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Short communication

Development of a simple capillary electrophoretic determination of glucosamine in nutritional supplements using in-capillary derivatisation with *o*-phthalaldehyde

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ABSTRACT

A simple capillary electrophoretic method was developed for the determination of glucosamine using in-capillary derivatisation. Glucosamine in commercial products was extracted with purified water. The CE separation was achieved on an uncoated fused-silica capillary using a 20 mM borate buffer (pH 9.2) containing 5 mM *o*-phthalaldehyde (OPA) and 5 mM 3-mercaptopropionic acid (MPA) at 25 kV, followed by UV detection at 340 nm. The detector response was linear ($r^2 > 0.999$) in the concentration range 10–1000 μg/mL. The limit of detection (LOD) was 1.3 mg/g. Spiked glucosamine recoveries at 50 and 100 mg/g level were 95.1% and 104.3%, respectively. The method was applied to 16 commercial products. The concentrations of glucosamine were 109–705 mg/g, and the ratios of detected glucosamine content to the labelled value were 88.8–124%. No significant bias was observed ($r^2 = 0.989$, $p < 0.01$), between results obtained by the proposed CE method and an official colorimetric method (Japanese Health Food & Nutrition Food Association).

1. Introduction

Glucosamine (2-amino-2-deoxyglucose), a key component of cartilage, is an amino sugar and distributed widely in tissues as a constituent of glycosaminoglycans (Roggi, Sottocornola, & Montani, 1979; Sietsma & Wessels, 1990). Several studies have reported that glucosamine was effective in easing osteoarthritis pain and in relieving arthritis (Dahmer & Schiller, 2008; Naito et al., 2010; Persiani et al., 2005; Qiu, Gao, Giacovelli, Rovati, & Setnikar, 1998; Setnikar, Pacini, & Revel, 1991; Tsi, Khow, Ono, Iino, & Kiso, 2003). The glucosamine products as nutritional supplements have been marketed worldwide, therefore the development of a simple analytical method is important to determine the content of these commercial products.

HPLC (AOAC Offcial Method 2005.01, 2011; Liang, Leslie, Adebowale, Ashraf, & Eddington, 1999; Nemati, Valizadeh, Ansarin, & Ghaderi, 2007; Shao, Alluri, Mummert, Koetter, & Lech, 2004; Zhou, Waszkuc, & Mohammed, 2004; Zhu, Cai, Yang, & Su, 2005), LC with mass spectrometry (Huang et al., 2006; Roda et al., 2006; Zhong, Zhong, & Chen, 2007), spectrophotometry (Wu, Hussain, & Fassihi, 2005), refractive index (El-Saharty & Bary, 2002; Way, Gibson, & Breite, 2000) and fluorescence detection (Ibrahim & Jamali, 2010; Shen, Yang, & Tomellini, 2007; Zhang et al., 2006) are often utilised for analysis of glucosamine in nutritional supplements or in human plasma. In addition, capillary electrophoresis (CE: Chen, Lee, Cheng, Hsiao, & Chen, 2006; Dutta & Dain, 2005; Jac, Los, Spacil, Pospisilova, & Polasek, 2008; Rammouz, Lacroix, Garrigues, Poinsot, & Couderc, 2007; Skelley & Mathies, 2006; Volpi, 2009) is more preferable because of its high resolution, low cost and short analysis time. However, derivatisation is necessary for UV detection, because glucosamine lacks any UV-absorbing chromaphore. *o*-phthalaldehyde (OPA: Chen et al., 2006; Nemati et al., 2007), *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (FMOC-Su: Zhou et al., 2004) and anthranilic acid (Volpi, 2009) are common as a derivatisation agent. OPA possesses high reactivity to primary amines, and the reaction occurs rapidly in room temperature to be easily automated.

In-capillary derivatisation (Hai et al., 2010; Oguri, Watanabe, & Abe, 1997; Ptolemy & McKibbin, 2006; Su, Lin, Cheng, & Jen, 2010; Taga, Nishino, & Honda, 1998), that the chemical reaction occurs in the inner space of capillary tube, is one of labelling techniques for CE. That is, this technique leads to derivatise and separate simultaneously. There have been few studies that have tried to determine glucosamine using an in-capillary derivatisation technique so far. Chen et al. (2006) have reported determination of glucosamine using an in-capillary derivatisation technique, in which the sample and OPA solution were sequentially introduced to the inlet of capillary. However, this procedure is cumbersome and complicated, furthermore, it required to a special instrument which allows a continuous injection.

The purpose of this study was to develop a simple CE method for the determination of glucosamine using an in-capillary

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derivatisation technique, in which the capillary was filled with an electrophoretic solution containing OPA to generate OPA derivative during electrophoresis. Furthermore, the proposed method was applied to the quantification of glucosamine in nutritional supplements on the market, and compared to the method based on Japan Health Food & Nutrition Food Association (Rondle–Morgan procedure: Rondle & Morgan, 1955).

