ANALYSIS OF FREE INOSITOL STEREOISOMERS IN DIETARY SUPPLEMENTS AND FOODS BY HYDROPHILIC LIQUID CHROMATOGRAPHY

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INTRODUCTION

Inositols are cyclic carbohydrates with nine stereoisomers (Figure 1), of which myo- and D-chiro-inositol are the most abundant in humans, while others (e.g., scyllo-, neo-, and epi-) are also present at lower levels. Inositols are associated with important health benefits, including the management of insulin resistance, metabolic syndrome, PCOS, and gestational diabetes. Adequate intake is important for babies whose digestive and metabolic systems are still developing, and for individuals with inositol deficiency. With increasing incorporation of inositol into supplement and food products, there is growing demand for analytical methods capable of profiling inositol stereoisomers across various food products.

HPLC and GC are commonly used for inositol analysis. While GC provides high resolution, it requires derivatization and long analysis times. HPLC enables faster analysis without derivatization, but standard methods are typically limited to individual isomers. There is no standard method for simultaneous determination of inositol stereoisomers in supplement and food products.

OBJECTIVES

This study aimed to develop HILIC–MS(/MS) methods for the simultaneous separation and quantification of free inositol stereoisomers in dietary supplements and food products.

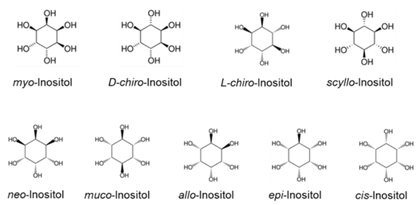
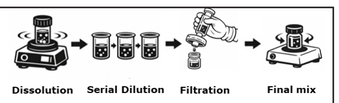


Figure 1. Structures of inositol stereoisomers

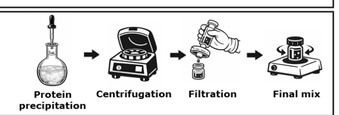
EXPERIMENTAL

Sample Prep.:

For dietary supplements:



For infant formula, milk, and grains:



LC-MS(/MS) Methods:

For dietary supplements

Software: Empower™ 3 CDS
ACQUITY QDa II Mass Detector setting:
Ionization mode: ESI- Capillary voltage: 0.5 kV
Probe Temp.: 600 °C Cone voltage: 7.0 V
SIR [M]⁻: 179 Da, 185 Da (ISTD)

LC conditions:
Column: ACQUITY UPLC™ BEH™ Amide Column, or ACQUITY Premier BEH Amide VanGuard™ FIT Column (1.7 µm, 2.1 × 150 mm).
Col. temp.: 25 °C
MP A: ACN:water (90:10 v/v) with 0.01% NH_3
MP B: ACN:water (50:50 v/v) with 0.01% NH_3, 20 mM NH_4HCO_3
Gradient elution Table:

Time	Flow Rate (mL/min)	A%	B%	Curve
Initial	0.35	91%	9%	Initial
5.7	0.35	91%	9%	6
13.1	0.35	79%	21%	6
17.4	0.35	79%	21%	6
18.0	0.35	91%	9%	6
23.0	0.35	91%	9%	6

For infant formula, milk, and grain

Software: MassLynx™
XevoTQ-S cronos MS setting:
Ionization mode: ESI- Capillary voltage: 2.50 kV
Source temp.: 150 °C Desolvation temp.: 350 °C
Cone gas flow: 40 L/Hr Desolvation gas flow: 650 L/Hr
MRM transitions and detection parameters:

Analyte	Dwell (secs)	MRM Transitions	Cone Voltage (V)	Collision Energy (eV)
inositol	0.1	179.0 > 86.9 (Quan)	20	12
	0.1	179.0 > 98.9 (Qual)	20	14
myo-inositol-d_6	0.1	185.0 > 88.9 (Quan)	20	12
	0.1	185.0 > 101.9 (Qual)	20	14

LC conditions: the same as those for dietary supplements

RESULTS AND DISCUSSION

Chromatographic separation optimization

- Column temperature
- Gradient elution conditions
- Injection volume
- QDa II optimization (capillary voltage and probe temperature)

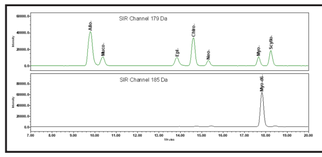


Figure 2. SIR chromatograms of inositol standards (allo-, muco-, epi-, chiro-, neo-, myo-, and scyllo-inositol) and an ISTD (myo-inositol-d6). Resolution for allo-/muco-, chiro-/neo-, and myo-/scyllo- are 1.6, 2.3, and 2.0, respectively.

