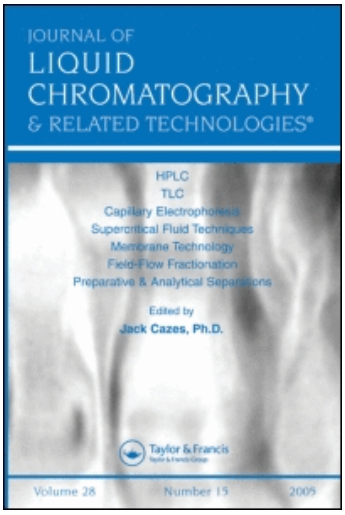


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# A SIMPLE AND VALIDATED LC METHOD FOR THE SIMULTANEOUS ANALYSIS OF GLUCOSAMINE AND CHONDROITIN SULFATE EQUIVALENT IN DIETARY PRODUCTS

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□ *A simple and reliable reversed-phase liquid chromatographic (RP-LC) method was developed and validated to determine simultaneously a glucosamine and chondroitin sulfate equivalent in dietary products. The procedure is based upon the reaction of o-phthaldialdehyde with glucosamine and galactosamine coming from the galactosaminoglycan hydrolysis. The hydrolysis reaction was carried out with hydrochloric acid (7.5 N) at 80°C for 8 hr, whereas, the pre-column derivatization reaction was carried out in alkaline media for 1 min at ambient temperature. The chromatographic separations were performed on a Phenomenex Synergi 4 μ fusion-RP 80 A (250 mm × 3.0 mm i.d.) using a mobile phase consisting of a mixture of sodium acetate buffer (pH 5.9; 0.05 M) and methanol (85:15, v/v). UV-DAD detection at λ = 340 nm was used. Linear responses were observed and the limit of quantitation for both aminosaccharides was about 60 pmol. The intra-day precision (RSD) was ≤1.8% and there was no significant difference between intra- and inter-day data. Recovery studies showed good results (99.3–101.0%) with RSD ranging from 1.1 to 2.1%.*

**Keywords** chondroitin sulphate equivalent, galactosaminoglycans dietary supplements, glucosamine, method validation, o-phthaldialdehyde OPA, reversed-phase liquid chromatography RP-LC

## INTRODUCTION

Glucosamine (GlcN) is a natural substance found in chitin, mucoproteins, and mucopolysaccharides. It is involved in the manufacture of glycosaminoglycan which forms cartilage tissue in the body. It is also present in tendons and ligaments, but the ability of the body to synthesize GlcN declines with age. Chondroitin sulfate (CS) belongs to the large family of

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glycosaminoglycans (GAGs), linear polysaccharides composed of alternating hexuronic acid, or hexose and hexosamine units that play important roles in all living organisms. Depending on the nature of hexosamine, the GAGs can be classified as glucosaminoglycans (heparin, heparan sulfate, and hyaluronic acid) and galactosaminoglycans (CS and dermatan sulfate, DS). Repeating disaccharide units of glucuronic acid (GlcA) and differently sulfated N-acetylgalactosamine (GalNAc) form CS, which is a constituent of most cartilaginous tissues. CS is used orally as sodium salt in reactive arthritis, such as gonococcal arthritis, and it is often given in association with GlcN for its supposed chondroprotective action in bone, joint, and connective tissue disorders.[1] CS is available from various sources such as bovine and shark which, in general, differ depending on the position of the sulfate group. The predominant form for CS from bovine sources is sulfated in 4-position over the hydroxylic group, whereas the 6-position is the form predominant in shark source.[2] Although the use of CS for the symptomatic treatment of osteoarthritis has become very popular, the oral bioavailability and efficacy of CS remains controversial. However, the majority of physiological benefits subsequent to administration of CS appear to be a direct result of increased availability of the monosaccharide/disaccharide residues of CS produced by the action of enzymes found in the intestine.[3]



