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# Capillary electrophoretic determination of glucosamine in osteoarthritis tablets via microwave-accelerated dansylation

Li Qi, Shu-Feng Zhang, Min Zuo, Yi Chen *

*Laboratory of Analytical Chemistry for Life Science, Department of Chemical Biology, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China*

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**Abstract**

A robust capillary electrophoretic method has been established to separate aminosaccharides including glucosamine, galactosamine, *N*-methyl-glucamine, *N*-acetyl-glucosamine and amino glucuronic acid. All the aminosaccharides were dansylated fast under microwave irradiation at 385 W for 6 min (about 50-fold faster than common methods) and detected via on-line UV adsorption at 214 nm. Baseline separation of the dansylatied products was achieved in 20 min using a running buffer of 320 mM borate at pH 9.50. Quantitation of glucosamine in osteoarthritis tablets was then conducted. A linear working range was found in between 2.00 μg/mL and 1.80 mg/mL with linear regression coefficient of 0.9964. The limit of detection reached 1.00 μg/mL glucosamine (signal-to-noise, S/N = 3). Recoveries were determined by spiking a known amount of glucosamine in tablet-extracted solutions, giving a range of 88.0–99.7%. The run-to-run relative standard deviation was 0.24% ($n = 5$) for migration time and 2.72% for peak area.


*Keywords:* Aminosaccharide; Osteoarthritis therapy tablets; Microwave dansylation; CE

## 1. Introduction

Aminosaccharides have involved in many life processes, they are hence widely used in the fields of biology and medicine [1–3]. For instance, glucosamine, which is a normal constituent of glycosaminoglycans in cartilage matrix, synovial fluid and human tissues, stimulates the synthesis of glycosaminoglycan, proteoglycan and hyaluronic acid, therefore it has various pharmacological actions in articular cartilage and joint tissues [2]. Several short- to medium-term clinical trials in osteoarthritis therapy showed that glucosamine hydrochloride had significant symptom modifying effect and was safe for human use. Kanurnyy [4] found that glucosamine labeled by *N*-carboxyphenyl anthranilic acid could alleviate emotional hypertension caused by high stress.

Separation and analysis of aminosaccharides have since become an interesting research topic for many years, and some powerful methods [5–7] have been inspected or explored such as mass spectrometry (MS), gas chromatography (GC), high performance liquid chromatography (HPLC) and spectrophotometry. HPLC analysis of aminosaccharides using silica-based packing materials with refractive index (RI) detection offers a general way for intact solutes but possesses only moderate sensibility. Glucosamine bulk materials and formulations in pharmaceutical products can be separated by amino columns but at a high cost [8]. Ion chromatography with integrated pulsed amperometric detection (IC-IPAD) is often used for sensitive detection of saccharides but some extra, interfering small peaks might appear unpredictably. GC is also very fast and sensitive, but needs to make the solutes volatile by complicated sample preparation process, resulting in sometimes multiple peaks for a single component. MS is a valuable tool for identification of especially unknown constituents but the costly apparatus prevents it from becoming a routine technique.

*Abbreviations:* CE, capillary electrophoresis; Rs, resolution; HPLC, high performance liquid chromatography; GC, gas chromatography; MW, microwave; UV, ultraviolet; LIF, laser-induced fluorescence; MS, mass spectrum

* Corresponding author at: P.O. Box 2709, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China.
Fax: +86 10 62559373.
*E-mail address:* chenyi@iccas.ac.cn (Y. Chen).

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In present work, capillary electrophoresis (CE) is considered and studied [9,10] because of its high efficiency, fast speed, small sample requirement, low cost, simplicity and flexibility. The main drawback in using CE is its low detection sensitivity. To improve the detection sensitivity, incorporation of a UV-absorbing [11–14] or fluorescing [15–17] group onto an aminosaccharide molecule is normally required. In this study, dansylation [18] was adopted and achieved under microwave (MW) irradiation [19–22]. A type of model compounds composed of glucosamine, galactosamine, *N*-methyl-glucamine, *N*-acetyl-glucosamine and amino glucuronic acid were successfully dansylated under MW oven at 385 W for 6 min, giving about 50-fold faster labeling speed than common methods (2–12 h [23–25]). Baseline CE separation of the dansylated products has been obtained within 20 min using a running buffer of 320 mM borate at pH 9.50. The method has since been applied to the determination of osteoarthritis tablets, a kind of glucosamine-contained oral administration tablets. The working curves for glucosamine covered a range from 2.00 μg/mL to 1.80 mg/mL, with a moderate linearity ($r$, 0.9964) and excellent recovery 88.0–99.7%. The limit of detection (LOD) for glucosamine reached 1.00 μg/mL. The relative standard deviation (R.S.D.) of migration time was 0.24% ($n = 5$) for the same solute.

