

Determination of Glucosamine in Dietary Supplements Using HPAE-PAD

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Key Words

CarboPac PA20, Eluent Generation, GlcN, Reagent-Free Ion Chromatography, RFIC

Introduction

Glucosamine (GlcN), an amino sugar, occurs naturally in the human body. It is a major structural component in the biosynthesis of glycosaminoglycans, compounds involved in normal joint function. Use of GlcN as a dietary supplement in the management of osteoarthritis has attracted considerable attention.¹ Results of the 2002 National Health Interview Survey showed that GlcN was one of the five nonvitamin, nonmineral herbal products/dietary supplements most frequently used by adults in the U.S.A.² Increased use in Canada was also noted.³ While the principal use for GlcN dietary supplements is for arthritis management, especially in older adults, its use as a preventive measure to maintain health⁴ and in veterinary medicine⁵ also has been reported.

The 1994 Dietary Supplement Health and Education Act granted the United States FDA authority to prescribe good manufacturing practices for dietary supplements.⁶ The final rule, published in June, 2007, established regulations requiring current good manufacturing practices (cGMP) for dietary supplements.⁷ 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.^{8,9} As GlcN lacks a chromophore, these methods require either pre- or postcolumn derivatization and are often limited to determining only the glucosamine. However, carbohydrates, glycols, alcohols, amines, and sulfur-containing compounds can be oxidized and therefore detected directly without derivatization using amperometry. Pulsed amperometric detection (PAD), a powerful detection technique with a broad linear range and very low detection limits, is ideally suited for determination of GlcN and related substances. This detection method is specific for those analytes such as GlcN that can be oxidized at a selected potential, leaving all other compounds undetected.



High-performance anion-exchange with pulsed amperometric detection (HPAE-PAD) chromatography is a sensitive, direct-detection technique capable of separating mono- and disaccharides rapidly and efficiently.^{10,11} At approximately pH 12, the Thermo Scientific™ Dionex™ CarboPac™ PA20 anion-exchange column will separate and elute neutral monosaccharides, aminosaccharides, and disaccharides while retaining oligosaccharides. The use of HPAE-PAD has been reported for the determination of saccharides in dietary glyconutritional products.¹²

Generating highly reproducible retention times for HPAE chromatographic systems relies on the use of a high purity hydroxide eluent mobile phase prepared with an accurate and precise concentration. An eluent generator (EG) produces such an eluent. The usual variability in hydroxide concentration associated with manual eluent preparation, and the variability of carbonate contamination due to absorption of atmospheric carbon dioxide, are essentially eliminated by the EG, leading to highly reproducible retention times.

In this application note, a rapid, rugged HPAE-PAD method for determining GlcN in dietary supplement tablets, gelatin capsules, and fortified liquids is described. Key performance parameters are evaluated including accuracy, precision, limits of detection/quantification, linearity, and ruggedness. The system setup (Figure 1) provides good sample throughput (7.5 min run time) while retaining the selectivity to resolve many other mono- and disaccharides that may be present in the supplement formulation.

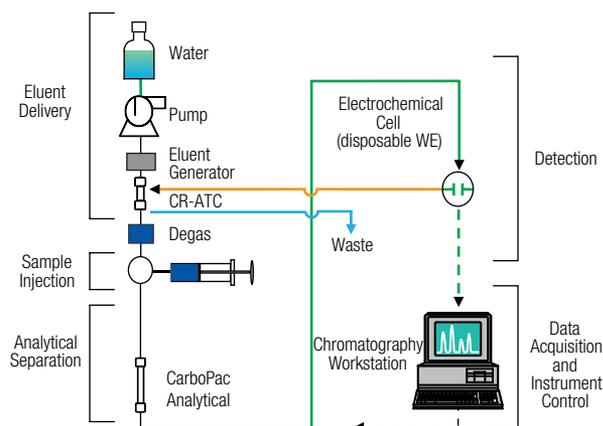


Figure 1. HPAE-PAD system for glucosamine determinations.

