

A Rapid Method for Determining Glucosamine in Dietary Supplements Using HPAE-PAD

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ABSTRACT

Glucosamine (GlcN) supplements are some of the most frequently used nonvitamin, nonmineral supplements in the United States. The US FDA regulates dietary supplements to ensure they are produced under cGMP. Using high-performance anion-exchange chromatography (HPAE) coupled with pulsed amperometric detection (PAD), carbohydrates such as GlcN can be determined without chemical derivatization. This poster reports a rapid, rugged HPAE-PAD method for determining GlcN in dietary supplement tablets, capsules, powders, and fortified liquids. Limits of detection (0.09 μM), linear range (0.30–340 μM), method accuracy (98–108% recovery), and retention time (< 2% change over 7 days) and peak area (< 0.5% over 7 days) precision were determined. The method was fast (with sample throughput as short as 7.5 min) while maintaining sufficient selectivity to resolve and detect many other mono- and disaccharides, sugar alcohols, and other electroactive compounds present.

INTRODUCTION

Glucosamine (GlcN), a major structural component in the biosynthesis of glycosaminoglycan compounds, is involved in normal joint function and is sold as a dietary supplement for joint health. The 1994 Dietary Supplement Health and Education Act granted the United States FDA authority to prescribe good manufacturing practices for dietary supplements.¹ The final rule, published in June, 2007, established regulations requiring current good manufacturing practices (cGMP) for dietary supplements.² Using the cGMP regulation model for foods, the rule ensures that dietary supplements are produced in a quality manner, do not contain contaminants or impurities, and are accurately labeled.

Previously-reported methods for the determination of glucosamine in dietary supplements have used HPLC with UV or fluorescence detection.³ As GlcN lacks a chromophore, these methods require either pre- or postcolumn derivatization and are often limited to determining only the glucosamine. Pulsed amperometric detection (PAD)—a powerful detection technique with a broad linear range and high sensitivity—is ideally suited for determination of GlcN and related substances without the need for chemical derivatization.

Here we report a method using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) to determine GlcN in dietary supplement tablets, capsules, powders, and fortified liquids—both those containing chondroitin sulfate (CS) and those without.^{4,5} Samples were quantified after being dissolved in DI water, centrifuged to remove particulates, and diluted to a target concentration of 10 μM (1.8 $\mu\text{g}/\text{mL}$) GlcN based on label information. Limits of detection and quantification, method accuracy, and method precision are reported. The method provides high sample throughput while maintaining the selectivity to resolve and detect many other mono- and disaccharides, sugar alcohols, and other electroactive compounds present.

EXPERIMENTAL

Instrumentation

Dionex ICS-3000 Reagent-Free™ Ion Chromatography system (Figure 1) with Eluent Generation (RFIC-EG™ system) consisting of:

DP Dual Gradient or SP Single Gradient Pump, with the EG/DP/SP Vacuum Degas Conversion Kit and GM-4 Gradient Mixer
Eluent Generator with EluGen® EGC II KOH eluent generator cartridge
Continuously Regenerated Anion Trap Column (CR-ATC)
DC Detector Chromatography module equipped with single or dual temperature zones, injection valve(s) and 10 μL injection loop
ED Electrochemical Detector, ED cell and spacer block with combination pH/Ag/AgCl Reference Electrode and Carbohydrate Disposable Au Working Electrodes

AS Autosampler (with diverter valve for dual systems) and 2 mL vial tray
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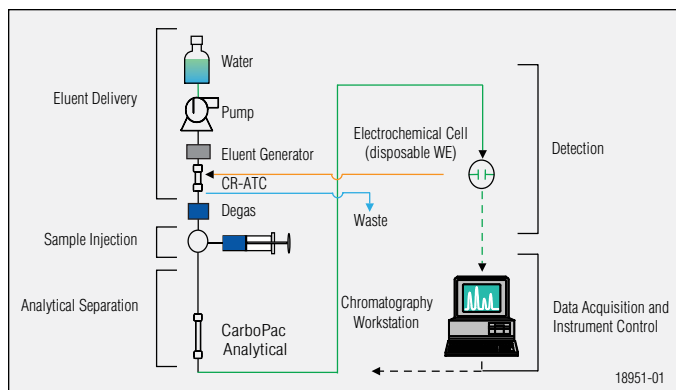


Figure 1. HPAE-PAD system for glucosamine determinations.