Well-known procedures concern the direct analysis of single GlcN or CS by LC with refractive index (RI)[4] or low-wavelength ultraviolet (UV) detection,[2,5,6] by colorimetric and dye binding assays[3,7,8] and cetylpyridinium chloride (CPC) titration.[2,6,9] These methods are often laborious, have poor selectivity and are insensitive when the available sample quantity is low. In particular, before CS analysis, many methods involved enzyme digestion of the polymer into the individual disaccharide monomers that are quantified by LC or CE.[3,10–18] Despite the high selectivity of this approach and due to the sequence-specific interactions of the enzymes with the glycosaminoglycan chains, the use of enzymatic hydrolysis involves some drawbacks such as the batch-to-batch variability and high costs of enzymes and standards. Conventional acid hydrolysis is a useful alternative. It gives the monosaccharide components of glycosaminoglycans that can potentially be useful to estimate both the type and relative amount of the parent polysaccharide.[19–21] Some methodologies for oligosaccharide analysis by direct mass spectrometry (MS) or LC-MS[22–24] were developed, but they require expensive instrumentation. On the other hand, several methods provided the derivatization in GC and GC-MS to obtain volatile saccharidic derivatives[25–28] or in LC, to obtain saccharide derivatives with chromophore or fluorophore groups increasing the detection. Different compounds reacting with aminic or carboxylic functional group were proposed for the analysis of mono- or oligosaccharides deriving from

glicoproteins and proteoglycans. 2-Aminopyridine (2-AP),[11,14,29–31] 2-aminoacridone (AMAC),[29–31] antranilic acid (ABA),[29,30] 8-aminonaphtalene-1,3,6-trisulfonic acid (ANTS),[30,31] 4-aminobenzoic acid,[31,32] 7-aminonaphthalene-1,3-disulfonic acid (ANDSA),[33] 1-phenyl-3-methyl-5-pyrazolone (PMP),[29,30,34] 9-fluorenylmethyl chloroformate (FMOC),[35] *o*-phthaldialdehyde (OPA),[6,21,36–39] phenylisocyanate,[40] phenylisothiocyanate (PITC),[41,42] and 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)[43] were the most used derivatization reagents, but some of them can give a variety of drawbacks. For example, PITC derivatization is long and involves several stages of drying under vacuum before the injection into chromatographic system. On the other hand, with FMOC, the excess of the derivatization reagent can give rise to problems if it is not removed by extraction with pentane or it is not derivatized with an amine whose derivative does not interfere.

Despite a variety of new oral preparations recently brought onto the market to better our knowledge, among several methods reported in literature, none addresses the determination of GlcN combined with CS in formulations. The first aim of the present study was the development and validation of a LC method for the simultaneous analysis of GlcN and CS or molecules derived from its potential modifications (CS equivalent) in dietary products. The proposed procedure is based upon the pre-column derivatization reaction of OPA (I) with GlcN and galactosamine (GalN) coming from galactosaminoglycan acidic hydrolysis (Figure 1). OPA is a fluorogenic reagent, which is prevalently used in post-column derivatization because of the adduct instability, but it has also been applied as pre-column labeling with reliable results.[6,21,37–39,44] With respect to other reagents, OPA has the advantage to react rapidly (1 min) in mild conditions utilizing a primary amino group without removing the reagent excess prior to the analysis. The pre-column labeling allows decreasing the polarity of



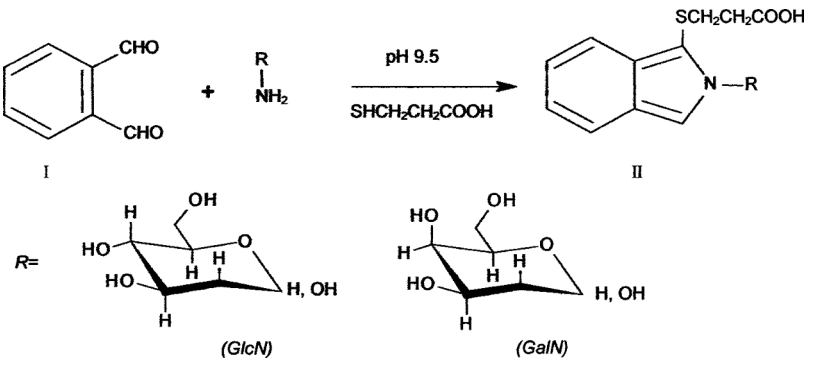


**FIGURE 1** Scheme of derivatization reaction of GlcN and GalN with OPA (I).

the compounds so they can be retained on the reversed stationary phase. In addition, the combination of the pre-column derivatization with UV detection makes the method easily applicable in common analytical laboratories. A subsequent objective of the research was the achievement of reproducible hydrolysis conditions that are essential for routine analysis of dietary supplements.

