Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 1009–1014

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Development and validation of a HPLC–ES-MS/MS method for the determination of glucosamine in human synovial fluid

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ARTICLE INFO

Article history:
Received 5 June 2009
Received in revised form 2 July 2009
Accepted 3 July 2009
Available online 31 July 2009

Keywords:
Glucosamine
HPLC–ES-MS/MS
Knee osteoarthritis
Method validation
Synovial fluid

ABSTRACT

A new HPLC method for the determination of glucosamine (2-amino-2-deoxy-D-glucose) in human synovial fluid was developed and validated. Synovial fluid samples were analyzed after a simple protein precipitation step with trichloroacetic acid using a polymer-based amino column with a mobile phase composed of 10 mM ammonium acetate (pH 7.5)–acetonitrile (20:80, v/v) at 0.3 mL/min flow rate. D-[1-^{13}C]glucosamine was used as internal standard. Selective detection was performed by tandem mass spectrometry with electrospray source, operating in positive ionization mode and in multiple reaction monitoring acquisition (m/z 180 → 72 and 181 → 73 for glucosamine and internal standard, respectively). The limit of quantification (injected volume = 3 μL) was 0.02 ng, corresponding to 10 ng/mL in synovial fluid. Calibration curves obtained using matrix-matched calibration standards and internal standard at 600 ng/mL were linear up to 2000 ng/mL. Precision values (%R.S.D.) were ≤14% in the entire analytical range. Accuracy (%bias) ranged from −11% to 10%. The recoveries measured at three concentration levels (50, 800, and 1500 ng/mL) were higher than 89%. The method was successfully applied to measure endogenous glucosamine levels in synovial fluid samples collected from patients with knee osteoarthritis and glucosamine levels after oral administration of glucosamine sulfate (DONA®) at the dose of 1500 mg/day for 14 consecutive days (steady-state).

1. Introduction

The amino monosaccharide glucosamine (2-amino-2-deoxy-D-glucose, GLcN) is a natural component of chitin, glycoproteins and glycosaminoglycans, such as hyaluronic acid and heparin sulfate. It acts as building block of the amino sugars, hence being an important component of the cell wall structure and interstitial proteins [1], and is physiologically present in blood at relatively low concentrations (average values ~60 ng/mL) [2]. Glucosamine is widely used in Europe as a prescription drug and in the USA as a dietary supplement in the treatment of knee osteoarthritis (OA) [1,3–6]. The European League Against Rheumathism (EULAR) guidelines assigned to GLcN sulfate a ranking of "highest level of evidence and strength of recommendation" for its use as symptom-modifying drug in knee OA based on randomized clinical trials [7]. Despite the perceived benefits [8–16], information on the absorption and pharmacokinetics of GLcN in OA patients is limited and only recently its pharmacokinetics has been described [17].

The action mechanism of GLcN is still unclear [1,8]. It is assumed that GLcN acts as a precursor for the production of cartilage glycosaminoglycans, such as chondroitin sulfate [1,18]. Glucosamine sulfate was found to inhibit in vitro NFkB nuclear translocation and thus interleukin-1 (IL-1)-induced gene expression [19]. In IL-1β stimulated human chondrosarcoma cells, GLcN sulfate produced a significant inhibition of the IL-1 intracellular signalling pathway and consequent gene expression of inflammatory and matrix degradation markers [20]. The calculated IC_{50} values for GLcN were in the 10 μM range, i.e., in the concentration range found in plasma following oral administration of therapeutic doses of crystalline GLcN sulfate in humans [2].

An accurate determination of GLcN concentration at its site of action (i.e., the joint) is necessary to assess whether the drug levels achieved after oral administration of GLcN preparations were or were not pharmacologically relevant, to correlate the symptomatic and therapeutic effects with exposure of the joint tissues to GLcN in

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doi:10.1016/j.jpba.2009.07.008

the pharmacologically effective concentration range (10 μM), and to investigate the relationship between plasma and synovial fluid GLcN concentrations.

