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Short communication

Direct pharmaceutical analysis of bisphosphonates by capillary electrophoresis

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Abstract

Bisphosphonate compounds have been studied as a class of potential drugs for the treatment of various bone diseases. However, the analyses of these compounds are problematic because most of them do not contain strong chromophores. Based on the unique structures of these compounds, we have employed a capillary electrophoresis (CE) technique for the characterization of these compounds in pharmaceutical dosage formulations. In this study, two CE methods were developed for the determination of a bisphosphonate compound, 2-thioethane-1,1-bisphosphonic acid. The first method involved the use of an uncoated column, a phosphate buffer, and hydrostatic injection with direct UV absorbance detection. The method showed excellent resolution and precision with a reasonable detection limit of 30 μg/ml. Sensitivity was further improved using a glycerol-coated column, together with a phosphate buffer of higher concentration and electrokinetic injection under sample stacking conditions. This modified method revealed a significant improvement in sensitivity with a detection limit of about 50 ng/ml. Both methods demonstrated high simplicity and excellent reproducibility and were successfully applied to the quantitative analyses of pharmaceutical dosing solutions.

Keywords: Bisphosphonates

1. Introduction

Bisphosphonate-containing compounds have been identified as an important class of drugs for the treatment of a variety of bone diseases [1,2]. Therefore, the development of sensitive analytical methods for the determination and characterization of bisphosphonate-containing compounds is crucial for the development of this class of drugs. Since many of these compounds do not have strong chromophores typically used for detection in HPLC, the analyses of the compounds are problematic. The measurement of bisphosphonate compounds without chromophores by HPLC–UV has been accomplished by introducing a chromophore into these molecules through pre- and post-column derivatization with molybdate via photochemical reaction [3]. Gas chromatography with mass spectrometry detection has also been employed through sample derivatization [4]. In addition, other methods without derivatization have been developed utilizing ion-exchange chromatography with on-line flame photometric detection [5], conductivity detection [6], and mass spectrometry detection [7]. However, all those methods are relatively complex. As capillary electrophoresis (CE) has gained its popularity for the determination of charged molecules, we explored this technique for the analysis of this class of compounds since these com-

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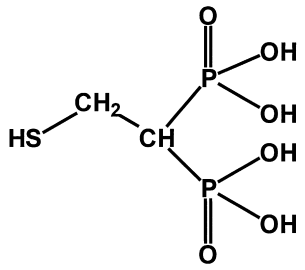

Fig. 1. Molecular structure of 2-thioethane-1,1-bisphosphonic acid (PGE-7787262).

pounds are highly negatively charged. Compared to HPLC, CE provides high resolution and requires much less sample and reagents. A CE method involving on-line formation of a chromophoric complex has been published before [8]. Here, we report two simple and sensitive CE methods that we have developed and utilized for the quantitative determination of a bisphosphonate-containing compound, 2-thioethane-1,1-bisphosphonic acid (PGE-7787262, see Fig. 1 for its molecular structure), in pharmaceutical dosage formulations.

2. Experimental

2.1. Materials

PGE-7787262 ($C_2H_8O_6P_2S$, M_r 222.1) was synthesized by Procter & Gamble Pharmaceuticals (Cincinnati, OH, USA). Monobasic sodium phosphate and dibasic sodium phosphate were obtained from J.T. Baker (Phillipsburg, NJ, USA) and used without further treatment. Deionized water purified with a Milli-Q Ultra-Pure Water System (Millipore, Bedford, MA, USA) was utilized for all sample preparations. All electrolyte solutions were filtered through a 0.45-μm Millex-HA filter (Millipore, Bedford, MA, USA).

2.2. Electrolyte, standard and sample preparations

The electrolyte solutions were made by dissolving appropriate ratios of mono- and di-basic sodium phosphates in Milli-Q water to give 3 and 45 m*M* solutions at pH 7.1. The pharmaceutical dosing solutions of PGE-7787262 were prepared by dissolving the compound in dosage formulations. The standard solutions were prepared the same way as the dosing solutions to yield a stock solution of 10 mg/ml. The stock solutions were diluted in series to give a series of standard solutions in a concentration range of 100 μg/ml to 50 ng/ml.