## EXPERIMENTAL

### Materials

Glucosamine (GlcN) hydrochloride, galactosamine (GalN) hydrochloride, chondroitin sulfate (CS) sodium salt (≥99%) obtained from bovine cartilagine and methanol for chromatography (HPLC grade) were purchased from Sigma-Aldrich (Milan, Italy). CS ex shark (≥98%), coming from two different batches, was purchased from K.W. Pfannenschmidt GmbH (Hamburg, Germany). The effervescent table formulation was provided from E-Pharma Trento S.p.A (Ravina, Italy), whereas the formulations (capsules) are commercially available. The *o*-Phthaldialdehyde (OPA), hydrochloric acid, and salicylic acid (internal standard, IS) were obtained from Fluka (Milan, Italy). The pH indicator bromothymol blue was from Carlo Erba (Milan, Italy). All the other chemicals were of analytical reagent grade. Deionized, double distilled water was used for all solution and mobile phase preparation.



### Preparation of Solutions

The reagent OPA solution (20 mg/mL) was prepared solubilizing the compound in 250 μL of methanol and adding 10 μL of 3-mercaptopropionic acid and 2.24 mL of sodium borate buffer (pH 9.5; 0.2 M). The mixture was stored in the dark at 4°C and was allowed to stand for 24 hr before use. The standard solutions of GlcN, GalN, and CS were prepared in hydrochloric acid 7.5 N (concentration under calibration graphs). The internal standard solution (IS) (7.5 mg/mL) was prepared in water. Borate buffer (pH 9.5, 0.2 M) solution was prepared according to USP method.[6] The pH indicator bromothymol blue solution was prepared as previously described.[45]

### Apparatus

The liquid chromatograph consisted of a PU-1580 pump equipped with the LG-1580–02 ternary gradient unit and a diode-array detector (DAD)

model MD-910 (Jasco Corporation, Tokyo, Japan). The data were collected on a PC equipped with the integration program Borwin-PDA. Manual injections were carried out using a Rheodyne model 7125 injector with 20 µL sample loop. A column inlet filter (0.5 µm × 3 mm i.d.) model 7335 Rheodyne was used. The solvents were degassed on line with a degasser model DG 2080–53 (Jasco, Tokyo, Japan). Sonarex Super RK 102 (35 KMZ) Bandelin (Berlin, Germany) equipment with thermostatically controlled heating (30–80°C) was used for ultrasonication. The hydrolysis reaction was carried out on a heating and stirring apparatus Reacti-Therm™ (Pierce, Rockford, IL, USA).

### Hydrolysis Procedure

A 2 mL aliquot of CS standard chloridric solution (or sample chloridric solution) was hydrolyzed at 80°C for 8 hr in a micro reaction vessel (3 mL) under magnetic stirring. The mixture was left to stand all night at ambient temperature and then was transferred quantitatively in 10 mL volumetric flask. The resulting solution was neutralized with 10 N sodium hydroxide, which used a bromothymol blue solution as the pH indicator, and was diluted to volume with water after the addition of a 4 mL aliquot of IS solution. A 200 µL aliquot of the solution was subjected to the derivatization procedure.



### Derivatization Procedure

A 200 µL aliquot of standard solution of GlcN and GalN (or sample solution) coming from hydrolysis procedure was treated with 400 µL of borate buffer (pH 9.5; 0.2 M) solution and 100 µL of the reagent solution was added. The reaction was carried out at ambient temperature for 1 min in a micro centrifuge tube (2 mL). Then, to reaction mixture 500 µL of mobile phase were added and 20 µL aliquots of the resulting clear solution were injected into the chromatograph.

### Chromatographic Conditions

The routine LC separations were performed at 33 ± 2°C on a Phenomenex Synergi 4 µm fusion-RP 80 A (150 × 3.00 mm id) stainless steel column with a guard column packed with the same stationary phase. Isocratic elution conditions were used with a mobile phase consisting of a mixture of sodium acetate buffer (pH 5.9; 0.05 M) and methanol (85:15, v/v) at a flow-rate of 0.4 mL/min. UV-diode array detection, setting the wavelength at 340 nm, was employed.

## Method Validation

### *Specificity*

GlcN, GalN (1.17 μmol/mL, 0.29 μmol/mL, respectively) standard solutions, blank (water), placebo (a mixture of excipients and other ingredients, except the analytes) after hydrolysis, and sample solutions, before and after hydrolysis, were prepared according to the described procedure for standard solution and sample. All the solutions were subjected to the reported derivatization reaction.

