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Determination of Glucosamine in Chondroitin Sulfate-Containing Dietary Supplements Using HPAE-PAD

INTRODUCTION

Glucosamine (GlcN), a major structural component in the biosynthesis of glycosaminoglycan compounds, and chondroitin sulfate (CS), a glycosaminoglycan, are involved in normal joint function and are sold as dietary supplements for joint health. Accurate determinations of GlcN in dietary supplements by high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAE-PAD) were reported in Application Note 197.¹ In that note, the dietary supplements analyzed did not contain CS, a high molecular weight, polyanionic polysaccharide that has the potential to overload the anion-exchange column and compromise GlcN determinations. This application update extends the determination of GlcN to dietary supplements containing CS. Key performance parameters are evaluated including accuracy, precision, and ruggedness. The feasibility of using this method to monitor the stability of GlcN in dietary supplements is also evaluated.

Despite the presence of CS, the capacity of the CarboPac® PA20 column is not exceeded, and the method is as rugged as that reported in Application Note 197. The system has good sample throughput (7.5 min) while retaining the selectivity to resolve many other mono- and disaccharides that may be present in the supplement formulation. Electrolytic eluent generation assures eluent purity and consistency while reducing operator labor.

EQUIPMENT

Dionex ICS-3000 Reagent-Free™ Ion Chromatography system 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 (P/N 063353) and GM-4 Gradient Mixer (P/N 049135)

Eluent Generator with EGC II KOH cartridge (EluGen® II Hydroxide; P/N 058900) and Continuously Regenerated Anion Trap Column (CR-ATC; P/N 060477)

DC Detector/Chromatography module equipped with single or dual temperature zones, injection valve(s) and a 10 µL injection loop, ED Electrochemical Detector (P/N 061718), ED cell and spacer block (P/N 061756) with combination pH/Ag/AgCl Reference Electrode (P/N 061879) and Carbohydrate Disposable Au Working Electrode (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

Chromeleon® Chromatography Management Software
Helium, 4.5-grade, 99.995%, <5 ppm oxygen (Praxair)

Filter unit, 0.2 µm nylon (Nalgene® 90 mm Media-Plus, Nalge Nunc International, P/N 164-0020 or equivalent nylon filter)

Vacuum pump (Gast Manufacturing Corp., P/N DOA-P104-AA or equivalent; for degassing eluents)
 1.5 mL glass injection vials with caps (Vial Kit, Dionex 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, 18 MΩ-cm resistance or higher
 D(+)-Glucosamine (Sigma-Aldrich; P/N G4875)
 Sucrose (Thermo 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 (Mallinckrodt Baker; P/N M55605)
 Mannitol (Sigma-Aldrich; P/N M9546)
 Glycerol (EMD Chemicals; formerly EM Science; P/N GX0190-6)
 Propylene glycol (1,2-propanediol; Sigma-Aldrich; P/N P6209)
 L-Ascorbic acid (Thermo Fisher Scientific; P/N A6125)
 D-Mannosamine hydrochloride (Sigma-Aldrich; P/N M4670)

SAMPLES

Samples of GlcN- and CS-containing tablets, capsules, beverages, and powders were purchased from a retail grocery or a drugstore. Table 1 lists the expected amount per dosage or serving size, source of GlcN and CS, the salt form of GlcN in each sample, other ingredients listed on the label, and the amount used to prepare the sample. For accuracy studies, a supplement containing CS but no GlcN served as the sample matrix.

CONDITIONS

Column: CarboPac PA20 Analytical, 3 × 150 mm (P/N 060142)
 Eluent: 20 mM KOH, isocratic, 7.5 or 16 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 (P/N 060139, package of 6; P/N 060216, package of 24)
 Background: 19–25 nC
 Typical Noise (Peak to Peak): ~60 pC
 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 ICS-3000, but not used in older Dionex systems.

Reference electrode in Ag mode (Ag/AgCl reference). For instrument operational considerations, see Dionex Application Note 197.¹

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 and no biological contamination. Bottled water is not recommended. 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 update, a manually prepared NaOH eluent can be used (prepare 100 or 200 mM NaOH and allow the pump to proportion the 20 mM eluent). Follow the instructions in Dionex Technical Note 71² for proper preparation of the hydroxide eluent.