Equipment

- Thermo Scientific™ Dionex™ ICS-3000 Reagent-Free™ Ion Chromatography system with Eluent Generation (RFIC-EG™ system) consisting of the following:
 - DP Dual Gradient or SP Single Gradient Pump, with the EG/DP/SP Vacuum Degas Conversion Kit (P/N 063353) and GM-4 Gradient Mixer (P/N 049135)
 - Eluent Generator with EGC II KOH eluent generator cartridge (Thermo Scientific™ Dionex™ EluGen™ II Hydroxide; P/N 058900) and Continuously Regenerated Anion Trap Column (Thermo Scientific™ Dionex™ CR-ATC; P/N 060477)
 - DC Detector/Chromatography module equipped with single or dual temperature zones, injection valve(s), and 10 μ L injection loop, ED Electrochemical Detector (P/N 079830), ED cell and spacer block (P/N-061756) with combination pH/Ag/AgCl Reference Electrode (P/N 061879) and Carbohydrate Disposable Au Working Electrodes (P/N 060139, package of 6; 060216, package of 24)
 - AS Autosampler (with diverter valve for dual systems), and 2 mL vial tray
 - EO Eluent Organizer, including pressure regulator, and four 2 L plastic bottles for each system

- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Management Software
- Helium; 4.5-grade, 99.995%, <5 ppm oxygen (Praxair®)
- Thermo Scientific™ Nalgene™ Rapid-Flow™ sterile disposable filter unit, 0.2 μ m nylon, 90 mm (P/N 164-0020 or equivalent nylon filter)
- Vacuum pump (Gast™ Manufacturing Corp., P/N DOA-P104-AA or equivalent; for degassing eluents)
- Thermo Scientific Dionex Vial Kit, 1.5 mL glass injection vials with caps (P/N 055427)
- Microcentrifuge tubes with detachable screw caps (polypropylene, 1.5 mL, Sarstedt®, P/N 72.692.005; or equivalent)

Reagents and Standards

- Deionized water (DI), 18 M Ω -cm resistance or higher
- D(+)-Glucosamine (Sigma-Aldrich®; P/N G4875)
- Sucrose (Fisher Scientific™; P/N S5500)
- Glucose (Sigma-Aldrich; P/N G5250)
- D-Sorbitol (Sigma-Aldrich; P/N S1876)
- *myo*-Inositol (Sigma-Aldrich; P/N I5125)
- *N*-Acetyl-D-glucosamine (Sigma-Aldrich; P/N A8625)
- D(-)-Fructose (Avantor Performance Materials®; P/N M556-05)
- Mannitol (Sigma-Aldrich; P/N M9546)
- Glycerol (EMD Millipore®; P/N GX0190-8)
- Propylene glycol (1,2-propanediol; Sigma-Aldrich; P/N P6209)

Samples

Samples of GlcN-containing tablets, capsules, and beverages were purchased from retail groceries or drugstores. Table 1 lists the expected amount per serving size, source, the salt form of GlcN in each sample, other ingredients listed on the label, and the amount used to prepare the sample.

Table 1. Description of glucosamine-containing samples.

Sample	mg GlcN (Serving Size)	Size Used for Analysis	GlcN Salt Form	GlcN Source	Other Ingredients
Supplement A	1500 (2 tablets)	1 tablet	HCl	Shellfish	MSM*, cellulose, hypromellose, croscarmellose sodium, stearic acid, silicon dioxide, magnesium stearate, corn starch, povidone, polyethylene glycol
Supplement B	1500 (1 tablet)	1 tablet	HCl	Shellfish	Cellulose, hydroxypropyl cellulose, stearic acid, coating (titanium dioxide, polydextrose, hydroxypropyl methylcellulose, triacetin, polyethylene glycol, magnesium trisilicate), copolyvidone, croscarmellose sodium, silicon dioxide
Supplement C	750 (1 tablet)	1 tablet	HCl	Vegetarian	Sorbitol, dibasic calcium phosphate, stearic acid, modified cellulose gum, colloidal silicon dioxide, wheat/gluten
Supplement D	1000 (1 tablet)	1 tablet	HCl	Vegetarian	Cellulose, modified cellulose gum, stearic acid, magnesium stearate
Supplement E	1000 (1 tablet)	1 tablet	H ₂ SO ₄	Not disclosed	Potassium chloride, cellulose, modified cellulose gum, stearic acid, magnesium stearate
Supplement F	1500 (2 capsules)	1 capsule	H ₂ SO ₄	Shellfish	Potassium chloride, gelatin, magnesium stearate
Supplement G	1500 (1 can**)	1-237 mL can	HCl	Not disclosed	Sparkling water, orange juice concentrate, citric acid, mango juice concentrate, passionfruit juice, sodium hexametaphosphate, sucralose, potassium sorbate, coloring extracts