### *Linearity*

GlcN and GalN standard solutions and placebo solution spiked of both GlcN and CS were prepared in hydrochloric acid (7.5 N). The concentration ranges are reported in Table 1. A 200 μL aliquot of standard solution or fortified placebo solution, coming from hydrolysis procedure, were subjected to the derivatization reaction. Duplicate injections for each solution were made and the peak-area ratio of analyte to IS was plotted against the analyte concentration to obtain the calibration graphs.



### *Precision*

20 effervescent tablets were finely ground and 6 aliquots for each day corresponding to about 120 mg of powder were accurately weighted. The solutions were prepared according to the method (see *Analysis of dietary supplements*) and derivatized after hydrolysis.

### *Accuracy*

The accuracy of the method was determined as mean recovery on 9 solutions. The solutions contained known amounts of CS and GlcN corresponding to approximately 75, 100, and 125% of the claimed content in presence of effervescent tablet placebo. Each solution was subjected to hydrolysis and derivatization procedures as described and injected twice. The recovery was calculated with respect to the standard solutions.

## Analysis of Dietary Supplements

### *Sample Preparation*

*Effervescent Tablets.* 20 tablets were finely ground and an amount of powder equivalent to about 12 mg of CS and 15 mg of GlcN was introduced in a 25 mL volumetric flask filling up to volume with hydrochloric acid (7.5 N) and subjected to ultrasonication for 10 min to favor the complete dissolution.

*Capsules*. The contents of 20 capsules were mixed and an amount equivalent to about 17 and 10 mg of CS for type I and II, respectively, and about 18 and 25 mg of GlcN, for type I and II, respectively, was dissolved in a 25 mL volumetric flask with hydrochloric acid (7.5 N) under ultrasonication for 10 min to favor the complete dissolution.

### *Assay Procedure*

A 2 mL aliquot of each final solution was subjected to hydrolysis procedure, whereas an equivalent aliquot was directly diluted to 10 mL with water. Then, 200 μL aliquots of the same sample (i.e., before and after hydrolysis) were subjected to the described derivatization reaction and the analyte content in each sample was determined by comparison with an appropriate standard solution.

## RESULTS AND DISCUSSION



To determine GlcN and GalN simultaneously, the samples were subjected to the hydrolysis procedure. Then, the obtained amino-monosaccharides were submitted to pre-column derivatization with OPA reagent forming adducts that showed a good stability for about 2 hr.

### Hydrolysis of Galactosaminoglycans to GalN

The hydrolysis process involves the following acid-catalyzed steps: the hydrolysis of glycosidic linkage (depolimerization) and the N-acetyl linkages (de-N-acetylation) and the desulfation[19–21,46] that finally yielded the formation of the amino-monosaccharide GalN.

On the basis of the literature data,[19–21] the galactosaminoglycan hydrolysis procedure was carried out using hydrochloric acid that reflect the acidic conditions of the stomach. To assure the reliability of the CS hydrolysis procedure, both the effect of the acid concentration and the influence of the temperature on the hydrolysis reaction were evaluated. The better conditions were found at 80°C using hydrochloric acid (7.5 N) for 8 hr and longer hydrolysis time (within 16 hr) did not affect the hydrolysis percentage (Figure 2). Lower temperatures brought poor results, and higher temperatures did not show to be advantageous. In the described conditions, the percentages of obtained GalN were in accordance with the data of the literature.[19,20] In particular, the found GalN percentages of three analyzed CS samples from different provenience corresponded to 26.71 (from bovine cartilage; RSD = 2.22%; n = 6), 26.70 (from shark cartilage, batch *a*; RSD = 1.50%, n = 6) and 26.64 (from shark cartilage, batch *b*; RSD = 1.94%; n = 6). The GalN percentage obtained