Chromatography Conditions

Column:	CarboPac® PA20 Analytical, 3 × 150 mm
Eluent:	20 mM KOH, isocratic, 7.5 or 15 min run time
Eluent Source	EGC II KOH
Flow Rate:	0.5 mL/min
Injection Volume:	10 µL (full loop)
Temperature:	30 °C
Detection:	Pulsed amperometry, using Carbohydrate Disposable Au Working Electrodes
ED Waveform:	Carbohydrate 4-Potential Waveform
Background:	19-25 nC
Noise (Peak-to-Peak):	~ 60 pC
Typical System Backpressure:	2580–2730 psi

GlcN Standards Preparation

1. Prepare the standard concentrate solution gravimetrically from dried GlcN.HCl and DI water.
2. Dilute the standard concentrate solution gravimetrically to produce ~ 1.0 mM (179 µg/mL) GlcN free base standard stock solution.
3. Prepare GlcN standard working solutions gravimetrically from standard stock solution.

Sample Preparation

1. Dissolve 1 unit* GlcN-containing sample in DI water to make a 1.00 L solution using a volumetric flask.
2. Centrifuge suspension in microcentrifuge tube.
3. Dilute supernatant gravimetrically in water to 1.00 mM (179 µg/mL) GlcN free base sample stock solution (based on product label information).
4. Dilute sample stock solution gravimetrically to a target concentration of 10 µM (1.8 µg/mL) GlcN free base for injection.

* 1 unit = 1 tablet, capsule, powder packet, or container of fortified liquid
See Table 1 for a description of the samples used in this study.

Sample (Dosage or Serving Size)	mg Per Serving		Size Used for Analysis	GlcN Salt Form
	GlcN	CS		
Supplement A (2 tablets)	1500	1200	1 tablet	HCl
Supplement B (2 caplets)	1500	300	1 caplet	HCl
Supplement C (4 capsules)	1500	1200	1 capsule	HCl
Supplement D (1 capsule)	1500	400	1 capsule	HCl
Supplement E (1 can*)	1200	250	1 can	HCl
Supplement F (1 packet**)	500	400	1 packet	H ₂ SO ₄ ·2KCl
Supplement G (2 capsules)	0	1200	1 capsule	Not Applicable

* 1 can contains 250 mL liquid
** 1 packet contains 8.7 g powder

RESULTS AND DISCUSSION

Chromatographic Separations

Figure 2 shows chromatograms for six GlcN- and CS- containing dietary supplements and one CS supplement that did not contain GlcN. The CarboPac PA20, combined with PAD, yielded simple chromatograms for most of the supplements tested.

Mono- and disaccharides, and sugar alcohols present in significant amounts in some of the supplements, were sufficiently separated from GlcN and did not interfere with its determination.

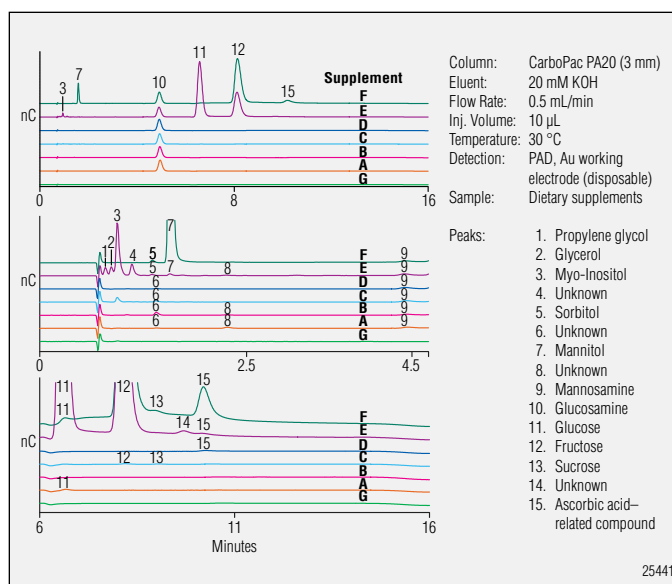


Figure 2. HPAE-PAD analysis of GlcN- and CS-containing dietary supplements. Seven dietary supplement samples diluted to approximately 10 µM (1.8 µg/mL) GlcN, 10 µL injection. A) Full chromatogram, B) expanded early RT region of the chromatogram, C) expanded later RT region of the chromatogram.

Linearity, Limit of Detection, Quantification

Figure 3A presents the relationship of GlcN peak area (nC*min) to concentration of the GlcN injected (10 µL) over a wide range of concentrations (0 to 1000 µM; or 0–179 µg/mL). Table 2 summarizes the results for limit of detection (LOD), lower limit of quantification (LOQ), upper limit of linearity, and linear range.

