Liquid Chromatographic Analysis of Glucosamine in Commercial Dietary Supplements Using Indirect Fluorescence Detection*

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Abstract

A method of using indirect fluorescence detection is evaluated for the analysis of glucosamine in commercial dietary supplements following chromatographic separation. In this method, the eluting analyte, glucosamine, was detected by monitoring an increase in the fluorescence signal for L-tryptophan (L-Trp) or DL-5-methoxytryptophan (5-MTP) after glucosamine complexed with a copper(II) ion and released either L-Trp or 5-MTP from a copper(II) complex, which is introduced postcolumn. The fluorescence of L-Trp and 5-MTP are quenched when complexed with the copper(II) ion. The results obtained using indirect fluorescence detection are compared with the results obtained for precolumn derivatization with phenylisothiocyanate. Statistical analysis is performed to compare the results obtained for the two postcolumn interaction components, $Cu(L\text{-}Trp)_2$ and $Cu(5\text{-}MTP)_2$, as well as the results obtained using the indirect fluorescence detection method and a precolumn derivatization method. The indirect fluorescence detection method provided an alternative to precolumn derivatization for determining the concentration of glucosamine in commercial dietary supplements. The stability of the glucosamine–*o*-phthalaldehyde–3-mercaptopropionic acid derivative is also evaluated to investigate the applicability of the popular precolumn derivatization reagent, *o*-phthalaldehyde–3-mercaptopropionic acid, for this analysis.

Introduction

Glucosamine-containing products are used widely as dietary supplements to relieve the symptoms of osteoarthritis (1,2). Glucosamine has been reported to decrease joint pain, improve joint strength, enhance joint function, and to rebuild and maintain connective tissue (1,2). Several liquid chromatographic (LC) methods have been studied for the determination of glucosamine in raw materials and dietary supplements (3–8). The lack of a suitable chromophore or fluorophore in the glucosamine molecule makes optical detection challenging. Glucosamine can be detected after elution by measuring changes in the refractive index (3) or UV absorbance at 195 nm (4,5). Often, however, glucosamine is derivatized to incorporate a suitable chromophore or fluorophore before performing the LC separation to improve analyte detectability (5–8). Possible derivatization reagents include *o*-phthalaldehyde (OPA)–3-mercaptopropionic acid (MPA) (5,6), phenylisothiocyanate (PITC) (7,8), and *N*-(9-fluorenyl-methoxycarbonyloxy) succinimide (FMOC-Su) (9).

A simple, indirect, fluorescence detection method has been demonstrated to be effective for monitoring the elution of the amino sugars, glucosamine, galactosamine, and mannosamine, following chromatographic separation (10). The indirect detection method for glucosamine utilized here is based on measuring the fluorescence signal of either L-tryptophan (L-Trp) or DL-5-methoxytryptophan (5-MTP). Either L-Trp or 5-MTP is added postcolumn as a copper complex. The fluorescence of each of these compounds is quenched when they are bound to a copper(II) ion in the solution. In the presence of a solute that is capable of complexing with the copper(II) ion, such as glucosamine, some fraction of the L-Trp or 5-MTP is released from the copper complex. The fluorescence of L-Trp or 5-MTP is thus recovered, as shown by:

$$Cu(L)_2 + n\text{GlcN} \rightarrow Cu(\text{GlcN})n + 2L$$

where L represents the fluorescent ligand, L-Trp or 5-MTP, GlcN is glucosamine, and n is the number of glucosamine molecules coordinated to the copper(II) ion [n = 2 for glucosamine according to the literature (11)].

The use of this method is explored for measuring the amount of glucosamine in several formulations of commercially available dietary supplements. The use of L-Trp and 5-MTP is compared for the indirect fluorescence detection of glucosamine following LC separation. The results obtained using indirect fluorescence detection are compared with those obtained for precolumn

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derivatization of the glucosamine with PITC because of questions about the stability of the glucosamine–OPA–MAP derivative, which becomes evident as part of this study.