Some investigations have been conducted in large animals, especially horses. Laverty et al. [21] demonstrated that in healthy horses GLcN enters into the synovial fluid following oral administration, but the GLcN concentrations in the synovial fluid are less than 10% of those in serum. In a very recent paper the same authors reported that in horses affected by joint inflammation the GLcN concentrations in synovial fluid were four times higher than those found in healthy horses [22]. It has been also reported that oral administration of a clinically relevant dose of GLcN sulfate (20 mg/kg) to female horses led to synovial fluid GLcN concentrations significantly higher then those obtained after administration of an equimolar dose of GLcN hydrochloride [23]. Persiani et al. [17] measured the synovial GLcN concentration in OA patients, showing that after administration of crystalline GLcN sulfate its concentration in synovial fluid was only 23.5% lower than the concentration in plasma.

However, to our knowledge none of the bioanalytical methods developed for the determination of GLcN in synovial fluid have been validated according to currently accepted guidelines. Method validation is fundamental to obtain robust analytical results, suitable for studying GLcN pharmacological activity and in general GLcN bioavailability at the site of action after oral administration.

The relatively low concentrations of GLcN in synovial fluid and the presence of other related aminosugars require highly sensitive and selective analytical techniques. Since GLcN levels in plasma and synovial fluid are quite similar each other and these biological fluids contain the same potentially interfering compounds, the HPLC methods used for the determination of GLcN in plasma and serum can be adapted for the analysis of synovial fluid samples. Most of these methods use GLcN derivatization to achieve adequate analyte retention (due to its high polarity, GLcN is poorly retained on the commonly used C18 columns) and to perform GLcN detection by spectrophotometric [24], fluorimetric [25,26] or mass spectrometric detection [27]. Direct HPLC analysis of GLcN requires polar columns, such as amino- [28], ciano- [29], or ion exchange-columns [30–32], and electrochemical or mass spectrometric (MS) detection. The combination of HPLC with MS using electrospray (ES) ion source offers the undoubted advantage of allowing both confirmatory and quantitative analyses, owing to the high sensitivity and selectivity obtained with triple quadrupole tandem MS (MS/MS) and operating in the multiple reaction monitoring (MRM) mode. Therefore, this technique is highly recommended for pharmacokinetic studies requiring the quantitative determination of the analyte(s) of interest in complex matrices. Several papers have been recently published dealing with the determination of GLcN in plasma by HPLC–ES-MS/MS with detection and quantification limits down to 3 and 4 ng/mL GLcN, respectively [28,29,33]. Direct determination of GLcN in horse plasma and synovial fluid by HPLC–ES-MS/MS has been also performed using a C18 HPLC column, but a poor retention factor was achieved [33].

In this paper, a HPLC–ES-MS/MS method for the determination of GLcN in human synovial fluid is described. Samples were subjected to a simple and rapid protein precipitation step, then analyzed using D-[1-^{13}C]glucosamine as the internal standard. The method was validated according to the criteria reported in internationally accepted guidelines for drug analysis in clinical studies [34] by evaluating its overall performance (selectivity, linearity, precision, accuracy, recovery) as well as the stability of the analyte in standard solutions and sample matrix. The method was used to measure GLcN in synovial fluid samples obtained from patients affected by knee OA and treated with GLcN sulfate at a 1500 mg/day dose for a period of 14 days.

2. Experimental

2.1. Chemicals and reagents

D-Glucosamine hydrochloride (GLcN·HCl, purity > 99.8%) was purchased from Sigma–Aldrich (Milan, Italy), while the ^{13}C-labelled internal standard (IS) D-[1-^{13}C]glucosamine hydrochloride (^{13}C-GLcN·HCl, isotope purity 99%) was obtained from Omicron (South Bend, IN). All other reagents were of analytical grade and were purchased from Carlo Erba Reagents (Milan, Italy). HPLC-grade solvents methanol (RS plus) and acetonitrile (LiChrosolv®) were from Carlo Erba Reagents and Merck (Darmstadt, Germany), respectively. HPLC-grade water was prepared using a Millipore Milli-Q Synthesis A10 system (Molsheim, France).