2.3. Equipment and capillary electrophoretic conditions

A Waters CIA capillary electrophoresis system (Milford, MA, USA) consisting of an automated sampler, a separation apparatus, and an ultraviolet absorbance detector was employed for all experiments. A Waters Millennium workstation was used to collect, process, and report data. An uncoated Waters AccuSep fused-silica capillary column of 55 cm length (48 cm to the detector) and 75 μm I.D. was used for the first method and an ISCO CE200/glycerol modified silica capillary column of 55 cm length (48 cm to the detector) and 75 μm I.D. for the modified method. The first uncoated-column method was developed utilizing a hydrostatic sample injection (10 cm height) of various durations, a positive run voltage of 30 kV, and a 3 m*M* phosphate electrolyte solution at pH 7.1. The capillary was sequentially treated with 0.1 *M* NaOH for 2 min, rinsed with Milli-Q water for 2 min, and conditioned with the electrolyte solution for 3 min. The second coated-column method employed an electrokinetic injection under a negative voltage of 15 kV with various durations, a negative run voltage of 15 kV, and a 45 m*M* phosphate electrolyte solution at pH 7.1. The coated capillary was washed with Milli-Q water for 2 min, followed by conditioning with the electrolyte solution for 3 min. All experiments were conducted at 25°C. The detection wavelength was set to 185 nm.

3. Results and discussion

The first CE method we developed for the analyses of bisphosphonate compounds utilizes an uncoated fused-silica column with hydrostatic injection and positive power supply. In an uncoated fused-silica column, an electric field-induced solvent flow, i.e., electroosmotic flow (EOF), occurs within the

column. Electroosmosis is often strong enough to pull anions toward the cathode, which is the case in this experiment. Here, sample injection is carried out at the anodic (positive) electrode and detection is made at the cathodic (negative) electrode. The electropherogram of 30 μg/ml PGE-7787262 is shown in Fig. 2. The detection limit ($S/N=2$) for the compound is about 30 μg/ml. Using this method, we were able to quantitatively determine the drug levels in the dosing solutions at concentrations between 10 mg/ml and 50 μg/ml. However, we were unable to analyze dosing solutions at drug concentrations of lower than 50 μg/ml. Therefore, a more sensitive CE method was required for drug samples of low concentrations, especially for the determination of dosing solutions at drug levels in the ng/ml range.

In capillary electrophoresis, there are generally three ways to improve sensitivity. The first one is to preconcentrate samples off-column, which is non-specific and often labor-intensive. The second is to use larger inner diameter capillaries, which is limited by increased heat production and loss of resolution. The third and better approach is to use on-column sample concentration techniques, such as sample stacking, sample focusing, and isotachophoretic sample enrichment. In this work, we chose an on-column sample stacking technique for its overall simplicity. To achieve sample stacking conditions, we modified the first method by using a glycerol-coated column, a phosphate buffer of higher concentration and electrokinetic injection. Here, samples were introduced into the capillary by briefly applying a voltage to the samples. Since the electrolyte concentration (45 m*M* phosphate buffer) was much higher than the sample concentration, the specific conductivity of the sample was much lower than that of the surrounding phosphate buffer. The electric field strength is inversely proportional to the specific conductivity of the solution. Therefore, the field strength of the sample plug in the capillary was higher than that of the running phosphate buffer. The higher the field strength, the greater the electrophoretic velocity. In this way, the ionic analyte zone in the sample plug was narrowed, leading to the sharpening of the analyte peak and thereby the improvement of the sensitivity or detectability of the method. In this modified method, a glycerol-coated column was used to eliminate electroosmotic flow. Fig. 3 shows the electropherogram of a 30 μg/ml dosing solution of PGE-7787262. Here, electrokinetic injections with a negative voltage were employed. Therefore the sample injection was carried out at the cathodic (negative) electrode and the detection was made at the anodic (positive) electrode. Under these conditions, the detection limit for the compound was about 50 ng/ml, a substantial improvement in sensitivity.

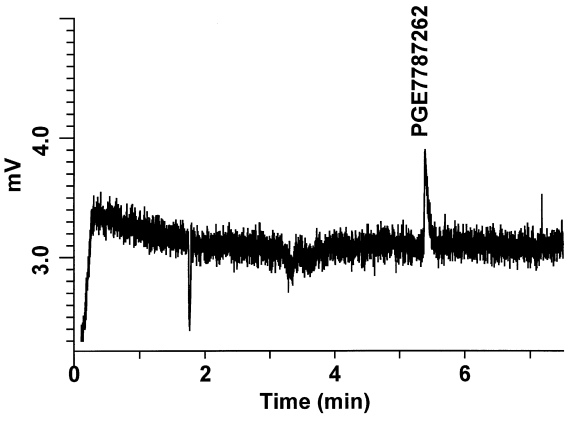

Fig. 2. Electropherogram of a 30 μg/ml dosing solution of PGE-7787262. Column, 55 cm×75 μm silica column; electrolyte, 3 m*M* phosphate buffer, pH 7.1; injection, hydrostatic, 10 s; separation potential, −30 kV (15 μA); detection, UV 185 nm.