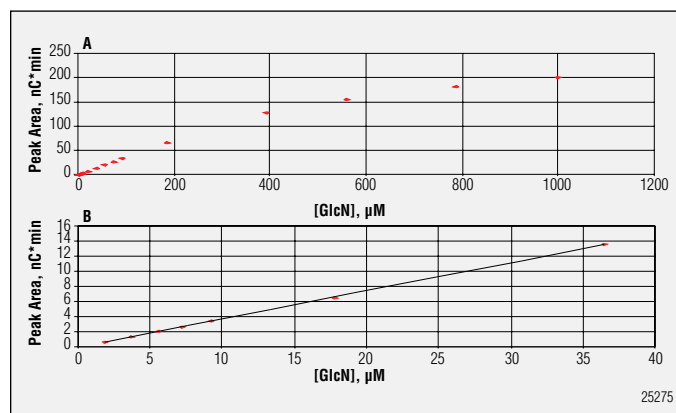


Figure 3. The relationship of peak area (mean) to glucosamine concentration injected for estimation of linear range (n = 8). A) Wide range curve. B) Narrower range used for GlcN quantification.

Table 2. Estimated Limits of Detection, Quantification, and Linearity for Glucosamine		
Noise (pC)	Mean ± SD; n=8	58 ± 11
	Range	41–73
Lower Limit Detection (n = 8) [†]	µM*	0.09 ± 0.02
	µg/mL	0.016 ± 0.003
Lower Limit Quantification (n = 8) ^{††}	µM*	0.30 ± 0.06
	µg/mL	0.054 ± 0.010
Upper Limit Linearity ^{†††}	µM*	340
	µg/mL	61
Linearity (Full Linear Range)	Concentration Range	0.77 – 180 µM (0.14 – 32 µg/mL)
	r ²	0.9995
	Slope (nC*min/µm)	0.3636
	Y-axis Intercept (nC*min)	0.13
Linearity (Target Range) ^{**}	Concentration Range	1.8 – 36 µM (0.32 – 6.4 µg/mL)
	r ²	0.9998
	Slope (nC*min/µm)	0.3746
	Y-axis Intercept (nC*min)	-0.057

[†] [GlcN] where peak height S/N = 3

^{††} [GlcN] where peak height S/N = 10

^{†††} see Ref. 4 for method used to calculate Upper Limit Linearity

* 10-µL injections

** Target [GlcN] = 10 µM (1.8 µg/mL)

While the full linear range for GlcN quantification covered more than three orders of magnitude (0.30–340 µM, 0.06–61 µg/mL for a 10 µL injection) for routine GlcN determination, we recommend a dilution scheme that targets a 10 µM (1.8 µg/mL) concentration of GlcN (Figure 3B) for dietary supplements that covers a narrower concentration range of 1.8–36 µM (0.32–6.4 µg/mL). The r² value in this range is >0.9998.

Accuracy

Method accuracy was determined from recovery of known amounts of GlcN spiked into either DI water or GlcN-free Supplement G suspension. Table 3 summarizes the results from both sets of spiking experiments.

Table 3. GlcN Spike Recovery from Dietary Supplement Containing CS		
Matrix Spiked	GlcN Expected	% Spike Recovery ± SD [†]
DI Water	4.9 µM (0.88 µg/mL)	98.3 ± 0.5
	9.9 µM (1.78 µg/mL)	99.8 ± 0.5
	14.6 µM (2.61 µg/mL)	99.6 ± 0.5
Supplement G	6.1 µM (1.09 µg/mL)	103 ± 0.5
	8.6 µM (1.54 µg/mL)	106 ± 0.4
	13.5 µM (2.42 µg/mL)	108 ± 0.5

[†] Expected amount derived from Supplement Facts on product label

^{††} Based on 0.015 µM detection limit (3x baseline noise)

Precision

GlcN retention time, peak area, number of theoretical plates, and peak asymmetry RSDs were determined for replicate injections of the prepared Supplement A sample over 7 days (1030 injections). This preparation was chosen because the label listed several cellulosic compounds, and high amounts of CS. GlcN concentration was targeted to be 10 µM (1.8 µg/mL) based on product label information using a 10 µL injection. The column was washed for 2 h with 100 mM KOH prior to this study, but no column wash was performed during the 7 day experiment.

Among the four chromatographic parameters monitored, only GlcN retention time showed a statistically significant trend, with a measured decrease of 1.9% after 7 days of continuous operation (Table 4). Despite 1030 injections of a challenging sample—with no column washes during the 7 day period—the small loss of retention time and high reproducibility indicates the method is rugged for the analysis of these samples.

Table 4. Method Ruggedness			
	All 7 Days		Percent Change Over 7 Days
	Mean	RSD	
Retention Time (min)	Mean	4.604	-1.86
	RSD	0.67*	
Peak Area (nC*min)	Mean	5.747	-0.40
	RSD	0.70	
Theoretical Plates	Mean	5458	0.09
	RSD	0.56	
Asymmetry	Mean	1.125	0.18
	RSD	1.70	

[†] 1030 injections over the 7-day period

* Because the retention time trends down with a small slope over 7 days, this RSD value is not strictly a true measurement for relative standard deviation (%)

Application

Table 5 shows the measured amounts of GlcN in the seven supplements analyzed for this study. The determined amounts of GlcN for all supplement samples containing GlcN ranged from 98%–119% of the GlcN label value. The CS formulation without GlcN (Supplement G) contained no measurable GlcN.