Experimental

Materials and reagents

The glucosamine hydrochloride (GlcN · HCl) (USP reference standard) was purchased from US Pharmacopeia (Rockville, MD). L-Trp (minimum purity, 98%), PITC (minimum purity, 99%), OPA (minimum purity, 99%), and MPA (minimum purity, 99%) were purchased from Sigma (St. Louis, MO). 5-MTP (minimum purity, 95%) was purchased from Aldrich (Milwaukee, WI). Reagent-grade copper sulfate and high-performance liquid chromatography-grade methanol were purchased from Fisher Scientific (Pittsburgh, PA). Reagent-grade sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) and potassium phosphate monobasic were purchased from J.T. Baker (Phillipsburg, NJ). Reagent-grade glacial acetic acid was purchased from VWR Scientific (San Francisco, CA). The copper complexes of L-Trp and 5-MTP were prepared by adding aqueous solutions of copper sulfate and the corresponding ligand at a 1:2 (mole:mole) ratio. The deionized water used to prepare the solutions was obtained from a Milli-Q water system (Millipore, Bedford, MA). All mobile phases were vacuum filtered through a 0.45-µm nylon filter (Whatman, Hillboro, OR) prior to use. The pH of the mobile phases and other solutions was adjusted using dilute solutions prepared from certified sodium hydroxide solution (50%, w/w) and reagent-grade hydrochloric acid (36.5%, w/w) purchased from Fisher Scientific (Fair Lawn, NJ).

Dietary supplement samples

Fourteen commercial glucosamine-containing samples (A–N) were analyzed. Two commercial chondroitin-containing samples (P and Q), which do not contain glucosamine, were used to evaluate potential interference by chondroitin or other compounds because many commercially available products are mixtures of glucosamine and chondroitin. The data presented are the average of triplicate injections of solutions prepared from each sample.

Apparatus

The LC system consisted of a Varian 9010 solvent delivery system (Palo Alto, CA) and a Rheodyne Model 7125 injector (Cotati, CA) with a 10-µL injection loop. A Kratos Spectroflow 980 fluorescence detector was employed for the indirect fluorescence detection method. A Waters 484 tunable absorbance detector (Milford, MA) was employed for the precolumn derivatization method. A Hamilton PRP-X100 column (250 × 4.1 mm, 10 µm, Reno, NV), containing a polymer-based strong anion-exchanger, was used for the indirect fluorescence detection method, and a Waters Xterra MS C18 column (50 × 4.6 mm, 3.5 µm) was used for the precolumn derivatization method. A Hitachi 655A-11 LC pump (Tokyo, Japan) delivered the post-column interaction components via a mixing tee for the indirect fluorescence detection method. A Varian flow control dampener was placed between the postcolumn solution pump and the mixing tee to improve flow stability. The stability of the reaction product of glucosamine with the derivatization reagent, OPA–MPA, was investigated with the use of a Varian Cary 50 Bio UV–vis Spectrophotometer.

Preparation of glucosamine standard solutions

USP glucosamine hydrochloride standard was accurately weighed to prepare standard stock solutions in water at concentrations of 0.3065 and 1.063 mg/mL. Glucosamine hydrochloride standard solutions were prepared from the stock solutions by serial dilution. The concentrations of the standards used for comparing the content of glucosamine in four commercial samples (A–D) with the use of L-Trp and 5-MTP ranged from 30 to 155 µg/mL. The concentrations of the standards used for comparing the content of glucosamine in the other 10 commercial samples (E–N) ranged from 50 to 265 µg/mL.

Preparation of glucosamine-containing sample solutions

Solid samples (tablet, capsule, and powder)

For tablet or capsule samples, between three and six tablets or capsules were transferred, as they were obtained, into a beaker filled with 300 mL of deionized water and dissolved with stirring. For powder samples, an appropriate amount of the powder was transferred, as it was obtained, to a beaker filled with 300 mL of deionized water and dissolved with stirring. The solution was then sonicated for approximately 30 min. The solution was transferred to a 500-mL volumetric flask and filled to the mark with deionized water, resulting in a stock solution containing approximately 3 mg/mL glucosamine. An aliquot of this stock solution was filtered through a 0.45-µm syringe filter and diluted to make a solution for analysis containing approximately 0.15 mg/mL glucosamine.

Liquid sample

An appropriate volume of liquid sample was transferred to a 25-mL volumetric flask and diluted, resulting in a solution containing approximately 0.15 mg/mL glucosamine. This solution was filtered through a 0.45-µm syringe filter.