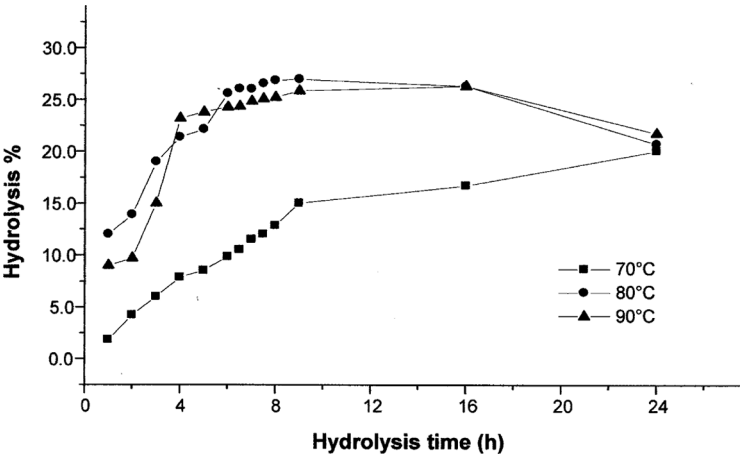


**FIGURE 2** Effect of temperature on the CS hydrolysis procedure. Hydrolysis %: GalN/CS (p/p) percentage.



from the hydrolysis process was used as a conversion factor for the CS equivalent determination in the samples. Both GlcN and GalN were stable in the developed hydrolysis conditions by comparison with hydrochloric (7.5 N) and aqueous standard solutions which were not submitted to the hydrolysis procedure. On the contrary, at higher hydrolysis temperature (100°C) the percentage of GlcN and GalN decreased about 50% after 1 hr; whereas, using hydrochloric acid (4 N, 100°C), GlcN and GalN response was already reduced by 10% after 4 hr.

## Chromatography

To obtain better separation conditions, on the basis of partly hydrophilic structure of the derivatized amino-monosaccharide, columns having selectivity to polar compounds, such as Phenomenex $C_{18}$ Synergi 4 μm fusion-RP, and Supelco Discovery RP-amide $C_{16}$, were considered. Synergi fusion-RP used a polar embedded and hydrophobic ligand to achieve improved selectivity. The $C_{18}$ ligand gives good hydrophobic retention and selectivity, while the polar embedded group provides enhanced polar retention. This dual-phase selectivity allowed balanced polar, acidic, basic and hydrophobic compound retention, and resolution. The ability to operate in an extended pH range of 1.5–10 is the direct result of an exhaustive end-capping procedure that is highly protective of the silica surface; pH stability is an indication of column ruggedness. Discovery RP-amide $C_{16}$ is a reversed-phase column with lower hydrophobicity than conventionally bonded C18 stationary phases, which provides a good retention and resolution for polar compounds. That column exhibits a high level of silanol

deactivation. In this stationary phase, the alkyl chains contain an amido group that electrostatically shields silanols from highly polar analytes. In addition, different mobile phases were studied examining the influence of composition and buffer pH on the amino-monosaccharide resolution. $C_{18}$ Synergi 4 μm fusion-RP was preferred for its better ability to separate the peaks in a shorter chromatographic run. On the basis of obtained data, a mobile phase consisting of acetate buffer (pH 5.9) and methanol (85:15, v/v) (Figure 3a) was chosen. Better results were found at pH 5.9 in respect to those obtained at lower (pH 5) and higher (pH 7) values in terms of peak symmetry, separation, and chromatographic run times. As it can be seen in Figure 3b, the reagent did not interfere with the analysis as there was no significant degradation products. In addition to retention time, the on-line UV-DAD spectrum was used as further information on the identification of the derivatized GlcN and GalN; an example (detail of the Figure 3), which is also explicative in regard to the wavelength choice at 340 nm, was reported.



## Method Validation

### *Specificity*

The specificity was evaluated in the effervescent tablet sample. The retention times of GlcN, GalN and IS in standard solution have been