Table 5. Determination of Glucosamine in Dietary Supplement Samples Containing Chondroitin Sulfate				
Samples	Dilution Factor (DF)	Measured Amount, mg/unit	Expected Amount, mg/unit ^a	Percent GlcN Found \pm SD
Supplement A	352	872 \pm 2.9	750	116 \pm 0.4
Supplement B	367	894 \pm 8.7	750	119 \pm 1.1
Supplement C	159	367 \pm 1.4	375	98.0 \pm 0.4
Supplement D	229	577 \pm 3.6	500	115 \pm 0.7
Supplement E	597	1290 \pm 5.5	1200	107 \pm 0.5
Supplement F	161	571 \pm 2.3	500	114 \pm 0.5
Supplement G	201	<0.6 ^{††}	0	

Glucosamine Stability

A GlcN-related compound with a retention time of approximately 4.5 min forms gradually at room temperature (Figure 4). This peak, which has the same retention time as mannosamine, was not present in Supplement G, a CS-containing supplement without GlcN. Abundance of this compound increased when GlcN or GlcN-containing products were incubated at room temperature for an extended period. When exposed to elevated temperature, this peak significantly increased in amount together with a decrease in GlcN amount. Therefore, it seems feasible that this technique could be used as a GlcN stability–indicating method using this peak.

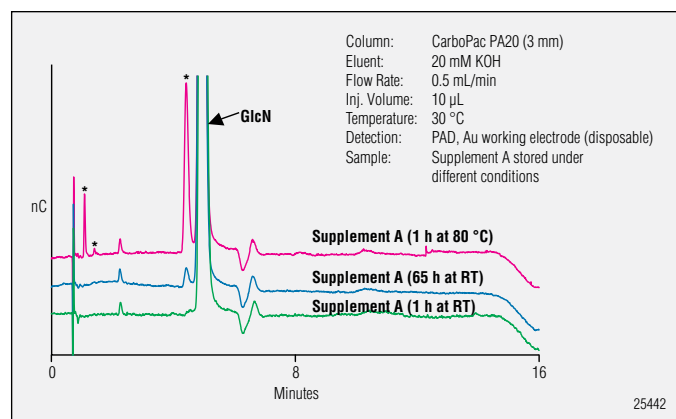


Figure 4. Additional peaks in GlcN-containing Supplement A. (*designates peaks that increase with exposure to elevated temperature.)

SUMMARY

- HPAE-PAD with eluent generation can be used to determine glucosamine in dietary supplements without the pre- or postcolumn chemical derivatization.
- Sample preparation consists of simply dissolving samples in DI water and diluting the resulting solution to a target concentration within the linear range.
- Despite the presence of CS, the capacity of the CarboPac PA20 column is not exceeded, and this method was proven rugged.
- The use of eluent generation enables isocratic analysis of >100 samples per day for 7 days with high peak area precision.
- The system provides high sample throughput while retaining the selectivity to resolve many other mono- and disaccharides that may be present in the supplement formulation.

REFERENCES

1. Dietary Supplement Health and Education Act of 1994, Public Law 103-417. U. S. Food and Drug Administration Center for Food Safety and Applied Nutrition. **1995**. <http://www.fda.gov/opacom/laws/dshea.html>.
2. U.S. Department of Health and Human Services, Food and Drug Administration, 21 CFR Part111 Current Good Manufacturing Practice in Manufacturing, Packaging, Labeling, or Holding Operations for Dietary Supplements; Final Rule. *Fed.Regist.* **2007**, 72, 34751–34958. <http://www.fda.gov/OHRMS/DOCKETS/98fr/07-3039.pdf>.
3. Ji, D.; Zhang, L.; Chen, J.; Peng, E. Precolumn Derivatization Liquid Chromatography Method for Analysis of Dietary Supplements for Glucosamine: Single Laboratory Validation Study. *J. AOAC Int.* **2005**, 88, 413–417.
4. Dionex Corporation. *Determination of Glucosamine in Dietary Supplements Using HPAE-PAD*; Application Note 197, LPN 2001, Sunnyvale, CA, **2008**.
5. Dionex Corporation. *Determination of Glucosamine in Chondroitin Sulfate-Containing Dietary Supplements Using HPAE-PAD*; Application Update 164, LPN 2035, Sunnyvale, CA, **2008**.

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