Procedure for reaction of glucosamine with OPA–MPA

The procedure followed to prepare the glucosamine–OPA–MPA derivative was adapted from the literature (5,6). The following were mixed at ambient temperature: 320 µL of a solution of OPA in methanol (10.798 mg/mL), 100 µL of MPA, 800 µL of the aqueous solution of glucosamine hydrochloride (1.023 mg/mL), and 9580 µL of borate buffer (80mM, pH = 9.5). To monitor the stability of the glucosamine–OPA–MPA derivative, 600 µL of this solution was mixed with 2400 µL of borate buffer (80mM, pH = 9.5) and transferred to a UV–vis spectrophotometer cell.

Procedure for precolumn derivatization reaction of glucosamine by PITC

The procedure followed was adapted from the procedures of Liang et al. (7) and Ji et al. (8). One milliliter of the solution containing approximately 0.15 mg/mL glucosamine, 1 mL of 0.3M phosphate buffer at a pH of 8.3, and 1 mL of 5% (v/v) phenylisothiocyanate in methanol were transferred to a 20-mL glass vial and mixed. The glass vial was capped tightly and placed

in a water bath held at 60°C. During the derivatization reaction, the glass vial was removed from the water bath and vortexed for 1 min every 20 min. After 120 min, the glass vial was removed from the water bath, put into an ice bath for 10 min, and then restored to room temperature. The solution was filtered through a 0.45-µm syringe filter. A blank sample for the pre-column derivation reaction was prepared by following the procedure previously described, except that 1 mL of water was transferred instead of 1 mL of the glucosamine solution.

Chromatographic conditions

Indirect fluorescence detection method

For the indirect fluorescence detection method, the following conditions were used: mobile phase, 1.6mM sodium borate, pH 9.0; flow rate, 1 mL/min; postcolumn interaction component, 2×10^{-5} M Cu(L-Trp)$_2$ in 40mM sodium borate at pH 9.0, or 2×10^{-5} M Cu(5-MTP)$_2$ in 40mM sodium borate at pH 8.4; flow rate, 1 mL/min; column, strong anion-exchange column, PRP-X100 (250 × 4.1 mm, 10 µm); detector, spectroflow 980 fluorescence detector, excitation wavelength = 280 nm; and the emission wavelength selection was provided by a longpass glass filter (λ_{cutoff} = 320 nm or 340 nm).

Precolumn derivatization method

For the precolumn derivatization method, the following conditions were used: mobile phase, methanol–water–acetic acid (10.00:89.96:0.04, v/v/v) (7); flow rate, 1 mL/min; column, reversed-phase, Waters Xterra MS C18 (50 × 4.6 mm, 3.5 µm); detector, Waters 484 tunable absorbance detector, λ = 254 nm.

Calculations

The content of glucosamine in the commercial samples in the form of glucosamine hydrochloride is calculated as follows:

$$GlcN \cdot HCl = \frac{h - b}{a} \times D \qquad \text{Eq. 1}$$

where $GlcN \cdot HCl$, in mg, is the content of glucosamine in the commercial samples in the form of glucosamine hydrochloride, h is the peak height in the sample chromatogram, b is the y-intercept of the calibration curve, a is the slope of the calibration curve, and D is the dilution factor.

For commercial samples, such as samples A, F, and N, which state that the content of glucosamine is in the form of a glucosamine base, the content of glucosamine is calculated as follows:

$$GlcN = GlcN \cdot Hcl \times \frac{179.17}{215.63} \qquad \text{Eq. 2}$$

where $GlcN$, in mg, is the content of glucosamine in the form of a glucosamine base, $GlcN \cdot HCl$ is the content of glucosamine in the form of glucosamine hydrochloride, 179.17 is the formula mass (in amu) of the glucosamine base, and 215.63 is the formula mass (in amu) of glucosamine hydrochloride.

For commercial samples, such as samples C, D, G, H, I, and L, which state the content of glucosamine is in the form of glucosamine sulfate, the content of glucosamine is calculated as follows:

$$(GlcN)_2 \cdot H_2SO_4 = GlcN \cdot HCl \times \frac{456.42}{431.26} \qquad \text{Eq. 3}$$

where $(GlcN)_2 \cdot H_2SO_4$, in mg, is the content of glucosamine in the form of glucosamine sulfate, $GlcN \cdot HCl$ is the content of glucosamine in the form of glucosamine hydrochloride, 456.42 is the formula mass (in amu) of glucosamine sulfate, and 431.26 is twice of the formula mass (in amu) of glucosamine hydrochloride.