## 2. Experimental

### 2.1. Materials and solutions

Dansyl chloride, *N*-methylglucamine and *N*-acetylglucosamine were purchased from Sigma Chemical Co. (St. Louis, USA). D-Galactosamine was from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). D-Amino glucuronic acid was a product of Beijing Chemical Factory (Beijing, China) and D-glucosamine a product of Shanghai Chemical Factory (Shanghai, China). Indomethacin Entric (IME, containing 75.0 mg glucosamine hydrochloride per tablet) was purchased from Hebei Huan Hai Pharmaceutical Inc. (Hebei, China) and Viartril-S (VTS, containing 250.0 mg glucosamine sulfate per tablet) from Rottapharm Ltd. (Ireland). Dimethyl sulfoxide (DMSO) and other chemicals were the products of Beijing Chemistry Factory (Beijing, China).

CE running buffer was composed of borate adjusted to a desired pH by addition of crystal boric acid or NaOH pellets. Before use, all running buffer solutions were filtered through a membrane with 0.45 μm pores and degassed by sonication for 2 min. The water used was doubly distilled using a distillation apparatus model SZ-93 (Yarong Biochemical Instrument Co., Shanghai, China).

### 2.2. Capillary electrophoresis

CE was performed using P/ACE model 2050 (Beckman Coulter Co, USA). A bare fused-silica capillary (Yongnian Optical Fiber Factory, Hebei, China) of 75 μm i.d. × 57 cm (50 cm to detector) was mounted. Prior to injection, the capillary was sequentially rinsed with 0.1 M NaOH, 0.1 M $HNO_3$ and running buffer for 2 min each. Samples were then injected at 0.5 psi for 2 s and separated at +18 kV and 20 °C. The separated bands were detected by UV absorption at 214 nm. Peaks were identified by spiking standard aminosaccharides in sample solutions. The target peaks will be observed to have an increase.

### 2.3. MW-assisted derivatization of aminosaccharides

Aminosaccharides were separately dissolved in 80 mM borate buffer at pH 9.5 to form 2 mM solutions. Labeling reagent solution was freshly prepared by dissolving 10.0 mg dansyl chloride in 10.0 mL acetone. The dansyl chloride solution was mixed with an aminosaccharide solution in a capped-vial at a volume ratio of 1:20, after vortex for 1 min, the vial was placed at the center of turntable of microwave oven (model Mei Di PJ21F-B, 2.45 GHz, Guangdong, China) and irradiated at 119–700 W for 1–16 min. Before CE, each labeled solution was diluted with water by 10 times.

It may worth of mentioning that with this simple condition only the amino group on the sugars will be dansylated while the hydroxy groups did not undergo dansylation which will happen when special reagent, such as trifluoromethanesulfonic acid, presents as a catalyst. It was also found that, once HCl (1–2 M, pH < 2.0) was added into *N*-acetyl-glucosamine, multiple products were measured because the *N*-acetyl-glucosamine partially decomposed to glucosamine. The dansylation was checked by MS for sure that no by-products were produced.

### 2.4. Classical derivatization of aminosaccharides

Aminosaccharide solution was mixed with dansyl chloride as in Section 2.3 and allowed to react in darkness at room temperature for 5 h. Before CE, the labeled solutions were also diluted with water by 10 times.

The labeling yield produced by classical method was set as 100%. The derivatization yield by MW was measured by comparing the average peak areas with those of the classical method.

