type and volume, and ultrasound frequency. After extraction, the suspension was centrifuged to remove insoluble components and filtered through qualitative filter paper, followed by filtration using a membrane syringe filter to remove any residual particulates.

The blank sample used for method evaluation and validation was a dietary supplement (Berocca effervescent tablet), which was confirmed by HPAEC-PAD analysis to contain no detectable levels of the target analytes.

2.3.3. Method validation

The method was validated according to AOAC requirements [11]: Specificity was assessed using blank samples consisting of the sample extraction solvent mixture and standard solutions. The limits of detection (LOD) and quantification (LOQ) were determined based on signal-to-noise (S/N) ratios. Method precision (repeatability) and accuracy (recovery) were evaluated using actual dietary supplement samples.

Specificity/Selectivity: Specificity was evaluated by analyzing standard solutions, blank matrix samples (samples without analyte), spiked blank samples, and actual test samples using the HPAEC system. The signals of the target analyte were compared across sample types to confirm the absence of interference.

Limit of Detection (LOD): A low-concentration sample - estimated to be 5 to 7 times the expected LOD - was analyzed in ten replicates. The standard deviation (SD) was calculated, and the LOD was determined using the formula:

$$\mathbf{LOD = 3 \times SD}$$

Limit of Quantification (LOQ): The LOQ was calculated using the formula:

$$\mathbf{LOQ = 10 \times SD}$$

Repeatability: Repeatability was assessed by performing at least six replicate measurements. The relative standard deviation (RSD%) of the analyte concentration was calculated to assess method precision.

Recovery: Recovery studies were conducted by spiking the analyte at three concentration levels into blank matrix samples. The spiked concentrations were chosen to fall within the previously validated analytical range.

3. RESULTS AND DISCUSSION

3.1. Separation efficiency

Due to its specificity in carbohydrate analysis, the Dionex CarboPac™ MA1 IC column was selected to separate *myo*-inositol from the sample matrix. To accommodate the column's operating pressure and reduce analysis time, the flow rate was fixed at 0.4 mL/min. Based on previous studies [9], the selected mobile phases for investigation included: 240 mM NaOH under isocratic conditions; 50 mM NaOH and 1 M NaOH, applied using gradient program 1 and 2.

When using the isocratic mobile phase of 240 mM NaOH, myo-inositol could not be separated from interfering peaks present in the sample matrix. In contrast, with the mobile phase consisting of 50 mM NaOH – 1 M NaOH under gradient program 2, analyte was effectively separated from matrix interferences, resulting in a sharp and symmetrical peak, enhanced analyte signal, and minimal baseline noise.

Moreover, when applying gradient program 1 (**Figure 1**), the chromatographic peak of myo-inositol appeared as a doublet. This phenomenon led to poor peak shape, lower signal intensity, and decreased quantification reliability. Conversely, gradient program 2 provided well-separated, symmetrical, and sharp peaks with improved baseline stability.

Therefore, gradient program 2 was selected as the optimal chromatographic condition for subsequent analyses. Therefore, this mobile phase composition was selected as the optimal condition for chromatographic separation. **Figure 2** shows a representative chromatogram of a dietary supplements sample containing *myo*-inositol and D-*chiro*-inositol under the optimized conditions.

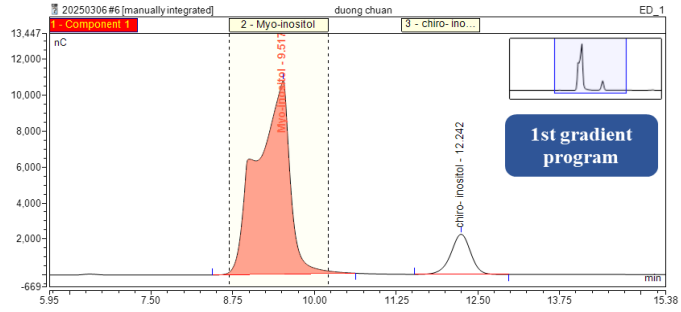

***Figure 1**. Chromatogram of a dietary supplement sample containing myo-inositol and D-chiro-inositol using gradient program 1*

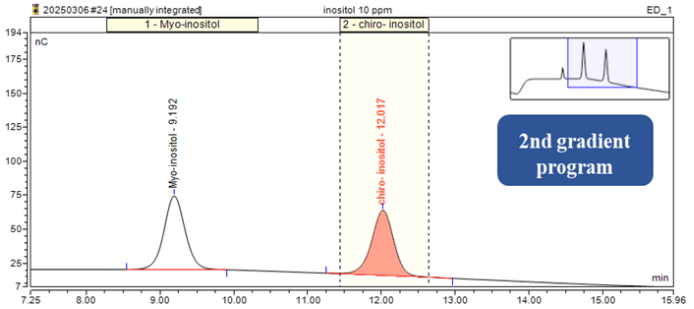

Figure 2. *Chromatogram of a dietary supplement sample containing myo-inositol and D-chiro-inositol using gradient program 2*

3.2. Sample preparation conditions

3.2.1. Optimization of ultrasonic extraction temperature for the sample

Overall, there were no significant differences in the concentrations of *myo*-inositol and D-*chiro*-inositol obtained under different temperature conditions, indicating that the analytes can be effectively extracted from the dietary supplement matrix using ultrasound-assisted water extraction regardless of temperature variation. Therefore, the ultrasonic extraction temperature of 2°C was chosen in this study. The results are shown in **Figure 3**.