For commercial sample E, which states the content of glucosamine is in the form of glucosamine sulfate potassium chloride, the content of glucosamine in the form of glucosamine sulfate potassium chloride salt is calculated as follows:

$$(GlcN)_2 \cdot H_2SO_4 \cdot 2KCl = GlcN \cdot HCl \times \frac{605.52}{431.26} \qquad \text{Eq. 4}$$

where $(GlcN)_2 \cdot H_2SO_4 \cdot 2KCl$, in mg, is the content of glucosamine in the form of glucosamine sulfate potassium chloride salt, $GlcN \cdot HCl$ is the content of glucosamine in the form of glucosamine hydrochloride, 605.52 is the formula mass (in amu) of glucosamine sulfate potassium chloride, and 431.26 is twice of the formula mass (in amu) of glucosamine hydrochloride.

Results and Discussion

Separation of glucosamine from other compounds

Several ingredients are found in the formulation of glucosamine-containing samples. Chondroitin is one of the ingredients commonly found in these formulations. Potential interferences in the quantitative analysis of glucosamine because of the presence of these ingredients must be considered. Therefore, chondroitin-containing samples were mixed with a representative glucosamine sample before performing the chromatographic separation. Figure 1 shows the separation of glucosamine from the unknown compounds in the chrondroitin-containing samples under the chromatographic conditions employed here with the use of indirect fluorescence detection. A higher than usual concentration of chondroitin-containing sample solution was used to enhance the detection signal of the unknown compounds, which may interfere with glucosamine.

Comparison of using either of two postcolumn interaction compounds, Cu(L-Trp)$_2$ and Cu(5-MTP)$_2$, for indirect fluorescence detection of glucosamine

A previous study (10) demonstrated the successful application of both Cu(L-Trp)$_2$ and Cu(5-MTP)$_2$ for indirect fluorescence detection of glucosamine, galactosamine, and mannosamine following LC separation. Here, a study was conducted to compare the results obtained by using these two postcolumn interaction compounds for the determination of glucosamine in the commercial samples. The purpose of this study was to evaluate the use of different postcolumn interaction compounds for quantitative analysis of glucosamine. The calibration curves produced using Cu(L-Trp)$_2$ and Cu(5-MTP)$_2$ in the postcolumn solution with r^2 = 0.997 and 0.999, respectively, were used to evaluate the

content of glucosamine in four samples, A–D. The results obtained are presented in Table I. The confidence limits (CL) for the content of glucosamine in the samples were calculated using an equation described in the literature (12). This value expresses the uncertainty of estimating the content of glucosamine in the samples using the calibration curve at a chosen confidence level. A *t*-test was performed to compare the results obtained for these two different postcolumn reagents resulting in $t_{calculated}$ = 2.71, which is less than the value of $t_{critical}$, 3.18, for 95% confidence and 3 degrees of freedom (13). This $t_{calculated}$ value indicates that the results obtained using the two methods, which employed two different postcolumn interaction compounds, $Cu(L\text{-}Trp)_2$ and $Cu(5\text{-}MTP)_2$, are not significantly different at the 95% confidence level. In the following study, $Cu(L\text{-}Trp)_2$ was used for the indirect detection of glucosamine for the commercial samples E–N.

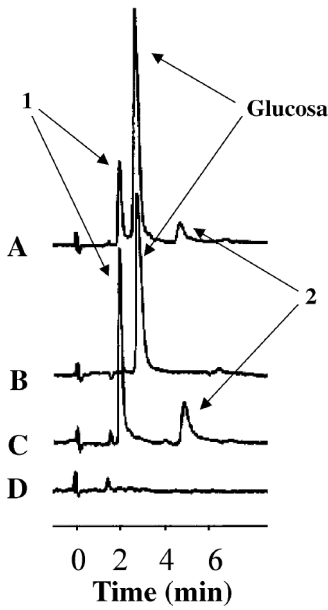

Figure 1. Separation of glucosamine from other compounds. The conditions were: mobile phase, 1.6mM sodium borate, pH = 9.0, 1 mL/min; postcolumn interaction component, 2×10^{-5} M $Cu(L\text{-}Trp)_2$ in 40mM sodium borate at pH = 8.4; flow rate, 1 mL/min; column, Hamilton PRP-X100, anion exchange; excitation wavelength, 280 nm; longpass filter, λ_{cutoff} = 340 nm. The chromatograms are: a mixture of glucosamine standard (GlcN · HCl, 0.0766 mg/mL) and chondroitin-containing sample P (sample P: 2.04 mg/mL) (A); glucosamine-containing sample B (sample B, 0.21 mg/mL) (B); chondroitin-containing sample P (sample P: 4.08 mg/mL) (C); and chondroitin-containing sample Q (sample Q: 4.46 mg/mL) (D). Peaks 1 and 2 are unknown compounds in chrondroitin-containing sample P.