Selectivity

- no interference from common monosaccharides (glucose, fructose, galactose, and mannose)

Ruggedness

- No statistically significant difference across 3 columns (different batches and format)

Table 1. Comparison of results across three columns (different batches and formats).

	myo-Inositol results (µg/mL)			*D-chiro*-Inositol results (µg/mL)		
	Col. #1	Col. #2	Col. #3	Col. #1	Col. #2	Col. #3
Measurement 1	0.586	0.614	0.623	0.568	0.627	0.624
Measurement 2	0.616	0.608	0.634	0.629	0.596	0.600
Measurement 3	0.640	0.600	0.633	0.593	0.606	0.645
Average	0.614	0.607	0.630	0.597	0.610	0.623
SD	0.027	0.007	0.006	0.031	0.016	0.023
One-way ANOVA P-value	0.30			0.45		
Comments	The difference among the averages are not statistically significant at α=0.05			The difference among the averages are not statistically significant at α=0.05		

Linearity and sensitivity

Table 2A. Calibration results and limit of quantitation (LOQ) for dietary supplements.

Analyte	Equation	R^2	Conc. Range (ppm)	LOQ (ppm)
Scyllo-Inositol	$Y = -3.73 \times 10^{-3} X^2 + X - 5.32 \times 10^{-3}$	0.9993	0.2 - 15	0.2
Myo-Inositol	$Y = -9.50 \times 10^{-4} X^2 + 0.616 X - 8.02 \times 10^{-2}$	0.9995	0.3 - 15	0.3
Neo-Inositol	$Y = -1.30 \times 10^{-3} X^2 + 1.16 X - 3.46 \times 10^{-1}$	0.9993	0.9 - 15	0.9
Chiro-Inositol	$Y = -6.27 \times 10^{-3} X^2 + 1.47 X - 5.00 \times 10^{-3}$	0.9981	0.1 - 15	0.1
Epi-Inositol	$Y = -9.41 \times 10^{-4} X^2 + 0.891 X - 1.36$	0.9978	0.9 - 15	0.9
Muco-Inositol	$Y = -6.02 \times 10^{-3} X^2 + 1.94 X - 7.25 \times 10^{-1}$	0.9990	0.9 - 15	0.9
Allo-Inositol	$Y = -1.81 \times 10^{-2} X^2 + 2.19 X - 1.11 \times 10^{-1}$	0.9993	0.09 - 15	0.09

Table 2B. Calibration results and LOQ for foods.

Analyte	Equation	R^2	Conc. Range (µg/mL)	LOQ* (µg/mL)	LOQ in matrix** (µg/mL)
Myo-Inositol	$Y = 4.62 \times 10^{-3} X^2 + 1.66 X - 2.59 \times 10^{-2}$	0.9998	0.15 - 15	0.09	0.3
D-Chiro-Inositol	$Y = -4.54 \times 10^{-2} X^2 + 4.82 X + 1.13 \times 10^{-2}$	0.9991	0.05 -15	0.05	0.2

Note: *LOQ estimated in solvent; **LOQ estimated in whole milk.

Accuracy and precision

Table 3A. Summary of accuracy and precision results in spiking experiments for dietary supplements.

myo-Inositol						
Dietary Supplement	Mean Conc. (µg/mL)	RSD (n=3)	Spiked Conc. (µg/mL)	Spiked level*	RSD (n=3)	Recovery
DS-1	2.71	0.5%	4.56	168%	0.3%	105.0%
DS-2	3.72	0.4%	7.60	204%	0.1%	101.1%
DS-3	2.40	0.6%	7.60	317%	0.1%	103.4%
DS-4	2.35	0.3%	1.52	65%	0.2%	106.0%
D-chiro-Inositol						
Dietary Supplement	Mean Conc. (µg/mL)	RSD (n=3)	Spiked Conc. (µg/mL)	Spiked level*	RSD (n=3)	Recovery
DS-1	0.00	-	4.479	-	0.7%	99.9%
DS-2	0.10	1.1%	7.465	-	0.7%	97.9%
DS-3	0.06*	7.4%	7.465	-	0.8%	97.9%
DS-4	0.03*	6.9%	1.493	-	0.7%	99.3%

Note: * spiked level relative to the native level; * close to LOQ.