Stock Standards

Prepare concentrate solutions of GlcN (typically 589.1 mM, 105.5 mg/mL) and other ingredients in the dietary supplements by accurately weighing standards into tared plastic vials, adding DI water, and then weighing the resulting solution. Prepare stock standard solutions to approximately 1.0 mM (179 μ g/mL). Store concentrate and stock standards at -15 °C until further dilutions are made. Dilute stock solutions with DI water to yield the desired working mixture concentrations (concentrations used in this update ranged from 2.1–38 μ M, 0.38–6.8 μ g/mL). For this update, all concentrates, working standards, and dilutions were made gravimetrically to ensure high accuracy, and concentrations were reported as GlcN free base.

SAMPLE PREPARATION

Place solid samples in a 1.0 L volumetric flask and add approximately 500 mL of DI water to the flask. Place the flask into an ultrasonic bath until the sample is fully dispersed and then bring to volume with DI water. Pour liquid dietary supplements into a 1.0 L volumetric flask, carefully degas under vacuum, and dilute to volume with DI water. Place 1 mL aliquots in 1.5 mL plastic microcentrifuge vials with detachable screw caps and centrifuge at 16,000 X g in a microcentrifuge for 20 min.

Prepare stock sample solutions by diluting the supernatant gravimetrically to 1.00 mM (179 μ g/mL) GlcN free base (based on product label information). Further dilute the stock sample solutions gravimetrically to produce working sample solutions with a target concentration of 10 μ M (1.8 μ g/mL) GlcN free base for injection into the HPAE-PAD system.

Prepare spiked samples by adding an accurately weighted concentrated GlcN standard solution to either DI water or uncentrifuged, GlcN-free Supplement G suspension. Centrifuge the spiked Supplement G suspension and follow the procedure described above, using the same dilution factor for all spiked solutions. Prepare the GlcN-spiked samples to have between 50% and 150% of the 10 μ M (1.8 μ g/mL) GlcN targeted in the Supplement A-F solutions (e.g., 4.89 μ M GlcN was produced in Supplement G for the 50% of target spiked sample).

Calculations

Measured molar concentrations of GlcN and of the other putatively identified ingredients were converted to the masses of these compounds in the original sample (one tablet, capsule, packet, 250 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 Working Sample solution concentrations (e.g., if a 5-fold dilution was needed to produce the Stock Sample solution and a 100-fold dilution was needed to produce the Working Sample solution, the DF = 500). The CF represents the factor that converts concentrations found for GlcN and other putatively identified ingredients from molar units to mass of the analyte in the original sample. As Supplement F contains GlcN as its sulfate potassium chloride salt, CF is 303 (half the FW of 2GlcN·H₂SO₄·2KCl). Other supplements contain GlcN as its chloride salt; their CF is 216 (the FW of GlcN·HCl). For other substances, CF is the compound's MW. To convert the measured GlcN free base concentration (expressed as μ M) 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 352-fold diluted sample of Supplement A is determined to be 10.0 μM , the amount of GlcN·HCl (CF = 216) in the tablet dissolved in 1.0 L water is:

$$\frac{\text{mg GlcN}\cdot\text{HCl}}{\text{tablet}} = \frac{10 \mu\text{mol}}{\text{L}} \times 352 \times 216 \frac{\mu\text{g GlcN}\cdot\text{HCl}}{(\mu\text{mol GlcN free base})} \times 1.0 \frac{\text{L}}{\text{tablet}} \times 0.001 \frac{\text{mg}}{\mu\text{g}} = 760.3 \frac{\text{mg}}{\text{tablet}}$$

RESULTS AND DISCUSSION

Separation

Figures 1A–C show chromatograms for the six GlcN- and CS-containing dietary supplements dissolved and diluted to the target 10 μM (1.8 $\mu\text{g}/\text{mL}$) GlcN concentration and one CS dietary supplement not containing GlcN, dissolved and diluted 201-fold. The CarboPac PA20, combined with PAD, yielded simple chromatograms for most of the supplements tested. The

four GlcN-containing dietary supplements in tablet or capsule form showed only low amounts of other electroactive compounds eluting from the PA20 column. Trace amounts of glucose in Supplement A and ascorbic acid in Supplement D were detected. Both of these ingredients were listed on the label of their respective supplements (Table 1). Trace amounts of *myo*-inositol were found in Supplement C. In Supplement E (a liquid), glucose, fructose, and *myo*-inositol were sufficiently separated from GlcN, as were the fructose, mannitol, and ascorbic acid in Supplement F. Even though ascorbate readily decomposes in base to yield electrochemically inactive oxalate,³ ascorbic acid in Supplements E and F and in the standard as well as manganese ascorbate in Supplement D produced peaks at the same retention time (peak 15). Trace amounts of other, unidentified, ingredients can be seen in Figures 1B–C. Supplement G produced a chromatogram yielding no electroactive components.