Stability of the glucosamine–OPA–MPA derivative

A precolumn derivatization method was employed to compare the results obtained with the indirect fluorescence detection method. The most commonly used precolumn derivatization reagents for glucosamines are OPA combined with thiols, including MPA (5,6,14), and PITC (7,8,15), though another derivatization reagent, FMOC-Su, has been developed recently (9,16). An experiment was conducted to investigate the stability of the glucosamine–OPA–MPA derivative under the conditions utilized here because questions about the stability of the amine-OPA–MPA derivatives have been raised in the literature (17). To evaluate the stability of the glucosamine–OPA–MPA derivative, the UV absorption of the glucosamine–OPA–MPA derivatization reaction solution was monitored over time. According to the reports in the literature, the use of a derivatization reagent with an OPA–MPA molar ratio of 1:50 result in increased derivative stability for selected mono-, di-, and polyamines, as well as amino acids (17). For this reason, the stability of the glucosamine–OPA–MPA derivative was investigated under similar conditions. A derivatization reaction of glucosamine with an OPA–MPA molar ratio of 1:50 was performed according to a published method (5,6). The maximum absorption of the glucosamine–OPA–MPA derivative in borate buffer (80mM, pH = 9.5) was found to occur at 335 nm. The absorbance for the glucosamine–OPA–MPA derivative solution at 335 nm was monitored for over four and half hours after mixing the reagents. The results, presented in Figure 2, show that the absorbance of the glucosamine and OPA–MPA reaction solution reached a maximum after about 60 min and then declined. The absorbance of the glucosamine–OPA–MPA derivative decreased to almost half of the maximum absorbance after 5 h. These results are consistent with the glucosamine–OPA–MPA derivative being unstable over the time frame studied under the conditions of this reaction. The instability in the absorbance because of the glucosamine–OPA–MPA derivative resulted in a relative standard deviation (RSD) for the chromatographic analysis much higher than 2% for peak heights measured for triplicate injections of the glucosamine–OPA–MPA derivative. This observation led to the comparison of the results obtained using indirect fluorescence detection with the results obtained using a PITC precolumn

Table I. Comparison of Results Obtained by Using $Cu(L\text{-}Trp)_2$ and $Cu(5\text{-}MTP)_2$ as the Postcolumn Interaction Component

		Using $Cu(L\text{-}Trp)_2$			Using $Cu(5\text{-}MTP)_2$		
Sample	Stated ingredient*	Assay†	RSD‡	95% CL§	Assay†	RSD‡	95% CL§
A	GlcN	628 mg/tablet	1.5%	608–648 mg/tablet	625 mg/tablet	1.2%	616–633 mg/tablet
B	GlcN · HCl	466 mg/tablet	1.3%	442–491 mg/tablet	441 mg/tablet	1.2%	431–451 mg/tablet
C	$(GlcN)_2 \cdot H_2SO_4$	517 mg/capsule	1.2%	491–542 mg/capsule	500 mg/capsule	1.5%	489–511 mg/capsule
D	$(GlcN)_2 \cdot H_2SO_4$	294 mg/capsule	2.3%	268–319 mg/capsule	287 mg/capsule	2.1%	276–298 mg/capsule

* Abbreviations: GlcN, glucosamine; GlcN · HCl, glucosamine hydrochloride; $(GlcN)_2 \cdot H_2SO_4$, glucosamine sulfate.
† Average of triplicate injections.
‡ RSD: relative standard deviation of triplicate injections.
§ Confidence limit at 95% confidence level.

derivatization method, where the RSD was less than 2%.