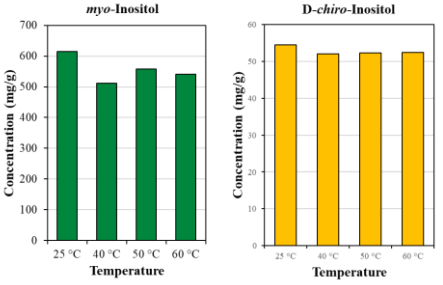

Figure 3. *Results of the ultrasonic extraction temperature optimization*

3.2.2. Optimization of ultrasonic extraction time for the sample

The effect of ultrasonic extraction time at various levels (5, 10, 20, and 30 minutes) was evaluated on a dietary supplement matrix. Overall, the extraction time showed no significant impact on the quantified levels of *myo*-inositol and *D*-chiro-inositol. To minimize analysis time while maintaining extraction efficiency, a 5-minute ultrasonic extraction was selected for subsequent experiments. The results are shown in **Figure 4.**

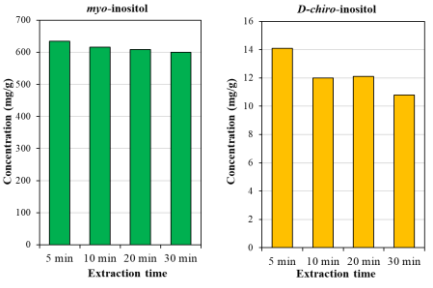

Figure 4. *Results of the ultrasonic extraction time optimization*

Based on the optimization results, the sample preparation procedure was as follows: Approximately 2 g of the homogenized dietary supplement sample was precisely weighed into a 50 mL centrifuge tube and thoroughly mixed. Ultrasonic extraction was performed at 25°C for 5 minutes. The extract was then adjusted to volume with distilled water, followed by centrifugation. The resulting supernatant was subsequently passed through a syringe filter. If necessary, the filtrate was diluted before HPAEC-PAD analysis.

3.3. Method validation results

3.3.1. Specificity/Selectivity

The specificity of the method is assessed through the analysis of blank samples, standard samples, and spiked samples. The results in **Figure 4** showed that there is no analyte signal in the blank sample, the analyte signals of the standard, and the spiked samples were at the same retention time with deviation under 1%. Therefore, the method has good specificity.

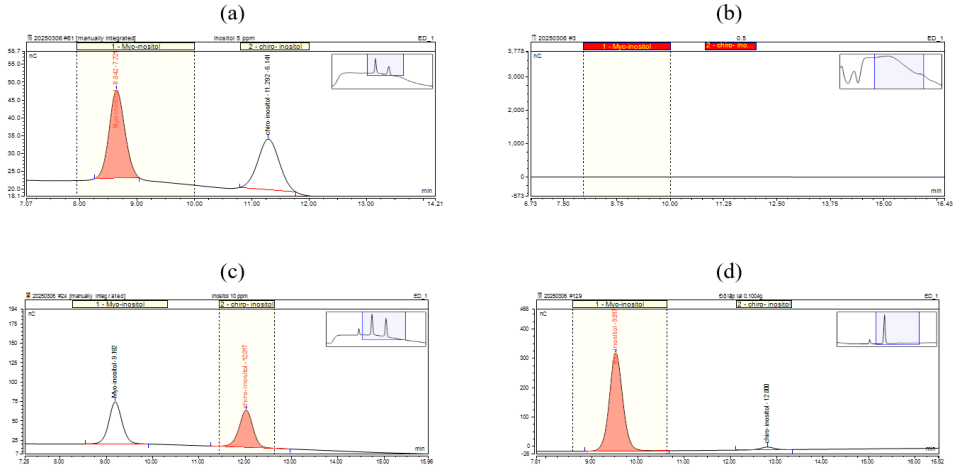

***Figure 4**. Chromatograms of the 5 mg/L standard sample (a), blank sample (b), blank spiked with 10 mg/L standard (c), and real sample (d).*

3.3.2. The calibration curves, LOD and LOQ

The calibration curves were constructed over the concentration range of 0.5–100 mg/L for both *myo*-inositol and D-*chiro*-inositol. The experiment was conducted using blank samples spiked with *myo*-inositol and D-*chiro*-inositol, as well as samples containing the analytes. The results of the calibration curves, correlation coefficients, limits of detection (LODs), and limits of quantification (LOQs) for the analytes are summarized in **Table 3**.