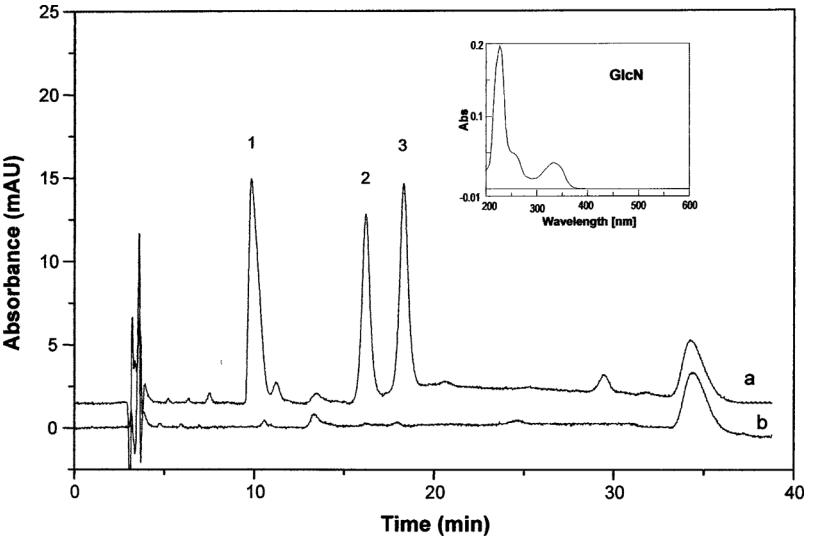


**FIGURE 3** Representative LC separation at 33 ± 2°C of: (a) equimolar (about 0.53 μmol/mL) standard mixture of GlcN and GalN derivatized with OPA; (b) solvent under derivatization conditions with OPA (blank). Peaks: 1 = salicylic acid (IS); 2 = GalN adduct; 3 = GlcN adduct. UV-DAD detection: $\lambda = 340$ nm. Detail: representative UV-DAD spectrum GlcN adduct.

compared with the placebo (after hydrolysis), sample (before and after hydrolysis), and reagent blank solutions prepared under the same conditions. No interferences with the analyte peaks due to placebo or blank have been observed (Figure 4). In particular, the formation of GalN after hydrolysis in the sample solution is confirmed by retention time (Figure 4e), GalN peak spectrum, and GalN standard addition. The comparison of the sample chromatograms before and after hydrolysis can be a useful tool to evaluate the quality of the preparation. GalN peak appearance, only after hydrolysis, excludes macroscopic adulteration such as the use of GalN monosaccharide, instead of the more expensive CS. In addition, no increase of the GlcN peak area under hydrolytic conditions can exclude the presence of other GAGs releasing GlcN, such as hyaluronic acid (HA), which can be present in variable amounts in CS raw materials and formulations[10] as impurity or in CS dietary supplements as co-ingredient for its joint lubricating properties. The method had specificity for the identification and assay of GlcN and GalN, but it was unable to distinguish between CS and DS, which are galactosaminoglicans with similar backbone structure: all of the uronic acid of CS is in the form of GlcA, while in DS is a mixture of GlcA and its C-5 epimer, iduronic acid (IdoA), in varying proportions. However, DS is commercially available in matrices (e.g., porcine intestinal mucosa) from different provenience respect to CS and it is considerably more expensive than CS; therefore, it cannot be considered a source of economic adulteration.



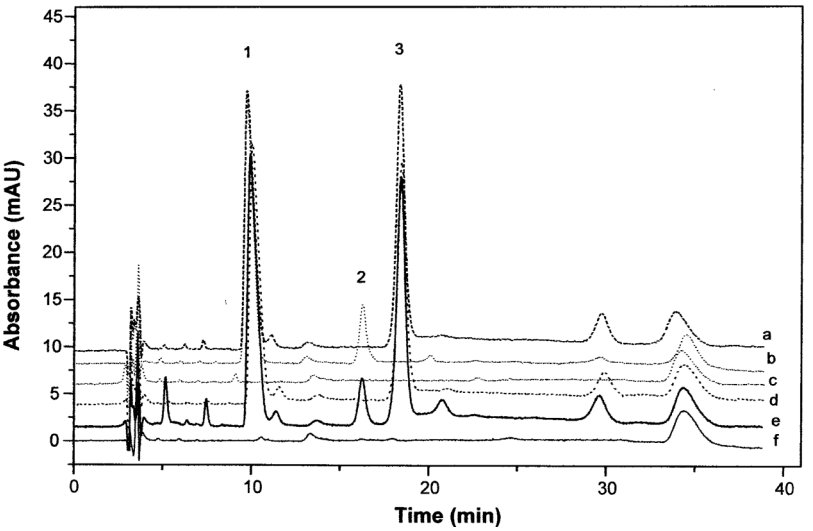


**FIGURE 4** Overlay of chromatograms: (a) GlcN standard, (b) GalN standard, (c) placebo after hydrolysis, (d) sample before hydrolysis, (e) sample after hydrolysis, and (f) reagent blank. Peaks: 1 = salicylic acid (IS); 2 = GalN adduct; and 3 = GlcN adduct. Detection as in Figure 3.