*MSM - Methylsulfonylmethane (dimethylsulfone) present at 1500 mg/serving

**One can contains 237 mL of liquid

Conditions

Column:	Dionex CarboPac PA20 Analytical, 3 × 150 mm (P/N 060142)
Eluent:	20 mM KOH, isocratic, 7.5 or 15 min run time
Eluent Source:	Dionex EGC II KOH cartridge
Flow Rate:	0.5 mL/min
Injection Volume:	10 µL (full loop)
Temperature:	30 °C
Detection:	Pulsed amperometry, using Thermo Scientific Dionex Carbohydrate Disposable Au Working Electrodes (P/N 060139, package of 6; P/N 060216, package of 24)
Background:	40–65 nC
Typical System Backpressure:	2580–2730 psi

Carbohydrate 4-Potential Waveform for the ED:

Time (s)	Potential (V)	Gain Region*	Ramp*	Integration
0.00	+0.1	Off	On	Off
0.20	+0.1	On	On	On
0.40	+0.1	Off	On	Off
0.41	-2.0	Off	On	Off
0.42	-2.0	Off	On	Off
0.43	+0.6	Off	On	Off
0.44	-0.1	Off	On	Off
0.50	-0.1	Off	On	Off

*Settings required in the Dionex ICS-3000 system, but not used in older Dionex systems.

Reference electrode in Ag mode (Ag/AgCl reference).

Instrument Operational Considerations

Analyze a GlcN check standard at regular intervals to assess both retention time (RT) and peak area precision. When required, a column wash at 100 mM KOH will restore RT for GlcN. The column requires at least 2 h after the column wash to reequilibrate to 20 mM KOH and achieve the highest RT precision. Shorter reequilibrations may yield acceptable precision.

When the system is idle for short (1–2 week) periods, we recommend that the pump and eluent generator be left on at 0.5 mL/min and 20 mM KOH or at a reduced flow rate to allow rapid start-up, and the cell to be turned off to extend disposable electrode life. The use of a lower flow rate, while maintaining the minimum backpressure of at least 200 psi, can extend the interval before water must be added to the eluent reservoir. When the system must be shut down for a period of several weeks, the pump, eluent generator, and electrochemical cell may be turned off. For shutdown periods exceeding several weeks, all plumbing lines should be resealed, and the reference electrode should be removed from the electrochemical cell and stored in the original solution in which it was shipped (3.5 M KCl). When the pump has been turned off for longer than 1 day, the column should be washed with 100 mM KOH for 1–2 h, and reequilibrated with 20 mM KOH for 2 h or less (see above) before analyzing samples.

Preparation of Reagents and Standards

Eluents

It is essential to use high-quality water of high resistivity (18 M Ω -cm) containing as little dissolved carbon dioxide as possible. Biological contamination should be absent. Source water must be obtained using a water purification system consisting of filters manufactured without electrochemically active surfactants or other leachable substances (e.g., glycerol). Prior filtration through 0.2 μ m porosity nylon under vacuum is recommended to remove particulates and reduce dissolved air. Keep the eluent water blanketed under 34–55 kPa (5–8 psi) of helium at all times to reduce carbonate contamination and opportunistic microorganisms.

Although not used to produce the data in this application note, a manually prepared NaOH eluent can be used. Follow the instructions in Thermo Scientific Dionex Technical Note 71 to prepare 100 or 200 mM NaOH and allow the pump to proportion the 20 mM eluent. Results obtained using manually prepared eluent may not be equivalent to the results reported here.

Stock Standards

Prepare stock solutions of GlcN and other ingredients in the dietary supplements by accurately weighing standards into tared plastic vials. Add filtered and degassed DI water and weigh the resulting solution. Prepare stock standard solutions at concentrations of approximately 1.0 mM. Store stock standards at -15 °C. Dilute stock standards with filtered, degassed water to yield the desired working mixture concentrations. For this application note, all dilutions were made gravimetrically to ensure high accuracy and concentrations reported as GlcN free base.