### 2.5. Preparation of tablet samples

One troche of IME or VTS was powered and transferred (15.0 mg for IME or 25.0 mg for VTS) into a 1.50 mL vial. An aliquot of 1.00 mL 80 mM borate buffer (pH 9.5) was added. The resulted solution was ultrasonically agitated for 10 min and centrifuged at 10 000 rpm for 5 min. The supernatant solution was filtered through a membrane with 0.45 μm pores, diluted with the borate buffer and dansylated as described above.

## 3. Results and discussion

### 3.1. MW-assisted derivatization

Fast derivatization of biological samples is becoming more and more important in many applications but often lack of simple and easy ways. MW-assisted technique is seemingly a promising candidate which has been shown to be able to largely accelerate

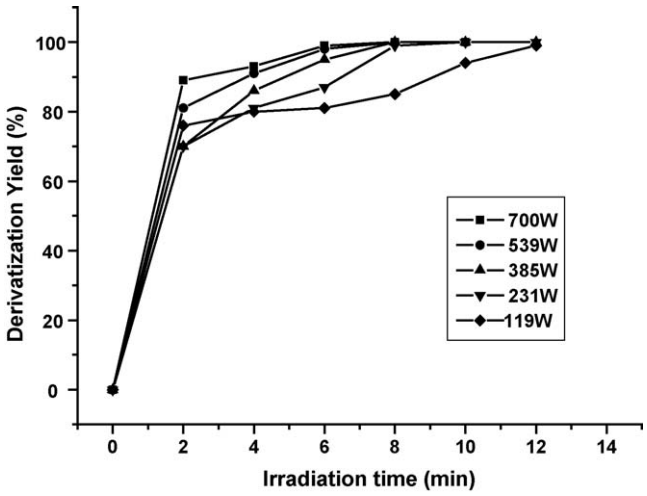


Fig. 1. Influences of microwave power and irradiation time on labeling yield.

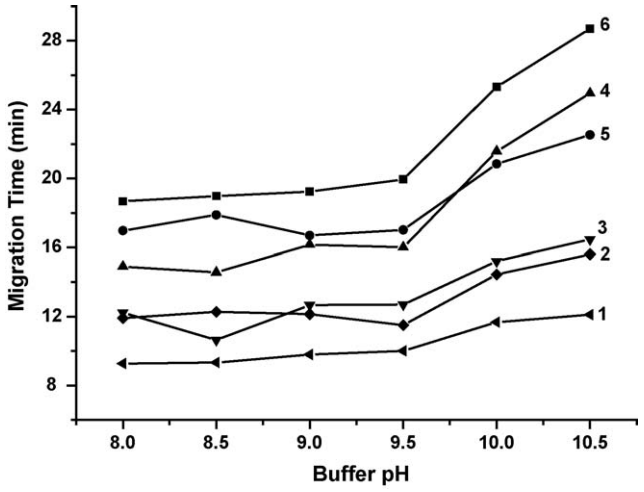


Fig. 2. Effect of buffer pH on migration time. Running buffer: 320 mM borate at pH 9.5; separation voltage: 20 kV; capillary: 75 μm i.d. × 57 cm (50 cm effective); injection: 0.5 psi for 2 s; detection: UV absorption at 214 nm; datum acquisition: 4 Hz. Peak identity: (1) *N*-acetyl-glucosamine; (2) *N*-methyl-glucosamine; (3) D-galactosamine; (4) dansyl chloride; (5) D-glucosamine; (6) amino glucuronic acid.

some reactions including labeling [25,26], but whether or not the dansylation of aminosaccharides can also be conducted in a MW oven remains untouched. It was thus studied and positive results were obtained.

A set of conditions were designed and inspected to optimize the labeling. As expected, MW power and irradiation time were found to be two critical factors in controlling the labeling yields. Fig. 1 shows that the yields increase very fast at the first 2 min and then level off gradually. Interestingly, the turning corner is nearly independent of the MW power. This means that, if the yield is not a concerned issue, the reaction time kept at 2 min can serve as a universal condition.

Nevertheless, for higher yield, the required MW irradiation time should be determined according to the power applied: It took 12 min to reach a 99.5% derivatization yield at 119 W but the time reduced to 10 min at 231 W. When the power was increased to 385, 539 and 700 W, respectively, the corresponding reaction time decreased to less than 6 min, with yields up to 98.0–99.5%. It should be noted that too high a power applied will cause sample solution boiling. Compromisingly, a power of 385 W was selected in this study and the reaction time was kept at 6 min to give a yield of >95.0%.