**TABLE 1** Data for Calibration Graphs ($n=5$)

| Compound | Slope[a] | Confidence Interval | y-Intercept[a] | Confidence Interval | Correlation Coefficient | Concentration Range (mg/mL) |
|---|---|---|---|---|---|---|
| GlcN[b] | 3.23 | ±0.24 | 0.01 | ±0.06 | 0.9992 | 0.02–0.48 |
| GlcN[c] | 3.37 | ±0.28 | 0.02 | ±0.07 | 0.9990 | 0.02–0.48 |
| GalN[b] | 1.91 | ±0.10 | −0.00 | ±0.08 | 0.9996 | 0.02–0.22 |
| GalN[c] | 1.82 | ±0.01 | −0.00 | ±0.01 | 0.9997 | 0.01–0.26 |

[a]According to $y=a\ x+b$, where $x$ is the analyte concentration and $y$ is the ratio of amino sugar peak-area to IS peak-area.

[b]Standard solution in HCl 7.5 N.

[c]GlcN and CS spiked in placebo solution and subjected to hydrolysis procedure. [GalN] = ([CS] × CF)/100, where CF = conversion factor (26.70).

### *Linearity*

The linearity was determined as linear regression with least-square method on standard solutions and spiked placebo solutions in the GlcN and GalN concentration range reported in Table 1. Good linearity was found for both aminosaccharides as indicated by the correlation coefficients ≥0.9990. The similar values of the standard and placebo slope and y-intercept attests that the matrix did not interfere with the GlcN and GalN determination, the analytes were not degraded during the hydrolysis procedure, the GalN percentage obtained is not affected by the galactosaminoglycan concentration submitted to hydrolysis reaction within the considered range. Figure 5 shows good linear correlation (r = 0.9997) between hydrolyzed CS and obtained GalN in the galactosaminoglycan concentration range (0.12–2.4 mg/mL) of a fortified placebo solution. In



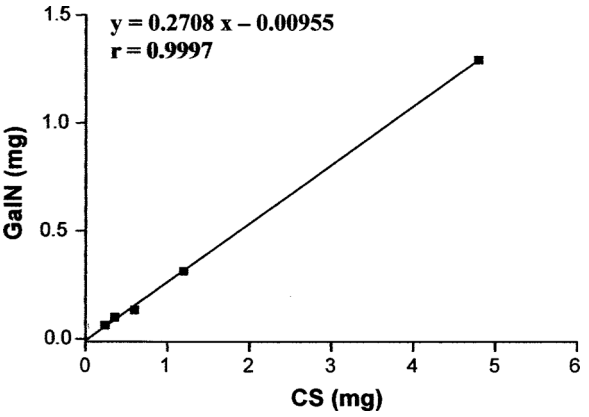


**FIGURE 5** Correlation graph between hydrolyzed CS and obtained GalN in spiked placebo solution.

particular, the slope value of about 0.27 confirms the value of hydrolysis percentage, which was used as the conversion factor.

### *Limit of Detection (LOD) and Quantitation (LOQ)*

LOD and LOQ were calculated by the signal/noise ratio of 3:1 and 10:1, respectively. The LOD and LOQ values were about 20 pmol and 60 pmol, respectively, for both aminosaccharides. The sensitivity of the method is adequate for a reliable quality control of formulations.

### *Precision*

The precision of the method, comprehensive of hydrolysis, derivatization procedures, and chromatographic separation, was expressed as repeatability and intermediate precision. The repeatability was calculated employing 6 test solutions; each one prepared starting from a homogeneous finished product sample. The intermediate precision of the method was determined on the sample with 12 solutions, prepared changing the parameters time-analyst: 6 solutions were prepared by the analyst A in day 1, while the other 6 solutions were prepared by the analyst B in day 2. The results of precision determined on the effervescent tablets were satisfactory as reported in Table 2.



### *Accuracy*

The accuracy was determined on the recovery of known amounts of analyte spiked in placebo. The samples were prepared in triplicate at three levels over a range of 75–125% of the target concentration of GlcN and CS.

**TABLE 2** Repeatability and Intermediate Precision (Effervescent Tablets)

| Compounds | Mean Corrected Area[a] (S.D.) | RSD (%) | Confidence (%)[b] | mg/tablet (S.D.) | RSD (%) | Confidence (%)[b] |
|---|---|---|---|---|---|---|
| Repeatability ($n=6$) | | | | | | |
| Analyst B/day 1 | | | | | | |
| GlcN | 0.82 (0.01) | 1.35 | 1.08 | 514.04 (5.38) | 1.05 | 0.84 |
| CS equivalent | 0.11 (0.00) | 1.31 | 1.05 | 403.56 (4.45) | 1.10 | 0.88 |
| Analyst B/day 2 | | | | | | |
| GlcN | 0.83 (0.01) | 0.74 | 0.60 | 518.62 (4.31) | 0.83 | 0.66 |
| CS equivalent | 0.11 (0.00) | 1.83 | 1.46 | 403.72 (5.97) | 1.48 | 1.18 |
| Intermediate precision ($n=12$) | | | | | | |
| GlcN | 0.82 (0.01) | 1.11 | 0.63 | 516.33 (5.23) | 1.01 | 0.57 |
| CS equivalent | 0.11 (0.00) | 1.52 | 0.86 | 403.64 (5.02) | 1.24 | 0.70 |

[a]Analyte to IS area ratio.
[b]Confidence percentage ($\alpha=0.05$).