Sample Preparation

Place tablet or capsule sample in a 1.0 L volumetric flask and add approximately 500 mL of filtered DI water. Place the flask into an ultrasonic bath until the sample is fully dispersed (20–30 min) and then bring to volume with filtered DI water. Pour liquid dietary supplement sample into a 1.0 L volumetric flask, carefully degas under vacuum, and bring to volume with filtered, degassed DI water. Make further dilutions by placing 1 mL aliquots in 1.5 mL plastic microcentrifuge vials with detachable screw caps and centrifuge at 16,000 \times g in a microcentrifuge for 20 min. Dilute the supernatant gravimetrically to produce sample stock solutions expected to have 1.0 mM (180 μ g/mL) GlcN free base concentrations based on product label information. Further dilute aliquots from the 1.0 mM solutions gravimetrically to produce solutions for injection into the HPAE-PAD system.

Quantitative results for GlcN concentration and for concentrations of other putatively identified ingredients were converted to the masses of these compounds in the original sample (one tablet or capsule or one 237 mL can of liquid). Two factors, the dilution factor (DF) and the molar conversion factor (CF) were needed for this calculation. The DF represents the factor required to dilute product solutions from their concentration in the 1.0 L volumetric flask to their injected target concentra-

tions. Dilutions used for this application note are listed in Table 3. The CF represents the factor that converts concentrations found for GlcN and other putatively identified ingredients to mass of the analyte in the original sample. For supplements containing GlcN as the sulfate salt, CF was 228 (half the FW of 2GlcN \cdot H₂SO₄). Supplements E and F contained GlcN as its H₂SO₄ salt. For Supplements A, B, C, D, and G, which contained GlcN as its chloride salt, the CF was 216 (the FW of GlcN \cdot HCl). For other substances, CF was the compound's MW. To convert the measured GlcN free base concentration (expressed as μ M, μ moles/L) to mg of GlcN as its appropriate salt form per unit dissolved in the original 1.0 L of water, the following equation was used:

$$\frac{\text{mg GlcN (salt form)}}{\text{unit}} = \frac{\mu\text{mol GlcN}}{\text{L}} \times \text{DF} \times \text{CF} \times 1.0 \frac{\text{L}}{\text{unit}} \times 0.001 \frac{\text{mg}}{\mu\text{g}}$$

A unit of supplement is a tablet, capsule, can, packet, or any other amount of product dissolved or diluted in 1.0 L of water to prepare the sample concentrate. For example, if the GlcN concentration in the diluted sample of Supplement A is determined to be 10.0 μ M, the amount of GlcN \cdot HCl in the tablet dissolved in 1.0 L water is:

$$\frac{\text{mg GlcN}\cdot\text{HCl}}{\text{unit}} = \frac{10 \mu\text{mol}}{\text{L}} \times 350 \times 216 \frac{\mu\text{g GlcN}\cdot\text{HCl}}{(\mu\text{mol GlcN free base})} \times 1.0 \frac{\text{L}}{\text{unit}} \\ \times 0.001 \frac{\text{mg}}{\mu\text{g}} = 756 \frac{\text{mg}}{\text{unit}}$$

Method accuracy was assessed from recovery of known amounts of GlcN spiked into either DI water or Supplement B previously diluted to an expected GlcN concentration of 9.9 μ M (1.8 μ g/mL). A 1.00 mM (179 μ g/mL) GlcN standard was used to accurately spike the Supplement B sample at 50% and 100% of the expected GlcN concentration in the supplement.

Results and Discussion

Separation

Figure 2A shows chromatograms for the seven GlcN dietary supplements diluted to the target 10 μ M (1.8 μ g/mL) GlcN concentration. The CarboPac PA20 column, combined with PAD, yielded simple chromatograms for most of the supplements tested. In Supplement A, the high concentration of methylsulfonylmethane (MSM), another active ingredient in this product, was not detected and did not interfere with the GlcN determination. Sorbitol in Supplement C, an inactive ingredient (preservative), was detected but did not interfere. In liquid Supplement G, glucose, fructose, sucrose, and *myo*-inositol were also observed and sufficiently separated from GlcN. The added non-nutritive sweetener, sucralose, was retained on the Dionex CarboPac PA20 column and was not eluted using this method. Sucralose can be determined using similar methods.^{13,14} Although we expected the possibility that *N*-acetyl-glucosamine might be present in some of the dietary supplements from shellfish sources, it was not detected.