2.2. Human synovial fluid samples

Synovial fluid samples were obtained from patients affected by knee OA and treated with crystalline GLcN sulfate (DONA®, Rottapharm, Milan, Italy) at the dose of 1500 mg/day for 14 consecutive days. The study protocol and related material were approved by the Local Ethics Committee of the Istituti Ortopedici Rizzoli in Bologna, Italy. The study was carried out in accordance with the current revision of the Declaration of Helsinki concerning medical research in humans, and following current Good Clinical and Laboratory Practice Guidelines (USA and EU). All subjects involved in the study gave written informed consent to participate in the study.

Synovial fluid was collected by aseptic arthrocentesis from knee joint into heparinised tubes. Samples were centrifuged at 2000 × *g* for 10 min to remove any dispersed solid matter, and then frozen at −20 °C in polypropylene tubes as three separate 600-μL aliquots. Aliquots of synovial fluid samples with a GLcN content below the limit of detection (LOD) of the method collected either during this study or in previous ones were pooled to obtain a large supply of synovial fluid used for preparing matrix-matched calibration standards and quality control (QC) samples.

2.3. Calibration standards and quality control samples

Stock solutions of GLcN (1 mg/mL free base) and ^{13}C-GLcN (150 μg/mL free base) were prepared in 10 mM ammonium acetate buffer, pH 7.5, and stored at −20 °C until use. Working solutions of GLcN for calibration standards (concentration levels in the range of 0.5–100 μg/mL) and QC samples (concentration levels 2.5, 40, and 75 μg/mL), as well as IS working solution (30 μg/mL), were prepared by further dilution of aliquots of stock solutions with ammonium acetate buffer. All stock and working solutions were stored at −20 °C in glass vials for no longer than 6 weeks.

Matrix-matched calibration standards at GLcN concentration ranging from 10 to 2000 ng/mL and QC samples at 50, 800, and 1500 ng/mL GLcN were prepared by spiking each working standard solution of analyte into human synovial fluid pool aliquots. A high-concentration sample spiked at 5000 ng/mL for assay parallelism assessment was also prepared. Volume of working standard solution spiked into synovial fluid samples was less than 3% of the synovial fluid volume.

2.4. Sample preparation

A 490 μL-aliquot of sample was put into disposable plastic tubes and mixed with 10 μL of the IS working solution to achieve a 600 ng/mL final concentration of ^{13}C-GLcN. Protein precipitation was performed by adding 250 μL of a 1.2 M water solution of trichloroacetic acid (TCA). The sample was vortexed and cen-

trifuged at 12,000 × *g* for 10 min. Then, 600 μL of the supernatant was transferred into disposable glass autosampler vials and 30 μL of 30% ammonia were added before HPLC–ES-MS/MS analysis.

2.5. HPLC–ES-MS/MS analysis

Liquid chromatography was performed using a 2695 Alliance system (Waters, Milford, MA) equipped with a built-in 120-position cooled autosampler. The analytical column was a Shodex® Asahipak NH2P-50 2D column (5 μm, 150 mm × 2.0 mm i.d.) protected by a Shodex® Asahipak NH2P-50G 2A guard column (5 μm, 30 mm × 2.0 mm i.d.); both columns were from Showa Denko K.K. (Kanagawa, Japan).

Mobile phases were 10 mM acetic acid in water adjusted to pH 7.5 with ammonia (solvent A) and acetonitrile (solvent B). Separation was achieved at 0.3 mL/min flow rate under isocratic elution conditions (6 min at 20% A), followed by column purge (6 min at 50% A) and column re-equilibration (8 min at 20% A). Injected sample volume was 3 μL. The analytical column was maintained at 28 °C to improve reproducibility or retention time values. The eluate was introduced directly into the ES source, operating in positive ionization (PI) mode, of a triple quadrupole mass spectrometer (Quattro LC, Micromass, UK). Nitrogen was used as nebulizer gas at 100 L/h flow rate and as desolvation gas at 610 L/h. Ion source block and desolvation temperatures were set at 120 °C and 250 °C, respectively. Capillary voltage was 3.1 kV and cone voltage was 12 V. Chromatograms were acquired using mass spectrometer in MRM mode, selecting the $180 \rightarrow 72$ and $181 \rightarrow 73$ m/z ion transitions for GLcN and ^{13}C-GLcN, respectively. To achieve high detectability the acquisition was performed at a relatively low resolution (∼0.8 *u* FWHM), which however still allowed the separate detection of the analyte and the IS. Dwell time and inter-channel delay values were set to 500 and 50 ms, respectively, for each selected ion transition. Relative collision energies (RCE), expressed as percent of the maximum instrument voltage difference value (200 V), were 7.5% for both analyte and IS.