Chromatographic run times were extended to 180 min to identify possible late eluting components. Supplements E and F produced several peaks at longer retention times (data not shown). All of these peaks

Table 1. Description of Chondroitin Sulfate-Containing Samples

Sample (Dosage or Serving Size)	mg per Serving		Size Used for Analysis	GlcN Salt Form	Source		Other Ingredients
	GlcN	CS			GlcN	CS	
Supplement A (two tablets)	1500	1200	1 tablet	HCl	Shellfish	Bovine, porcine, avian	Croscarmellose sodium, DI water, povidone, crospovidone, silicon dioxide, magnesium stearate, hypromellose, sodium carboxymethylcellulose, dextrin, dextrose, soy lecithin, sodium citrate
Supplement B (two caplets)	1500	300	1 caplet	HCl	Shellfish	Bovine, porcine, avian	MSM + hyaluronic acid, cellulose, croscarmellose sodium, hypromellose, polyvinyl alcohol, crospovidone, magnesium stearate, silicon dioxide, stearic acid, titanium dioxide, polyethylene glycol, talc, artificial vanilla cream flavor
Supplement C (four capsules)	1500	1200	1 capsule	HCl	Shellfish	Bovine cartilage	Gelatin, silica, magnesium stearate
Supplement D (one capsule)	500	400	1 capsule	HCl	Shellfish	Not disclosed	Manganese ascorbate, gelatin, water, magnesium stearate, titanium dioxide, FD&C Red #3, FD&C Blue #1
Supplement E (one can*)	1200	250	1 can	HCl	Non- shellfish	Not disclosed	Ascorbic acid, purified water, orange juice concentrate, white grape juice concentrate, citric acid, mandarin juice concentrate, natural flavorings, beta-carotene, apo-carotenal
Supplement F (one packet**)	500	400	1 packet	H ₂ SO ₄ ·2KCl	Shellfish	Not disclosed	Ascorbic acid, fructose, citric acid, natural flavors, tapioca maltodextrin, malic acid, silica, beta-carotene, annatto color, glycine, aspartic acid, tartaric acid, cysteine hydrochloride
Supplement G (two capsules)	0	1200	1 capsule	Not applicable	Not applicable	Bovine cartilage	Rice flour, gelatin, magnesium stearate

*One can contains 250 mL of liquid

**One packet contains 8.7 g of powder

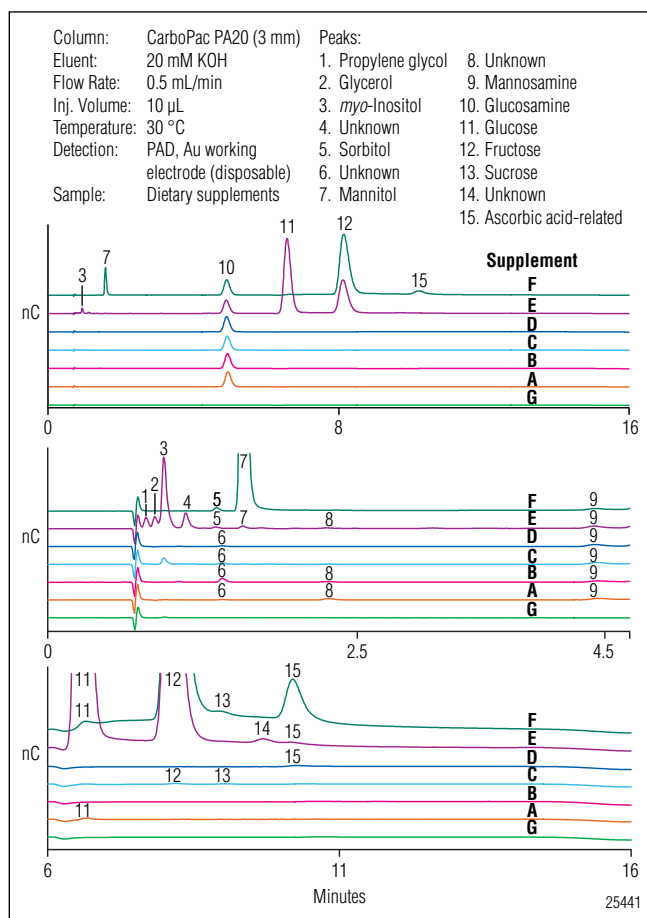


Figure 1. HPAE-PAD analysis of GlcN- and CS-containing dietary supplements. Seven dietary supplement samples diluted to approximately 10 μ M (1.8 μ g/mL) GlcN, 10 μ L injection. Top) Full chromatogram, Middle) expanded early RT region of the chromatogram, Bottom) expanded later RT region of the chromatogram. (See Table 3 for dilution factors.)