Since the MW power affected largely the reaction and its yield, the sample location in the MW oven should certainly be fixed to avoid yield fluctuation. In any case, the center point of the turntable was the best choice.

### 3.2. CE running buffer

It is known that buffer composition, its concentration and pH impact largely on CE separation, depending on solutes and operation mode. To find as a better buffer system as possible, several reagents were checked including carbonate, phosphate and borate, of which borate generated the best peak shape while the other two often produced peak shoulder at the buffer concentrations of 40–400 mM. Carbonate and phosphate were thus aborted while borate was adopted. Borate buffers allowed a long time of use.

Fig. 2 shows that pH has complicated influence on elution order and migration time. Commonly, the migration time increased with pH except for *N*-methyl-glucosamine, galactosamine, dansyl chloride and glucosamine. The former two changed their elution order twice at pH 8.1 and 8.8, respectively, and the latter two reversely eluted at pH 9.7. Serious overlapping happened at pH < 8.0 for *N*-acetylglucosamine, *N*-methylglucosamine and galactosamine. Since the elution time increased evidently at pH above 9.5 but kept nearly the same at the pH between 8.0 and 9.5, a working pH was suggested to be set in this range. In this work pH 9.5 was adopted to achieve the highest buffering capacity for borate. The elution time was thus confined within 20 min.

Fig. 3 shows that borate concentration (pH 9.5) has complicated influences on resolution, elution order and migration time. *N*-methylglucosamine and galactosamine could not be separated

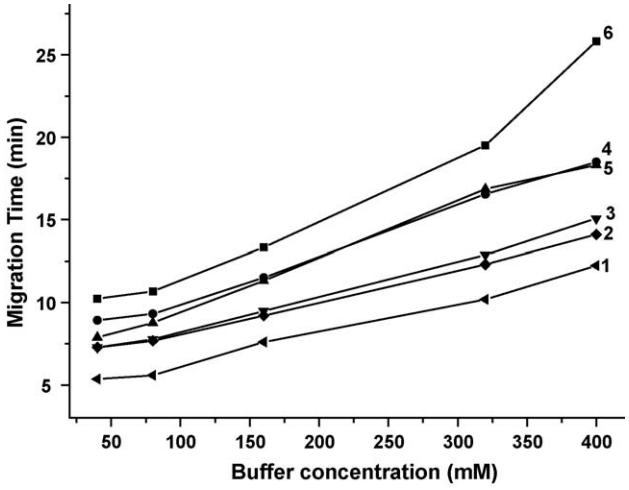


Fig. 3. Effect of buffer concentration on migration time. Electrophoretic conditions as in Fig. 2.

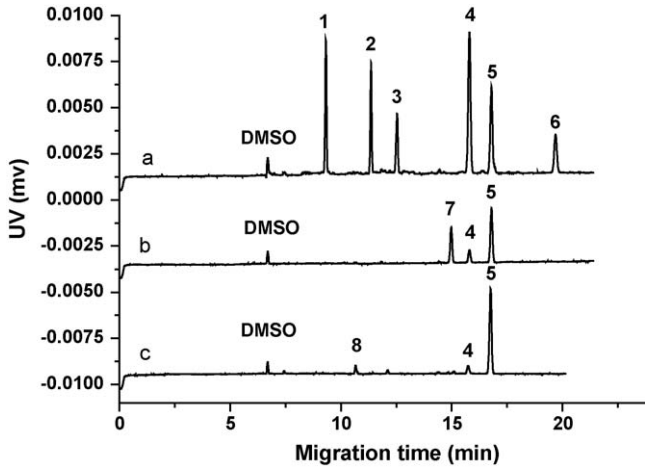


Fig. 4. Electropherogram obtained from aminosaccharide standard (a), IME extraction (b), and VTS extraction (c). Electrophoretic conditions as in Fig. 2. Peak identity: (1) *N*-acetyl-glucosamine; (2) *N*-methyl-glucosamine; (3) D-galactosamine; (4) dansyl chloride; (5) D-glucosamine; (6) amino glucuronic acid; (7, 8) unknown content.

when borate was below 160 mM. Glucosamine and dansyl chloride migrated very closely and changed their elution order twice at 320 and 400 mM borate. Also, they could not be well separated at a low concentration of borate: Their resolution was 1.31 at 320 mM borate but reduced to only 0.80 at 160 mM borate. Nevertheless, a running buffer of over 320 mM borate was not suggested to achieve fast separation. The elution commonly increased with buffer concentration and over 20 min was required for a buffer with >320 mM borate.