The Micromass Mass-Lynx Version 4.0 software was employed for instrument control, data acquisition, and processing.

2.6. Quantification

Seven-point calibration curves were obtained by analyzing the matrix-matched calibration standards prepared according to the procedure described above and plotting the analyte/IS peak area ratio vs. the GLcN concentration of the standards. The equations of the calibration curves were determined using weighted linear least-squares regression analysis; the weighting factor ($1/x$) was chosen to minimize deviation of back-calculated values from nominal concentrations.

2.7. Recovery study

The recovery of GLcN was determined at the concentration levels of the QC samples, while recovery of IS was measured at 600 ng/mL. Recovery was calculated by comparing the areas of the glucosamine chromatographic peaks obtained from analysis of synovial fluid pool samples spiked before and after protein precipitation with TCA to yield the same concentrations. Matrix effect on the GLcN and IS ionization processes was evaluated at the same concentration levels reported above. Ion suppression was assessed by comparing the GLcN and IS signals obtained from synovial fluid pool samples spiked after protein precipitation with those of solutions in ammonium acetate buffer.

3. Results and discussion

3.1. Sample preparation and HPLC–ES-MS/MS analysis

Sample preparation and HPLC–ES-MS/MS analysis procedures were a slight modification of those previously described for GLcN analysis in plasma [28]. The main aim of the further optimization of the procedures was the development of a robust analytical method including an efficient and reproducible sample clean-up step.

Protein precipitation with TCA confirmed to be the best choice according to the composition and protein content of synovial fluid. In addition, compared to other protein precipitation procedures (e.g., addition of acetonitrile or methanol) it determined a relatively low sample dilution. The polymer-based amino column allowed a higher GLcN retention without derivatization (capacity factor about 2.5) in comparison to the other stationary phases used [29,33]. Moreover, GLcN could be eluted in a mobile phase containing a quite large amount (80%) of organic solvent, thus assuring a high ionization efficiency. A supernatant neutralization step performed by addition of ammonia was introduced before HPLC–ES-MS/MS analysis to improve the long-term reproducibility of the chromatographic separation. In fact, injection of strongly acidic (pH < 2) samples caused shortening of the retention times after injection of 30–40 samples. This was probably due to the protonation of the amino groups of the polymer-based amino column and the consequent loss of retention power. The length of the chromatographic run was also reduced from 30 to 20 min by shortening the column purging and re-equilibration steps to 6 and 8 min, respectively. Such times were sufficient for the elution of the most retained sample components, such as other sugars (for example, the retention time of galactosamine was 6.8 min), and for re-equilibration of the column at the initial chromatographic conditions.

Using the modified analytical procedure, large sample batches requiring up to 80–100 injections could be analyzed assuring stable chromatographic conditions (column pressure and background signals) and reproducible retention times. In the optimized analytical conditions the mean retention time of GLcN was 4.80 ± 0.03 min (%R.S.D. = 0.6%, $n = 20$).

3.2. Selectivity

The selectivity of the method was evaluated by analyzing pretreatment synovial fluid samples from 10 different subjects. Fig. 1 shows representative MRM chromatograms of a human synovial fluid sample with GLcN content below the LOD of the method, which demonstrate the absence of significant interfering peaks due to other endogenous species.

3.3. Linearity

The linear range of the method was optimized according to the expected GLcN concentrations in synovial fluid after chronic oral administration of GLcN sulfate at a dose of 1500 mg/day. The linear calibration range of the method was extended to 2000 ng/mL with respect to that used for plasma analysis [28] by increasing the IS concentration up to 600 ng/mL. In fact, using ^{13}C-GLcN (that differs from the analyte by only $m/z = 1$) as IS, under our experimental conditions the linear calibration range is limited by the contribution to the IS peak area due to the naturally occurring ^{13}C-GLcN, which determines a nonlinear calibration curve [35]. The higher IS concentration allowed to achieve a satisfactory linear calibration up to 2000 ng/mL GLcN, as demonstrated by either simulated calibration curves (theoretical R^2 for linear fitting higher than 0.999) and the good determination coefficients obtained for the experimental calibration curves (see below).