***Table 3.** The results of calibration curves, LOD and LOQ*

Analyte	Calibration equation	R^2	LOD (mg/kg)	LOQ (mg/kg)
Myo-inositol	y =1.9086x - 0.8136	0.9997	0.5	1.5
D-*chiro*-inositol	y = 1.6217x + 0.5771	0.9992	0.5	1.5

3.3.3. The repeatability and recovery

The precision of the method was validated through the assessment of repeatability and recovery. Repeatability (RSD%) and recovery (R%) were evaluated using spiked blank samples at three concentration levels: 1 ppm, 40 ppm, and 200 ppm. Each level wasss analyzed in six replicates following the validated analytical procedure. The results for accuracy and precision are summarized in **Table 4**.

***Table 4.** The precision and trueness results*

Analyte	Precision (RSD%, n=6)	Trueness (R%, n=3)
Myo-inositol	6.627	94-104
D-*chiro*-inositol	8.564	102-104

As shown in **Table 4**, the repeatability (RSD%) was 6.627% for myo-inositol and 8.564% for D-chiro-inositol. The corresponding recoveries were 94–104% and 102–104%, respectively. According to AOAC guidelines [11], the method meets the required criteria for precision and accuracy and is suitable for analyzing myo-inositol and D-chiro-inositol in actual samples.

3.4. Analytical results of real samples

The analytical results of *myo*-inositol and D-*chiro*-inositol in 10 dietary supplements (M1-10) are tabulated in **Table 5**.

Table 5. *Analytical results of real samples*

Sample	Myo-inositol in analyzed content (mg/100g)	Myo-inositol in labeled content (mg/100g)	Bias (%)	D-chiro-inositol in analyzed content (mg/100g)	D-chiro-inositol in labeled content (mg/100g)	Bias (%)
M1	51.6	60	14	12.7	12	-5.83
M2	39.2	40	2	4.6	5	8
M3	40.8	50	18.4			
M4	36.2	32	-13.1			
M5	233	250	6.8			
M6	39.0	40	2.5			
M7	23.4	25	6.4			
M8	29.1	30	3			
M9	46.6	50	6.8			
M10	23.5	23	-2.17			

The analysis of dietary supplement samples revealed a wide variation in *myo*-inositol content, ranging from 23.5 mg/100g to 51.6 mg/100g. Overall, the measured values were in reasonable agreement with the labeled contents, indicating the accuracy of product labeling.

All tested samples were solid-form commercial dietary supplements (tablets, capsules, or powders). These were homogenized and subjected to ultrasonic water extraction prior to chromatographic analysis, thus reflecting the performance of the method on real-world solid matrices. According to reference [10], daily intake of myo-inositol above 12 g may cause adverse effects in healthy individuals, including nausea, bloating, insomnia, headache, dizziness, and fatigue. However, based on the concentrations found in this study, such high intake levels would require consuming impractically large quantities of the products, especially those with lower *myo*-inositol content.

In addition, D-*chiro*-inositol was detected in two samples, with concentrations ranging from 4.6 to 12.7 mg/100g. These levels also fall within safe consumption limits. Taken together, the presence of both *myo*-inositol and D-*chiro*-inositol in the analyzed dietary supplements is not expected to pose any health risks when used as directed.

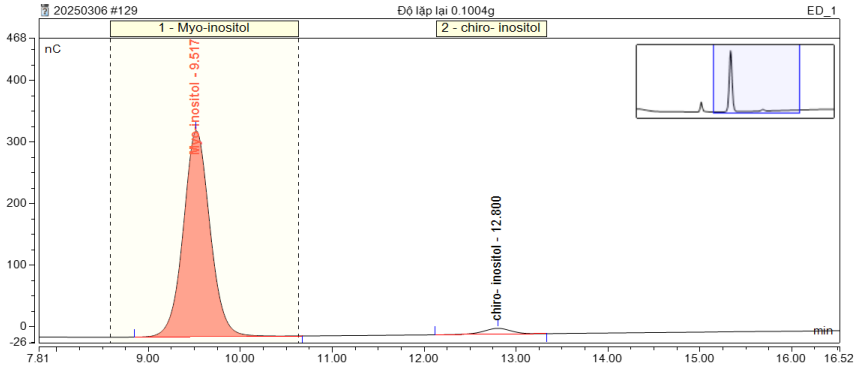

Figure 5*. Chromatogram of a representative real sample among the ten analyzed*

4. CONCLUSIONS

The study successfully determined the total content of *myo*-inositol and D-*chiro*-inositol in dietary supplements using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The sample preparation procedure for total *myo*-inositol and D-*chiro*-inositol was optimized specifically for dietary supplement matrices. The method was validated for specificity, calibration curve, repeatability, recovery, and detection limits. This procedure was applied to analyze ten commercially available dietary supplement products, with results showing that the total *myo*-inositol and D-*chiro*-inositol contents deviated by less than 10% from the values declared on the product labels.

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