(20.2 and 95.7 min in Supplement E and 38.9, 46.7, and 96.2 min in Supplement F) have areas < 1% of the GlcN peak. Table 1 lists the labeled ingredients in the seven dietary supplements evaluated in this update. The combined use of HPAE and the specificity of PAD yielded uncomplicated chromatograms for determination of GlcN.

Detection

Linearity

While the full linear range for GlcN quantification covered more than three orders of magnitude, 0.30–340 μ M, 0.06–61 μ g/mL (10 μ L injection),¹ for routine GlcN determination, we recommend a dietary supplement dilution scheme that targets a 10 μ M (1.8 μ g/mL) GlcN concentration. A calibration covering a GlcN concentration range of 2.1–38 μ M (0.38–6.8 μ g/mL) yielded a linear relationship between GlcN peak area and [GlcN], having an r^2 value of > 0.9999.

Table 2. Precision of Glucosamine Retention Time, Peak Area, Peak Efficiency, and Peak Asymmetry for Supplement A Injected Consecutively Over 7 Days

	Day							All 7 Days	% Change over 7 Days
	1	2	3	4	5	6	7		
Retention Time (min)									
Mean	4.666	4.620	4.604	4.591	4.583	4.580	4.579	4.604	-1.86
SD	0.020	0.007	0.005	0.006	0.004	0.004	0.004	0.031	
N	152	143	147	147	147	147	147	1030	
RSD	0.43*	0.16*	0.11*	0.14*	0.08*	0.09*	0.09*	0.67*	
Peak Area (nC*min)									
Mean	5.745	5.763	5.766	5.762	5.735	5.733	5.722	5.747	-0.40
SD	0.040	0.031	0.035	0.037	0.037	0.032	0.046	0.040	
N	152	143	147	147	147	147	147	1030	
RSD	0.70	0.54	0.61	0.65	0.64	0.56	0.80	0.70	
Theoretical Plates									
Mean	5464	5461	5452	5463	5453	5446	5469	5458	0.09
SD	26	26	16	32	19	30	48	31	
N	152	143	147	147	147	147	147	1030	
RSD	0.47	0.47	0.30	0.59	0.34	0.55	0.88	0.56	
Asymmetry									
Mean	1.123	1.124	1.128	1.125	1.125	1.123	1.125	1.125	0.18
SD	0.019	0.020	0.019	0.019	0.019	0.019	0.019	0.019	
N	152	143	147	147	147	147	147	1030	
RSD	1.68	1.75	1.67	1.69	1.69	1.68	1.73	1.70	

N = number of injections

*Because the retention time is trending down (though with a small slope) over 7 days, RSDs are not a meaningful measurement for this value.

Precision

Due to the concern about CS compromising GlcN determinations, 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). Supplement A was chosen for this study because it had a high amount of CS and several cellulosic compounds, and therefore was considered among the more challenging matrices of the products investigated in this update. Run time was 7.5 min (an injection made every 8.6 min). Table 2 shows the result of this study on a daily basis and the entire 7 day period. The column was washed for 2 h with 100 mM KOH prior to this study, but no 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 1.9% measured decrease after 7 days of continuous operation. Non-eluting sample ingredients and carbonate from the eluent can build up on the stationary phase and consume column capacity, eventually causing a decrease in GlcN retention time. Because an EG essentially eliminates carbonate contamination; only sample ingredients are likely to cause loss of column capacity. Despite 1030 injections of a challenging sample without any column washes during the 7 day period, the small loss of retention time and high reproducibility over the 7 days indicates the method is rugged for the analysis of these samples.

Application

Figure 1 presents chromatograms for the six GlcN- and CS-containing dietary supplements studied. Table 3 shows the measured amounts of GlcN in the seven dietary supplements analyzed for this update. The determined amounts of GlcN for all supplement samples containing GlcN were at or above the stated label amounts, ranging from 98%–119% of the GlcN label value. The CS formulation without GlcN (Supplement G) did not contain measurable GlcN.