The mean linear calibration curve, obtained by the statistical analysis of six independent 7-point calibration curves with $R^2 \geq 0.991$, was $y = (0.00165 \pm 0.00008)x + (0.011 \pm 0.008)$, where y and x were the ratio between the peak areas of GlcN and ^{13}C-GlcN and the GlcN concentration in ng/mL, respectively.

The back-calculated concentrations of the calibration standards showed %R.S.D. values ranging from 1.7% to 13.6%. The difference between the back-calculated and the nominal concentrations of the calibration standards ranged from −11% to 6.8%.

3.4. Limits of detection and quantification

The LOD of the method was estimated by measuring the S/N value of the GlcN signal in human synovial fluid samples spiked at 10 ng/mL GlcN and extrapolating GlcN concentration to the value correspondent to S/N = 3. The so-obtained LOD value was 5 ng/mL of GlcN.

The limit of quantification (LOQ) was evaluated according to the guidance for industry on the validation of bioanalytical methods, i.e., the LOQ was defined as the lowest analyte concentration for which (a) the analyte peak is identifiable and discrete, (b) the analyte response is at least five times the response of the blank sample, and (c) the analyte response is reproducible with a precision better than 20% and accuracy of 80–120% [34]. According to these criteria, the concentration value of 10 ng/mL was set as the LOQ of the method (Table 1 and Fig. 1).

3.5. Precision and accuracy

Table 1 reports the within- and between-batch precision and accuracy results obtained from the analysis of QC samples and of a synovial fluid sample spiked with 10 ng/mL GlcN, corresponding to the LOQ of the method.

As concerned the within- and between-batch precisions, the %R.S.D. values measured for all the tested concentration levels did not exceed 10% and 15%, respectively. The within- and between-batch accuracies (%bias) ranged from −8.0% to 9.2% and from −11% to 10%, respectively. The results reported in Table 1 for the analysis of a sample spiked at 5000 ng/mL GlcN indicate that over-range samples can be measured upon dilution with ammonium acetate buffer before spiking with IS and protein precipitation with precision and accuracy comparable to those achieved for samples within the calibration range. No significant differences were observed when synovial fluid pool was used for dilution of over-range samples instead of ammonium acetate buffer (data not shown). Dilution of over-range samples with ammonium acetate buffer allowed to reduce consumption of the synovial fluid pool, which was available in limited quantities.

Table 1
Within-batch and between-batch precision (%R.S.D.) and accuracy (%bias) of the method.[a].

Theoretical concentration (ng/mL)	Mean back-calculated concentration (ng/mL)	%R.S.D.	%Bias
Within-batch			
10	9.2	9.3	−8.0
50	49.9	6.5	−0.2
800	873.4	0.44	9.2
1500	1509	3.9	0.6
Between-batch			
10	8.9	14	−11
50	45.9	8.4	10
800	821	5.9	2.7
1500	1540	7.8	2.6
Within-batch, over-range sample[b]			
5000	5350	2.5	7.0

[a] Samples were obtained by spiking GLcN working standard solutions into aliquots of human synovial fluid pool with GLcN concentration below the LOD of the method. Data are the mean values of six experiments.

[b] Over-range samples were diluted 1:10 (v/v) with ammonium acetate buffer (pH 7.5) before spiking with IS and protein precipitation.

Table 2
Recoveries of GLcN and ^{13}C-GLcN.[a].

Theoretical concentration (ng/mL)	%Recovery (±S.D.)
GLcN	
50	89.6 ± 7.7
800	95.7 ± 6.2
1500	92 ± 12
^{13}C-GLcN (IS)	
600	95.4 ± 6.7

[a] Determined by comparing the signals obtained from the analysis of human synovial fluid pool samples spiked before and after protein precipitation with TCA. Data are the mean values of six experiments.