Table 3B. Summary of accuracy and precision results in spiking experiments for foods.

	myo-Inositol			*D-chiro*-Inositol		
	Spike Conc. (µg/mL)	RSD (n=3)	Recovery	Spike Conc. (µg/mL)	RSD (n=3)	Recovery
Solvent	0.61	4.4%	101%	0.61	5.2%	98%
	2.43	2.0%	96%	2.39	6.7%	102%
Soybean milk	0.61	3.6%	107%	0.61	2.5%	103%
	1.22	0.5%	118%	1.19	5.3%	108%
	2.43	1.6%	105%	2.39	1.9%	107%
Almond milk	1.22	2.2%	107%	1.19	1.0%	104%
Oat milk	1.22	3.2%	110%	1.19	7.5%	100%
Whole milk	1.22	2.2%	108%	1.19	2.5%	96%
	2.43	2.2%	102%	2.39	4.7%	107%
Cornmeal	0.61	3.2%	103%	0.60	3.5%	99%
Infant Formula	0.61	0.6%	89%	0.60	3.0%	97%
	0.61	1.7%	117%	0.61	5.0%	109%

Sample analysis results

Table 4A. Summary of dietary supplement analysis results.

Dietary Supplement	*myo-Inositol*			*D-chiro-Inositol*		
	Label value (mg/serving)	Mean (mg/serving)	Rel. to label	Label value (mg/serving)	Mean (mg/serving)	Rel. to label
DS-1	2000	1965	98.2%	50	0.0	0%
DS-2	2000	1893	94.6%	50	48.6	97%
DS-3	2000	2158	107.9%	50	58.2	116%
DS-4	2000	2049	102.4%	50	24.1	48%

Table 4B. Summary of food analysis results.

	myo-Inositol		*D-chiro*-Inositol	
	Mean Conc. (mg/100 g)*	RSD (n=3)	Mean Conc. (mg/100 g)	RSD (n=3)
Soybean milk	4.16	3.0%	3.10	2.3%
	4.08	3.8%	3.22	3.7%
	4.22	1.4%	3.29	1.8%
	Average (interday, n=9)		Average (interday, n=9)	
	4.16	2.9%	3.21	3.5%
Almond milk	2.46	0.6%	N.D.	
Oat milk	0.64	7.2%	N.D.	
Whole milk	3.13	1.7%	N.D.	
	2.88	3.2%		
	Average (interday, n=6)			
	3.01	5.1%		
Cornmeal	34.72	2.2%	N.D.	
Infant formula	83.45	1.1%	N.D.	
	77.18	0.2%		
	Average (interday, n=6)			
	80.32	4.3%	N.D.	

Note: * mg/100mL for cornmeal and infant formula.

CONCLUSION

- **We have developed fit-for-purpose solutions for analysis of inositol stereoisomers in dietary supplements and foods.**
- **Both analytical solutions demonstrated excellent linearity, sensitivity, specificity, accuracy, precision and ruggedness.**
- **These methods were successfully applied to commercial products, demonstrating their suitability for routine quantitation of free inositol stereoisomers across a variety of dietary and food matrices.**

Reference

1) Yang, J., Harden, S., and Rainville, P. HILIC-MS/MS Analysis of Free Inositol Stereoisomers in Foods, Application Note 720009200, Waters Corporation, 2026.
2) Yang, J., Harden, S., and Rainville, P. Analysis of Free Inositol Stereoisomers in Dietary Supplements by Hydrophilic Liquid Chromatography using the Arc™ Premier System and ACQUITY QDa II Mass Detector, Application Note 720009186, Waters Corporation, 2025.

Please scan the QR Codes on the right to download the application notes.

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