Comparison of the indirect fluorescence detection method and the PITC pre-column derivatization method

A representative chromatogram showing the isocratic elution of the glucosamine–PITC derivative is given in Figure 3. The column was washed with methanol–water (80:20, v/v) at 1 mL/min between injections of solutions containing glucosamine derivatized with PITC. Ten glucosamine-containing samples (E–N) were analyzed by the method based on indirect fluorescence detection described here and by the method based on precolumn derivatization of glucosamine with PITC. The calibration curves produced for the indirect fluorescence detection method and precolumn derivatization method with r^2 = 0.999 and 0.998, respectively, were used to evaluate the content of glucosamine in the samples (E–N). The results obtained based on these calibration curves are summarized in Table II and presented graphically in Figure 4. Though the goal of this study was to compare the results obtained for the two methods utilized and not to analyze the particular samples, the content of glucosamine in the samples analyzed is similar to the range observed for the analysis of commercially available glucosamine samples (15). For all of the samples, the results obtained using the method based on indirect fluorescence detection are close to those obtained using PITC precolumn derivatization. A *t*-test was performed to compare the results obtained for the two methods with a result of $t_{calculated}$ = 0.658, which is less than the value of

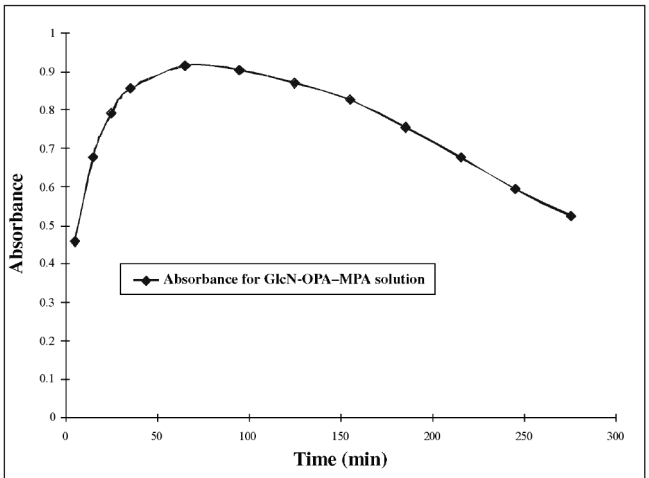

Figure 2. Absorbance at 335 nm for glucosamine–OPA–MPA derivative solution versus reaction time at room temperature.

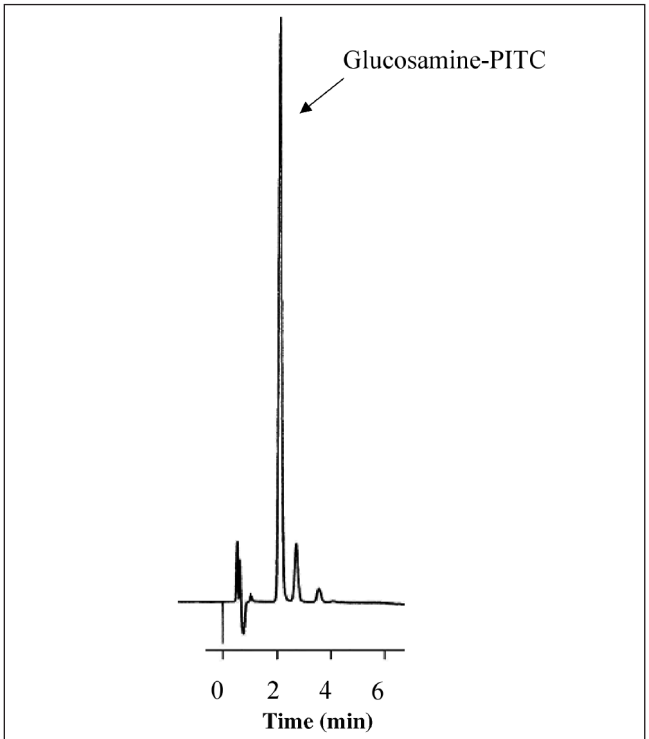

Figure 3. Representative chromatogram of glucosamine-PITC derivative. The conditions were: mobile phase, CH_3OH–H_2O–CH_3COOH = 10.00:89.96:0.04 (v/v/v), 1 mL/min; column, XTerra C18 (50 × 4.6 mm, 3 µm); detector, Waters 484 UV–vis detector, λ = 254 nm.