Column: Dionex CarboPac PA20, 3 mm
 Eluent: 20 mM KOH
 Temperature: 30 °C
 Flow Rate: 0.5 mL/min
 Inj. Volume: 10 μ L
 Detection: PAD, disposable Au electrode
 Sample: Dietary supplements, about 10 μ M (1.8 μ g/mL)

Peaks:	1. Propylene glycol	8. Mannitol
	2. Glycerol	9. Glucosamine
	3. <i>myo</i> -Inositol	10. Glucose
	4,5. Unknown	11. Fructose
	6. Sorbitol	12. Sucrose
	7. Unknown	

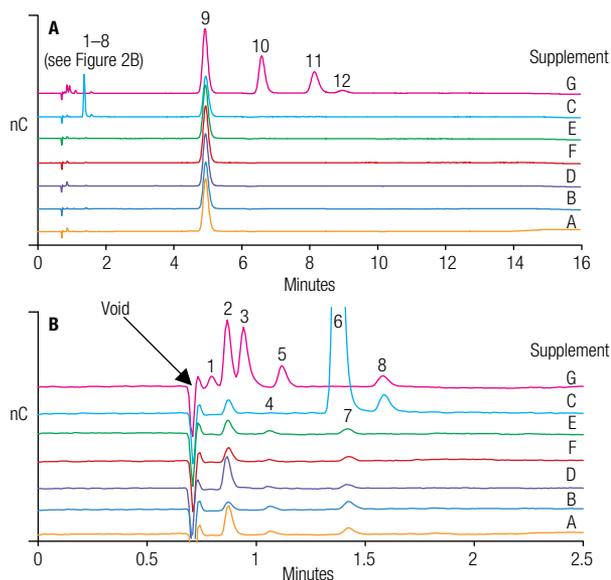


Figure 2. HPAE-PAD analysis of GlcN-containing dietary supplements. Seven dietary supplement samples diluted to approximately 10 μ M (1.8 μ g/mL) GlcN, 10- μ L injection. A) Full chromatogram. B) Expanded early RT region of the chromatogram.

Trace amounts of other, unidentified ingredients can be seen in Figure 2B. Peaks 4 and 7 are detected in all tablet and capsule samples analyzed, except in Supplement C, where peak 7 may be masked by sorbitol, peak 6. Neither peak was detected in the beverage (Supplement G). Table 1 lists the other ingredients present in the seven dietary supplements evaluated in this note. The combined use of HPAE and the specificity of PAD yields an uncomplicated chromatogram for determination of GlcN.

Eluent concentrations of 10–15 mM KOH caused the GlcN peak to coelute with a baseline dip, typically having a retention time of 6 min. Baseline dips associated with injections of water or samples are caused by the elution of non-electrochemically active trace organic impurities present in the sample. When these compounds elute, they exclude electrochemically active ions present in the eluent and appear as negative peaks. The “oxygen dip” (approximately 16 min retention time for the column used in this study) is due to oxygen present in the samples and appears as a function of the gas permeation volume of the column. The retention times of the “oxygen dip” and other baseline dips are constant for each column, but vary slightly from column to column, and many depend on the flow rate, not the eluent strength. Increasing the eluent strength to 20 mM KOH decreased the GlcN retention time to 5.0 min and thus removed any effect of the dip at 6 min on GlcN peak integration.

Eluting the baseline dips just prior to the end of the run, or timing their elution to occur at the end of the following injection, prevents the baseline dips from interfering with the peaks of interest. Using the overlapping sample preparation configuration (flushing the injection port, needle, and autosampler tubing for the next sample during the separation of the current sample), a run time of 7.5 min (total time between injections of 8.6 min) will produce a relatively flat baseline for integration of peaks having retention times between 1 and 6 min. For samples with compounds eluting later than GlcN, the run time can be set to 16 min without significant baseline interference from the oxygen dip.

Detection

Linearity

Figure 3A presents the relationship of GlcN peak area ($nC \times \text{min}$) to concentration of the GlcN injected (10 μ L) over a broad range of concentrations, 0 to 1000 μ M (0–179 μ g/mL). In this study, the lower limit of detection was estimated to be 0.09 μ M (0.02 μ g/mL). The full linear range in this study covered more than 3 orders of magnitude, 0.30–340 μ M, 0.06–61 μ g/mL, for a 10 μ L injection. For routine GlcN determination, we recommend a dietary supplement dilution scheme that targets a 10 μ M (1.8 μ g/mL) GlcN concentration. Figure 3B presents a plot covering a narrower concentration range of 1.8–36 μ M (0.32–6.4 μ g/mL) where the target concentration is near the middle of this range. The r^2 value in this range is >0.9998.