3.6. Recovery and matrix effect

The recoveries of GLcN and of ^{13}C-GLcN in the sample preparation procedure, measured by comparing the GLcN signals obtained for samples spiked before and after protein precipitation, were always greater than 89% (Table 2). It is well known and documented

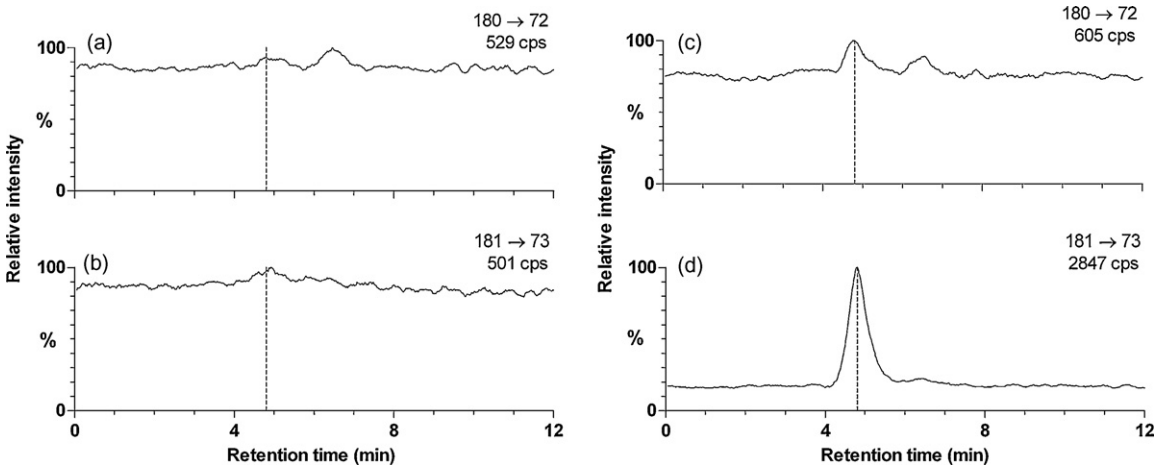

Fig. 1. Representative MRM chromatograms corresponding to the ion transitions m/z 180 → 72 (GLcN) and 181 → 73 (^{13}C-GLcN) obtained for (a and b) a human synovial fluid sample with GLcN content below the LOD of the method and (c and d) a synovial fluid pool sample spiked with 10 ng/mL GLcN and 600 ng/mL ^{13}C-GLcN. The dashed lines indicate the retention time of GLcN.

in HPLC–ES-MS analysis that coextracted and coeluted matrix components can affect the yield of analyte ion production during the ES ionization by competition processes [36,37]. Therefore, matrix effect on the GLcN ionization process was also studied, obtaining recoveries greater than 71% for both GLcN and ^{13}C-GLcN (data not shown).

3.7. Stability of stock solutions

The stability of GLcN (1000 μg/mL) and IS (150 μg/mL) stock solutions stored at −20 °C was tested along a period of 4 months. No significant decrease in the concentration was observed during this period of time (%bias values after 120 days of storage were −1.8% and 0.2% for GLcN and ^{13}C-GLcN, respectively).

3.8. Stability of samples

As recommended by the FDA Guideline, the stability of GLcN in synovial fluid under different experimental conditions was also studied. During validation, stored synovial fluid samples and QC samples were repeatedly analyzed to assess analyte stability in this matrix. Results confirmed that GLcN is stable for at least 4 months during storage at −20 °C and for 4 h at room temperature. In addition, no significant decrease in GLcN concentration was observed in synovial fluid samples subjected to up to three freeze/thaw cycles. To evaluate GLcN stability during a typical analysis cycle, processed QC samples were analyzed before and after a 30-h storage period at 7 °C in the autosampler carousel. No significant decrease in the measured GLcN concentrations nor modification of the chromatographic traces were observed (data not shown). After 30 h of storage at 7 °C, %bias values ranged from −2.8% to 9.2%.

3.9. Application to synovial samples

The validated HPLC–ES-MS/MS method was employed to measure GLcN levels in synovial fluid samples obtained from patients affected by knee OA and treated with GLcN sulfate at the dose of 1500 mg/day for 14 consecutive days. Synovial fluid samples were taken either before (for evaluation of basal GLcN levels) and at the end of the treatment. During treatment with GLcN sulfate, synovial fluid sampling was always performed 3 h after the last GLcN administration, i.e., in the correspondence of the highest GLcN plasma levels as determined in previous studies [2].