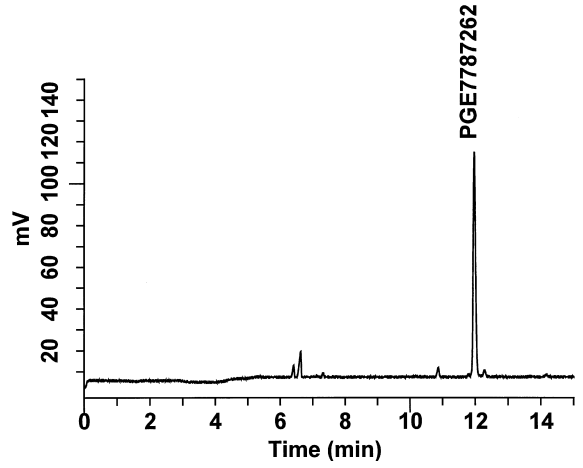

Fig. 3. Electropherogram of a 30 μg/ml dosing solution of PGE-7787262 under sample-stacking conditions. Column, 55 cm×75 μm glycerol-coated silica column; electrolyte, 45 m*M* phosphate buffer, pH 7.1; injection, electrokinetic, 10 s at +15 kV; separation potential, +15 kV (80 μA); detection, UV 185 nm.

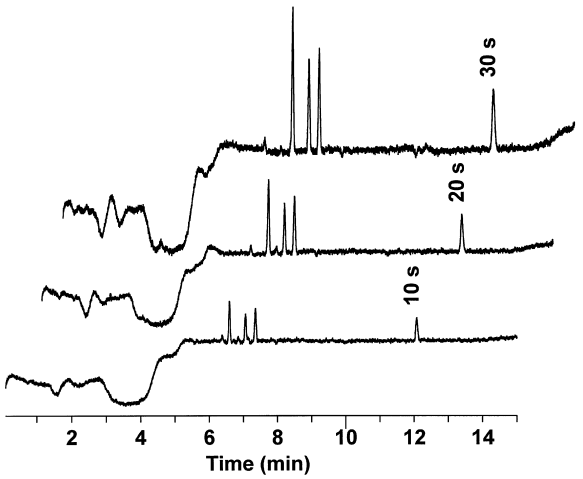

Fig. 4. Electropherograms of a 100 ng/ml dosing solution of PGE-7787262 at various injection times (under sample-stacking conditions). For separation conditions, see legend to Fig. 3, except electrokinetic injection, 10–30 s at +15 kV.

Using the coated-column method, we also examined the relationship among peak area, migration time and sample injection duration. Fig. 4 shows the representative electropherograms of 100 ng/ml PGE-7787262 at various injection times. The minor peaks around 7 min originated from the impurities in the dosage formulation. Based on our results, we found that both peak area and migration time increased with increasing injection time although they did not show good linearity. With a fixed injection time, however, both the peak area and migration time showed excellent reproducibility: the coefficients of variation were 2 and 0.4% with 15 intra-assay runs, respectively. The specificity of the modified method is also demonstrated in Figs. 3 and 4. Our analyte peaks were clearly separated from other component peaks. The calibration curve of the compound was linear from 0.1 to 50 μg/ml with a correlation coefficient greater than 0.999. The accuracy was found to be greater than 93% for drug levels ranging from 0.2 to 50 μg/ml. The detection limit at a signal-to-noise ratio of 2 was about 50 ng/ml.

The above uncoated and coated column CE methods have also been successfully applied to the determination of two other bisphosphonate compounds in dosage formulations. Both methods are simple and show excellent reproducibility. They are rugged, general and especially useful for routine stability studies of dosing solutions for this class of compounds. In addition, the coated-column method offers better sensitivity and should be preferred for the determination of drug samples of low concentrations.

In summary, the first CE method described here showed excellent separation and a reasonable detection limit of 30 μg/ml with the use of an uncoated column and direct UV absorbance detection. The second CE method was developed using a glycerol-coated column and sample stacking conditions. The modified method afforded a detection limit of 50 ng/ml, offering a substantial increase in sensitivity compared to the original method.

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