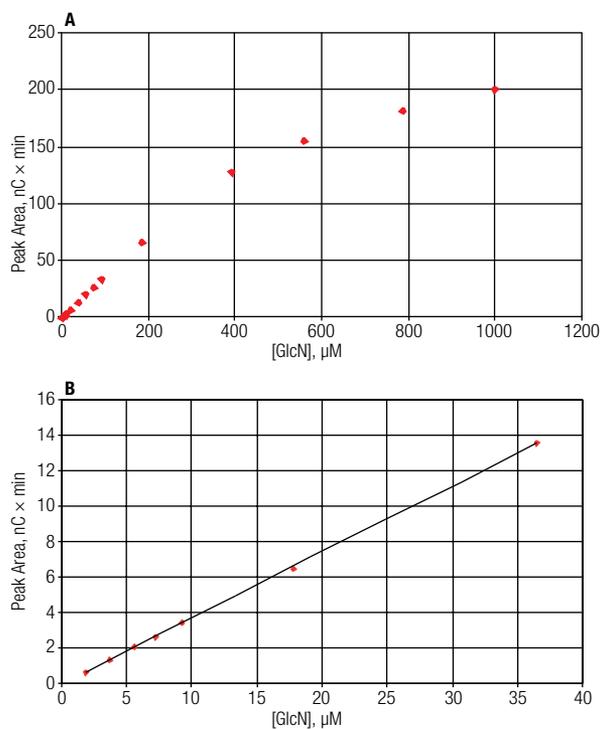


Figure 3. The relationship of peak area (mean) to glucosamine concentration injected for estimation of linear range ($n = 8$). A) Wide range curve. B) Narrower range used for GlcN quantification.

Precision

GlcN retention time and peak area RSDs were determined for replicate injections of Supplement B supernatant (GlcN concentration targeted to 10 μM [1.8 $\mu\text{g/mL}$] for 10 μL injection) over 5 days (718 injections). Supplement B was chosen for this study because the label lists several cellulosic compounds as part of this dietary supplement tablet and it was considered among the more challenging matrices of the products investigated in this note. Run times were 7.5 min (injections made every 8.6 min). Table 2 shows these results on a daily basis and for the 5-day period. The column was washed for 1 h at 100 mM KOH prior to this study, but no wash was performed during this 5-day period.

Table 2. Precision of glucosamine retention time and peak area for Supplement B injected consecutively over 5 days.

	Day					All 5 Days	% Change over 5 Days
	1	2	3	4	5		
Retention Time (min)							
Mean	4.972	4.937	4.908	4.899	4.896	4.922	-1.53
SD	0.011	0.011	0.007	0.007	0.009	0.03	
N	144	146	145	141	142	718	
RSD	0.22	0.22	0.14	0.14	0.18	0.61	
Peak Area (nC \times min)							
Mean	4.096	4.073	4.049	4.029	4.034	4.057	-1.51
SD	0.034	0.027	0.034	0.045	0.035	0.043	
N	144	146	145	141	142	718	
RSD	0.83	0.66	0.84	1.12	0.87	1.06	

Retention Time

Buildup on the stationary phase of non-eluting sample ingredients and carbonate contaminants from the eluent can result in decreasing capacity and eventually can decrease the retention time for GlcN. An EG essentially eliminates carbonate contamination; therefore, the only remaining concern is loss of column capacity due to sample ingredients. The data in Table 2 shows high retention time precision and little loss of retention time over the five days, despite injecting a challenging sample with no column washes during the five-day period.

Peak Area

Peak area precision is a measure of the ECD response stability and the variance in response for replicate injections. Table 2 shows there was good GlcN peak area reproducibility during the five-day study.

Accuracy

GlcN recovery from DI water and a diluted aqueous extract of a dietary supplement was evaluated in this application note. Percent recovery (mean \pm SD) from DI water at 5.1 μM (0.91 $\mu\text{g/mL}$) and 10.1 μM (1.81 $\mu\text{g/mL}$) was 101 \pm 1.3% and 102 \pm 0.3%, respectively. Recoveries from Supplement B supernatant spiked at 5.1 μM (0.91 $\mu\text{g/mL}$) and 9.9 μM (1.77 $\mu\text{g/mL}$) were 93.4 \pm 3.0% and 99.0 \pm 2.5%, respectively, indicating that the method was accurate.