**TABLE 3** Accuracy

| Compound | Level (%) | Spiked Amount[a] | Theoretical (μg/mL) | Found (μg/mL) | Recovery (%) | Mean Recovery (%, $n=3$) | RSD (%) | Mean Recovery (%, $n=9$) | RSD (%) |
|---|---|---|---|---|---|---|---|---|---|
| GlcN | 75 | 11.21 | 44.84 | 45.69 | 101.90 | 101.03 | 1.08 | 100.05 | 1.53 |
| | | 12.01 | 48.04 | 47.95 | 99.81 | | | | |
| | | 11.70 | 46.80 | 47.44 | 101.37 | | | | |
| | 100 | 14.60 | 58.40 | 59.30 | 101.54 | 99.83 | 2.13 | | |
| | | 15.11 | 60.64 | 60.74 | 100.50 | | | | |
| | | 15.30 | 61.20 | 59.64 | 97.45 | | | | |
| | 125 | 18.80 | 75.20 | 75.33 | 100.17 | 99.29 | 1.15 | | |
| | | 19.10 | 76.40 | 76.17 | 99.70 | | | | |
| | | 18.90 | 75.60 | 74.09 | 98.00 | | | | |
| CS | 75 | 9.41 | 37.64 | 37.04 | 98.41 | 99.68 | 1.36 | 99.91 | 1.35 |
| | | 9.28 | 37.12 | 36.95 | 99.54 | | | | |
| | | 9.10 | 36.40 | 36.80 | 101.10 | | | | |
| | 100 | 12.00 | 48.00 | 48.01 | 100.02 | 99.35 | 1.55 | | |
| | | 12.50 | 50.00 | 50.22 | 100.44 | | | | |
| | | 12.60 | 50.40 | 49.18 | 97.58 | | | | |
| | 125 | 15.30 | 61.20 | 62.21 | 101.65 | 100.70 | 1.23 | | |
| | | 15.62 | 62.48 | 63.20 | 101.15 | | | | |
| | | 15.70 | 62.80 | 62.36 | 99.30 | | | | |

[a] mg in about 120 mg of placebo.



Quantitative recovery was obtained in each instance with R.S.D. $\leq 2.13\%$ (Table 3).

## Analysis of Dietary Supplements

The results (Table 4) of GlcN and CS equivalent in commercial (capsules) and new formulations (effervescent tablets) were found in agreement with GlcN and CS nominal content and within the fixed range of USP (90.0–120.0%) for tablet dosage forms.[6] Other formulation ingredients did not interfere with the analysis.

**TABLE 4** Results for the LC Determination of GlcN and CS Equivalent in Dietary Supplements

| Dosage Forms | % Found[a] (RSD) | |
|---|---|---|
| | GlcN | CS Equivalent |
| Effervescent tablets | 102.79 (1.17) | 100.74 (1.16) |
| Capsules[b](type I) | 101.53 (1.04) | 101.83 (1.15) |
| Capsules[c](type II) | 102.24 (1.22) | 101.90 (1.18) |

[a]Mean of five determinations expressed as a percentage of the claimed content.
[b]Other ingredients: magnesium stearate, aerosil® 200 VV Pharma.
[c]Other ingredients: methylsulfonylmethane, magnesium stearate.

## CONCLUSIONS

The proposed UV-DAD LC method has shown to be suitable for the simultaneous analysis of GlcN and CS equivalent in dietary supplements. The hydrolysis procedure with hydrochloric acid was optimized allowing reliable analysis of CS equivalent. By using the chromatographic data before and after hydrolysis, the procedure can allow a rapid and preliminary screening for the presence of impurities or adulterants as glucosaminoglycans and GalN monosaccharide in CS raw materials. The method does not require preliminary extraction procedures and can be applied to real sample analysis using the instrumentation usually available in any analytical laboratory.

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