Fig. 4 shows that very clean and well-separated electropherograms can be generated with 320 mM borate at pH9.5. This is favorable for quantitative assay.

### 3.3. Determination of glucosamine in tablets

To determinate the content of glucosamine in commercial tablets, the quantitation features of the developed method were checked including LOD, linearity, recovery and reproducibility.

#### 3.3.1. Quantitation feature

Working curves were plotted via peak area ($y$) against concentration ($x$) and a linear regression equation was calculated for glucosamine in tablets:

$$y = 907.3 + 132862.2x$$

The equation covers a linear range of about three orders of magnitude, from 2.00 μg/mL to 1.80 mg/mL. Its linear regression coefficient is 0.9964. The LOD was 1.00 μg /mL (S/N = 3) measured by continuous dilution of a glucosamine standard.

Reproducibility was determined by five successive injections of a standard solution within 1 day. The relative standard deviations (R.S.D.) of migration time and peak area for glucosamine were 0.24 and 2.72%, respectively ($n = 5$), which were quite acceptable.

The recovery of the method was determined by using the standard addition technique, namely, by adding a known amount of glucosamine to a tablet-extracted sample. Overall mean recovery was determined by analyzing three osteoarthritis tablets, giving the data between 97.0–99.2% ($n = 5$) for IME and 88.0–99.7% ($n = 5$) for VTS (Table 1).

#### 3.3.2. Application

The established method has been successfully applied to the assay of two types of osteoarthritis therapy tablets at different content levels. Each content level of any type tablet was

Table 1
Recovery in CE assay of glucosamine tablets[a]

| Samples | Initial (μg/mL) | Added (μg/mL) | Found total[b] (μg/mL) | Mean recovery[c] (%) | R.S.D.[d] (%) |
|---|---|---|---|---|---|
| IME | 75.0 | 50.0 | 126.5 ± 1.2 | 97.0 | 2.2 |
| | 75.0 | 75.0 | 149.3 ± 0.5 | 99.1 | 1.3 |
| | 75.0 | 90.0 | 165.7 ± 4.1 | 99.2 | 2.0 |
| VTS | 150.0 | 125.0 | 267.4 ± 6.7 | 93.9 | 2.1 |
| | 170.0 | 62.5 | 225.0 ± 4.8 | 88.0 | 1.9 |
| | 170.0 | 250.0 | 420.7 ± 6.5 | 99.7 | 2.6 |

[a] By using the standard addition technique.
[b] With confidence limit of 95%.
[c] The mean recovery was averaged from three individual tablets but at the same indicated glucosamine content ($n = 5$, each tablet).
[d] Related standard deviation of mean recovery.

Table 2
Accuracy in assaying glucosamine tablets

| Samples | Content | | Accuracy[b] (%) | R.S.D. (%) |
|---|---|---|---|---|
| | Indicated (μg/mL) | Found[a] (μg/mL) | | |
| IME | 150.0 | 151.3 ± 2.3 | 100.9 | 2.2 |
| | 1500.0 | 1515.8 ± 6.4 | 101.1 | 0.3 |
| VTS | 150.0 | 149.9 ± 4.8 | 99.9 | 1.1 |
| | 1250.0 | 1240.2 ± 5.9 | 99.2 | 2.7 |

[a] Average from three tablets ($n = 3$, each tablet) with confidence limit of 95%.
[b] Accuracy (%) = 100 × found/indicated.

measured three times and averaged over three tablets. The final statistic data were listed in Table 2. As it shows, the measured contents agree well with the indicated data. Considering the satisfying recovery feature, it can be concluded that the method is accurate and reliable or highly applicable.

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