Application

Figure 2 presents chromatograms for the seven GlcN-containing dietary supplements studied. No other peaks were observed when run times were extended to 30 min. Table 3 shows the measured amounts of GlcN in the seven dietary supplements analyzed for this note, derived from a seven-point calibration over the 1.8 to 36 μM (0.32–6.4 $\mu\text{g/mL}$) range. The determined amounts of GlcN for all seven supplement samples were above the stated label amounts, ranging from 110% to 152% of the GlcN label value.

Table 3. Determination of glucosamine in dietary supplement samples.

Sample	Dilution Factor (DF)	Measured Amount, mg/unit ^a	Expected Amount, mg/unit ^b	% GlcN Found \pm SD
Supplement A	350	959 \pm 9.5	750	128 \pm 1.3
Supplement B	659	1650 \pm 2.5	1500	110 \pm 1.7
Supplement C	455	966 \pm 3.5	750	129 \pm 0.5
Supplement D	413	1130 \pm 4.1	1000	113 \pm 0.4
Supplement E	467	1370 \pm 2.5	1000	137 \pm 0.3
Supplement F	315	991 \pm 5.2	750	132 \pm 0.7
Supplement G	680	2270 \pm 11	1500	152 \pm 0.7

^aCalculated amount = [GlcN] found \times DF \times CF, converted to mg

^bExpected amount derived from Supplement Facts on label

Some dietary supplements showed significant amounts of PAD-responsive related substances using this method (Figure 2). The peaks for these related substances were putatively identified by matching their retention times with those of carbohydrate and glycol standards. Single-level calibrations were used to estimate the amount of these ingredients in the supplements. Table 4 shows the amounts of these related substances, expressed as mg/unit. Unknown ingredient peaks 4 and 7 (Figure 2B), present in all products except Supplement G, showed peak areas relative to GlcN ranging from 0.08 to 0.27% and 0.29 to 0.63%, respectively. This method can also be used to determine other carbohydrates or glycols present in dietary supplements. Higher concentration GlcN solutions can be injected for determination of trace mono- and disaccharide concentrations, if desired, for evaluation of GlcN quality.

Table 4. Determination of other substances detected in dietary supplements.

Sample	Analyte ^a	Calculated Amount/Unit (mg/unit) ^{b,c} ± SD	% Relative to Measured [GlcN] ± SD
Supplement A	Glycerol	28.1 ± 0.6	2.9 ± 0.1
Supplement B	Glycerol	17.6 ± 1.1	1.1 ± 0.1
Supplement C	Glycerol	16.8 ± 0.4	1.74 ± 0.04
	<i>myo</i> -Inositol	0.7 ± 0.4	0.07 ± 0.04
	Sorbitol	307 ± 1.5	31.8 ± 0.2
	Mannitol	43.9 ± 1.4	4.5 ± 0.1
Supplement D	Glycerol	37.3 ± 1.8	3.3 ± 0.2
Supplement E	Glycerol	18.5 ± 0.5	1.35 ± 0.04
Supplement F	Glycerol	12.7 ± 0.3	1.28 ± 0.03
Supplement G	Propylene glycol	5.44 ± 0.08	0.24 ± 0.01
	Glycerol	125 ± 2.7	5.5 ± 0.1
	<i>myo</i> -Inositol	61.7 ± 0.8	2.72 ± 0.04
	Mannitol	43 ± 2.2	1.9 ± 0.1
	Glucose	1380 ± 11	60.8 ± 0.6
	Fructose	1939 ± 4.5	85.4 ± 0.5
	Sucrose	300 ± 11	13.2 ± 0.5

n = 5 injections per sample

^aPutative identification based on retention time matches with standards

^bA unit is 1 tablet, 1 capsule, or 1 237-mL can of liquid

^cCalculated amount = [substance] found × DF × MW, converted to mg

Conclusion

HPAE-PAD with eluent generation can be used to determine glucosamine in dietary supplements without the pre- or postcolumn derivatization required when using UV or fluorescence detection. Sample preparation consists of simply dissolving samples in DI water and diluting the resulting solution to a target concentration within the linear range. The high capacity of the Dionex CarboPac PA20 column and the use of eluent generation enable the isocratic analysis of over 100 samples per day for 5 days with the analyst required to add only water and samples to the system. This method works for a variety of sample matrices, as demonstrated by the practical application of this method to the accurate determination of GlcN in seven dietary supplements.

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