Supplements E and F showed significant amounts of PAD-responsive related substances using this method (Figure 1). The peaks for these related substances were putatively identified by matching their retention times with those of appropriate standards. Single concentration standards were used to estimate the amount of these compounds in the supplements. Table 4 shows the

Sample	Dilution Factor (DF)	Measured Amount, mg/unit ^a	Expected Amount, mg/unit ^b	% 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 ^c	0	—

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

^bExpected amount derived from Supplement Facts on label

^cBased on 0.015 μ M detection limit (3 \times baseline noise) and CF for HCl salt

amounts of these related substances, expressed as mg/unit. Unknown peaks (Figure 1B and 1C), were present at less than 1% peak area relative to GlcN, except in Supplement E where peaks 4 and 14 show relative peak areas of 2% and 4%, respectively. This method can also be used to determine other carbohydrates or glycols

Table 4. Determination of Other Substances Detected in Dietary Supplements Containing Chondroitin Sulfate

Sample	Analyte ^a	Calculated Amount/Unit (mg/unit) ^{b,c} \pm SD	% Relative to Measured [GlcN] \pm SD
Supplement A	Mannosamine	0.74 \pm 0.06	0.08 \pm 0.007
	Glucose	9.0 \pm 0.7	1.03 \pm 0.08
Supplement B	Mannosamine	0.60 \pm 0.03	0.07 \pm 0.003
Supplement C	<i>myo</i> -Inositol	3.07 \pm 0.04	0.84 \pm 0.01
	Mannosamine	0.43 \pm 0.01	0.12 \pm 0.004
	Fructose	2 \pm 2	0.5 \pm 0.5
Supplement D	Sucrose	3.8 \pm 0.4	1.0 \pm 0.1
	Mannosamine	0.65 \pm 0.02	0.11 \pm 0.004
Supplement D	Ascorbic acid ^d	24 \pm 1	4.2 \pm 0.2
	Supplement E	Propylene glycol	13.9 \pm 0.2
Glycerol		34 \pm 1	2.64 \pm 0.08
<i>myo</i> -Inositol		142 \pm 0.2	11.0 \pm 0.02
Sorbitol		3.9 \pm 0.1	0.30 \pm 0.01
Mannitol		17.9 \pm 0.2	1.39 \pm 0.02
Mannosamine		1.88 \pm 0.05	0.15 \pm 0.004
Glucose		10330 \pm 21	800 \pm 2
Fructose		11700 \pm 21	907 \pm 2
Ascorbic acid ^d		96 \pm 3	1.70 \pm 0.05
Supplement F	Sorbitol	2.53 \pm 0.03	0.44 \pm 0.005
	Mannitol	860 \pm 3	151 \pm 0.5
	Mannosamine	0.34 \pm 0.01	0.06 \pm 0.001
	Glucose	6 \pm 4	1.1 \pm 0.8
	Fructose	5980 \pm 32	1048 \pm 6
	Sucrose	4.9 \pm 0.7	0.86 \pm 0.13
Supplement F	Ascorbic acid ^d	650 \pm 20	114 \pm 3
	Supplement G	— ^e	—

n = 5 injections per sample

^aPutative identification based on retention time matches with standards

^bA unit is 1 tablet, 1 capsule, 1 250 mL can of liquid, or 1 8.7 g packet

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

^dSignal due to ascorbic acid-related compound

^eNo quantifiable peaks

present in dietary supplements. Higher [GlcN] solutions can be injected for determination of trace mono- and disaccharide concentrations, if desired, for evaluation of GlcN quality.

Accuracy

GlcN recovery from DI water and a diluted aqueous spiked extract of GlcN-free Supplement G were evaluated in this application update. A 100% spike is considered 10 μM (1.79 $\mu\text{g/mL}$) GlcN. Percent recovery (mean \pm SD) from DI water at 4.89 μM (0.875 $\mu\text{g/mL}$), 9.92 μM (1.78 $\mu\text{g/mL}$), and 14.6 μM (2.61 $\mu\text{g/mL}$) was 98.3 \pm 0.5, 99.8 \pm 0.5, and 99.6 \pm 0.5 %, respectively. Recoveries from Supplement G supernatant spiked at 6.08 μM (1.09 $\mu\text{g/mL}$), 8.59 μM (1.54 $\mu\text{g/mL}$), and 13.5 μM (2.42 $\mu\text{g/mL}$) were 102.8 \pm 0.5, 106.2 \pm 0.4, and 108.8 \pm 0.5 %, respectively, indicating that the method was accurate.