2. Experimental

2.1. Instrumentation

2.1.1. Capillary electrophoresis

CE was performed using a CAPI-3300 instrument (Otsuka Electronics, Osaka, Japan) equipped with a photo-diode array (PDA) detector. Separation and analysis were performed on an uncoated fused-silica (75 μm I.D., 92 cm total length and 80 cm effective length) at 30 °C. The capillary was conditioned with 0.1 M NaOH for 3 min, Milli-Q water for 2 min, and then the operating buffer for 3 min between the runs. Samples were applied to the capillary using hydrodynamic injection (50 mbar) for 5 s at the anodic end. Electrophoresis was performed at 25 kV, and electropherogram was monitored at 340 nm.

2.1.2. Colorimetric method

In Rondle–Morgan procedure the absorbance was determined using a UV-2400PC spectrophotometer (Shimadzu, Kyoto, Japan) at 530 nm.

2.2. Chemicals

Glucosamine hydrochloride, OPA and 3-mercaptopropionic acid (MPA) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Sodium tetraborate anhydrous was from Kanto Chemical (Tokyo, Japan). Acetylacetone, sodium carbonate, hydrochloric acid and *p*-dimethylaminobenzaldehyde were purchased from Wako Pure Chemical (Osaka, Japan). These reagents were of analytical grade. Ethanol (Wako Pure Chemical, Osaka, Japan) was of liquid chromatography grade. Water was purified by a Milli-Q Water Purification System (Millipore, Billerica, MA, USA) and used for preparing buffers and sample solutions.

The stock standard solution (1000 μg/mL as glucosamine hydrochloride) was prepared by dissolving 100 mg of glucosamine hydrochloride in 100 mL water. The working standard solutions were prepared by dissolving in water.

The separating electrophoretic solution (pH 9.2) was prepared by dissolving OPA solution (33.5 mg in 1 mL of ethanol) and 22 μL of MPA in 50 mL of 20 mM sodium tetraborate solution.

2.3. Samples

Sixteen glucosamine products (13 were tablets, two capsules and one powder) were purchased on the market in Japan from October to November 2010. The blank sample for the method validation was prepared from commercial nutritional supplements containing chondroitin sulphates and hyaluronic acid, but contained no glucosamine.

2.4. Sample preparation for CE

A tablet sample was ground and capsule was opened to release the contents, and a 100 mg aliquot was accurately weighed in a 25 mL test tube, then 10 mL of water was added to extract at room temperature in an ultrasonic bath for 5 min. Supernatant fraction, obtained by centrifugation at 10,000 rpm for 5 min, was diluted with water to 10 times.

2.5. Rondle–Morgan procedure

This method is based on the theory that in alkaline solution at 100 °C the amino sugars react with acetylacetone to form a compound which gives a chromophore on treatment in acid solution with *p*-dimethylaminobenzaldehyde (Ehrlich's reagent).

In test tubes 1 mL of the test solution or standard working solution (10–50 μg/mL) were taken, then 1 mL of acetylacetone reagent and 1 mL of water were added. The tubes were heated for 20 min in a water bath at 100 °C, cooled to room temperature. A 6 mL of ethanol was added, followed by 1 mL of Ehrlich's reagent. The contents were thoroughly mixed and heated for 10 min in a water bath at 70 °C. After cooling to room temperature, the absorbance at 530 nm was measured. Water was used as blank sample in place of the test solution.

2.6. Accuracy and precision

A blank sample was spiked with glucosamine reference standard at 50 and 100 mg/g of the low-concentrated sample (sample No. 16 in Table 2). In a 25 mL test tube, 100 mg of blank sample containing 5.0 or 10 mg of glucosamine (50 mg/g level: 5.0 mg; 100 mg/g level: 10 mg) were prepared and analysed in five replicates on three consecutive days according to the method described in Section 2.4. The recovery was calculated as the ratio of contents of the spiked sample divided by amount of added glucosamine. The intraday precision and interday precision were calculated using one-way analysis of variance (ISO5725-3, 1994).

3. Results and discussion

3.1. Electrophoretic conditions

Glucosamine reacted readily with OPA in the presence of MPA at room temperature (Fig. 1). Glucosamine–OPA adduct (GlcN–OPA) was monitored at 340 nm, because both unreacted glucosamine and OPA had not absorption at this wave length. Therefore, GlcN–OPA was detected selectively without interference peak. GlcN–OPA was migrated as the anionic species due to dissociation of a carboxylic acid group derived from MPA, and separated from the other aliphatic primary amine–OPA adducts such as monoethanolamine. Glucosamine has two natural stereoisomers (alpha and beta), but one peak was shown because these isomers were migrated at the same time. The electropherograms and UV spectra of sample were shown in Figs. 2 and 3, respectively. Judging from UV spectra, the peak of GlcN–OPA at around 11 min in the electropherogram was high purity.

The peak of GlcN–OPA was broad and showed fronting due to the difference in electrophoretic velocity between glucosamine and GlcN–OPA. It is conceivable that the glucosamine zone is

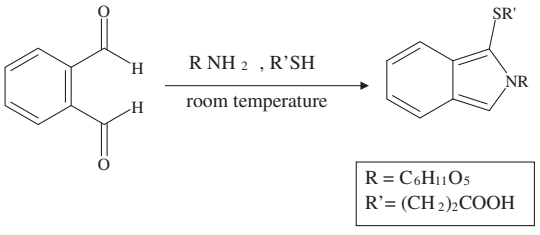

Fig. 1. Mechanism of OPA reaction with glucosamine.