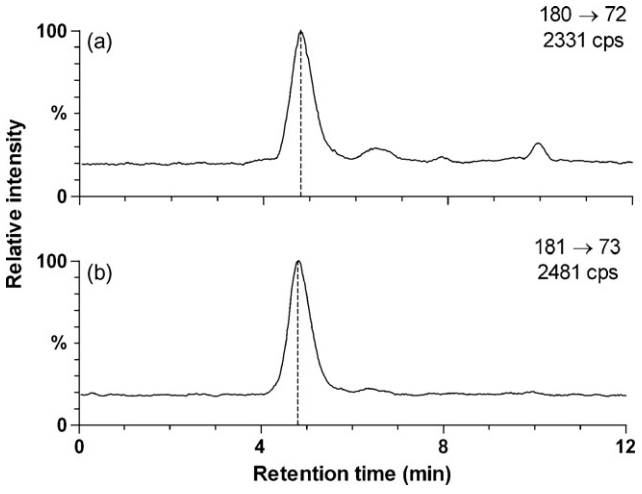

Fig. 2. Representative MRM chromatograms corresponding to the ion transitions (a) m/z 180 → 72 (GLcN) and (b) 181 → 73 (^{13}C-GLcN) obtained by analyzing a human synovial fluid sample collected after a 14-days treatment with 1500 mg/day GLcN sulfate. The dashed lines indicate the retention time of GLcN.

As concerned endogenous concentration at baseline, the concentrations of GLcN in synovial fluid showed a quite high variability, ranging from below the LOD of the method (5 ng/mL) to 51.6 ng/mL. Among the analyzed samples (n = 6), GLcN was undetectable in one case and below the LOQ of the method (10 ng/mL) in a second case. The mean basal GLcN concentration of the remaining samples was 32.3 ± 9.8 ng/mL (n = 4). The mean GLcN concentration in synovial fluid at the end of the treatment was 710 ± 210 ng/mL (n = 6). A representative MRM chromatogram obtained by analyzing a synovial fluid sample collected after the treatment (GLcN concentration 474 ng/mL) is shown in Fig. 2.

The synovial fluid GLcN levels at the end of the treatment have been compared with those obtained in plasma samples collected simultaneously to synovial fluid and analyzed according to the experimental procedure already described [28]. The GLcN concentrations measured in plasma samples (mean value 990 ± 180 ng/mL, n = 6) were slightly higher than those found in synovial fluid, as previously reported [17]. Nevertheless, the good correlation between synovial and plasma GLcN concentrations demonstrated that the drug efficiently reaches the site of pharmacological action, i.e., the joint. Therefore, plasma GLcN levels will be useful to monitor the relationship between therapeutic effect and exposure of the joint tissues to GLcN in patients with knee OA.

4. Conclusion

The new HPLC–ES-MS/MS method for determination of GLcN in synovial fluid fulfils the acceptance criteria generally established for bioanalytical assays in pharmaceutical analysis. In the explored concentration range (up to 2000 ng/mL GLcN) the method proved to be selective, accurate, precise, and sensitive enough to allow analysis of GLcN in 0.5-mL human synovial fluid samples. The method, which does not require pre-analytical derivatization, can be directly applied after a simple protein precipitation clean-up step, thus reducing analytical variability and shortening sample processing. The use of the internal standard ^{13}C-GLcN and of matrix-matched calibration standards allows to compensate ion signal suppression and improve accuracy. Furthermore, up to 60 samples/day can be analyzed with an autosampler system. The LOQ value of the method (10 ng/mL GLcN) is adequate to quantify endogenous GLcN. The validated method has been applied to the determination of endogenous GLcN levels in synovial fluid and of GLcN concentration after a treatment with the clinically recommended oral dose (1500 mg) of GLcN sulfate for 14 consecutive days. It was possible to demonstrate that after oral administration GLcN reaches the knee compartment. The synovial GLcN concentration increased 10–100-fold from baseline and was correlated with the GLcN levels in plasma. Furthermore, the concentrations of GLcN in synovial fluid after oral administration of GLcN were in the same range of those found to be pharmacologically effective in vitro.

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