Table II. Content of Glucosamine Determined using the Indirect Fluorescence Detection Method and the PITC Precolumn Derivatization Method

		Indirect fluorescence detection method			PITC precolumn derivatization method		
Sample	Stated ingredient*	Assay†	RSD‡	95% CL§	Assay†	RSD‡	95% CL§
E	$(GlcN)_2 \cdot H_2SO_4 \cdot 2KCl$	523 mg/tablet	0.8%	503–543 mg/tablet	488 mg/tablet	0.4%	468–509 mg/tablet
F	GlcN	310 mg/tablet	1.5%	298–321 mg/tablet	320 mg/tablet	0.1%	308–332 mg/tablet
G	$(GlcN)_2 \cdot H_2SO_4$	409 mg/capsule	0.4%	393–424 mg/capsule	407 mg/tablet	1.2%	392–422 mg/tablet
H	$(GlcN)_2 \cdot H_2SO_4$	879 mg/3tablets	1.1%	856–901 mg/3 tablets	936 mg/3 tablets	0.1%	913–956 mg/3 tablets
I	$(GlcN)_2 \cdot H_2SO_4$	823 mg/tablet	0.8%	800–845 mg/tablet	883 mg/tablet	0.3%	860–906 mg/tablet
J	GlcN · HCl	521 mg/capsule	1.8%	507–536 mg/capsule	572 mg/capsule	0.2%	557–586 mg/capsule
K	GlcN · HCl	503 mg/tablet	0.2%	489–518 mg/tablet	491 mg/tablet	0.5%	476–506 mg/tablet
L	$(GlcN)_2 \cdot H_2SO_4$	549 mg/tablet	0.2%	533–564 mg/tablet	567 mg/capsule	0.1%	551–583 mg/capsule
M	GlcN · HCl	1505 mg/11.2g	0.8%	1461–1548 mg/11.2 g	1496 mg/11.2g	0.3%	1452–1540 mg/11.2g
N	GlcN	947 mg/29.57mL	0.4%	923–971 mg/29.57 mL	889 mg/29.57 mL	0.3%	865–914 mg/29.57 mL

* Abbreviations: GlcN, glucosamine; GlcN·HCl, glucosamine hydrochloride; $(GlcN)_2 \cdot H_2SO_4$, glucosamine sulfate; $(GlcN)_2 \cdot H_2SO_4 \cdot 2KCl$, glucosamine sulfate potassium chloride salt.
† Average of triplicate injections.
‡ RSD = relative standard deviation of triplicate injections.
§ Confidence limit at 95% confidence level.

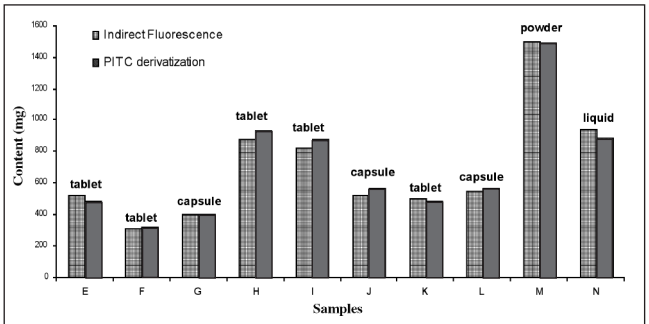

Figure 4. Comparison of the results obtained using the indirect fluorescence detection method and the PITC pre-column derivatization method for the analysis of glucosamine in 10 commercial products.

$t_{critical}$, 2.262, for 95% confidence and 9 degrees of freedom. This indicates that the results obtained by the method based on indirect fluorescence detection and those obtained by the method based on precolumn derivatization are not significantly different at the 95% confidence level.

Conclusion

It has been demonstrated that indirect fluorescence detection, using either $Cu(L\text{-}Trp)_2$ or $Cu(5\text{-}MTP)_2$ as the postcolumn interaction compound, is applicable for analyzing glucosamine in commercial dietary supplement samples. The results for 10 samples analyzed using the indirect fluorescence detection method were not significantly different from the results obtained using a PITC precolumn derivatization method at the 95% confidence level. The detection limit for glucosamine using the method based on indirect fluorescence detection is 0.15 nmol in 10 µL of solution injected (10), which corresponds to a concentration of 3.2 µg/mL. This detection limit is higher than the detection limit reported for the method based on precolumn derivatization of glucosamine by PITC, 0.075 µg/mL (8). However, advantages of using indirect fluorescence detection, such as avoiding a time-consuming precolumn derivatization step and possible stability problems of derivatization products, allow it to be a suitable alternative to methods based on precolumn derivatization for determining glucosamine concentration for analyses where the detection limit is not a significant consideration. For example, when analyzing commercial dietary supplements, the concentration of glucosamine in the analyzed samples may easily be maintained well above the detection limit for the method. The results presented here also indicate that caution should be exercised when using OPA–MPA for derivatization of glucosamine if the time between initiating the reaction and injection into the column varies.

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