Glucosamine Stability

A GlcN-related compound with a retention time of approximately 4.5 min forms at room temperature (Figure 2). This peak, which coelutes with 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 extended periods of time. When exposed to elevated temperature, this peak significantly increased in amount together with a decrease in GlcN amount. Degradation of GlcN in aqueous solution has been reported.^{4,5} It seems feasible that this method could be used to measure GlcN stability using the peak at 4.5 min as a stability indicator.

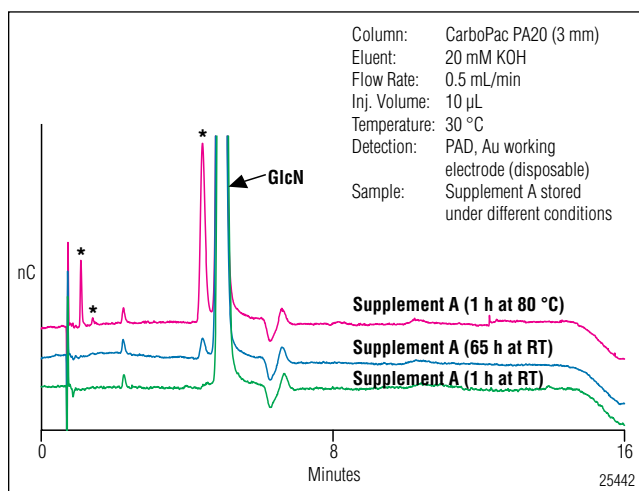


Figure 2. Additional peaks in GlcN-containing Supplement A. (* designates unknown peaks that increase with exposure to elevated temperature.)

CONCLUSION

HPLC-PAD with eluent generation accurately determines glucosamine in dietary supplements regardless of the presence of CS. Sample preparation consists of simply dissolving samples in DI water, centrifuging them, and diluting the resulting solution to a target concentration within the linear range. The high capacity of the CarboPac PA20 is not exceeded by CS levels present in the dietary supplements evaluated, and the use of eluent generation enables the isocratic analysis of more than 100 samples per day for 7 days. All the analyst has to do is add water and samples to the system.

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- 4) Jun, M.; Shao, Y.; Ho, C.T.; Koetter, U.; Lech, S. Structural Identification of Nonvolatile Dimerization of Glucosamine by Gas Chromatography-Mass Spectrometry, Liquid Chromatography-Mass Spectrometry, and Nuclear Magnetic Resonance Analysis. *J. Agric. Food Chem.* **2003**, *51*, 6340-6346.
- 5) Shu, C.K. Degradation Products Formed from Glucosamine in Water. *J. Agric. Food Chem.* **1998**, *46*, 1129-1131.

SUPPLIERS

EMD Chemicals Inc., 480 South Democrat Road, Gibbstown, NJ 08027, U.S.A. Tel: 1-800-222-0342 <http://www.emdchemicals.com>.

Gast Manufacturing Corp., 2550 Meadowbrook Road, Benton Harbor, MI 49022, U.S.A. Tel: 1-269-926-6171, <http://www.gastmfg.com>.

Mallinckrodt Baker, 222 Red School Lane, Phillipsburg NJ 08865, U.S.A. Tel: 1-800-582-2537 <http://www.mallbaker.com>.

Nalge Nunc International, 75 Panorama Creek Drive, Rochester, NY 14625, U.S.A. Tel: 1-800-625-4327, <http://www.nalgenunc.com>.

Praxair, 39 Old Ridgebury Road, Danbury, CT 06810-5113, U.S.A. Tel: 877-772-9247, <http://www.praxair.com>.

Sarstedt AG & Co., Rommelsdorfer Straße, Postfach 1220, 51582 Nümbrecht, Germany Tel.: +49-2293-305-0, <http://www.sarstedt.com>.

Sigma-Aldrich Chemical Company, P.O. Box 14508, St. Louis, MO 63178, U.S.A., Tel: 1-800-325-3010, www.sigma-sial.com.

Thermo Fisher Scientific, 4500 Turnberry Drive, Hanover Park, IL 60133, U.S.A. Tel: 1-800-766-7000 www.fishersci.com.

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