*Journal of Chromatography*, 626 (1992) 245–250
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 557

# Application of capillary electrophoresis to pharmaceutical analysis

# Determination of alendronate in dosage forms☆

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(First received June 2nd, 1992; revised manuscript received August 13th, 1992)

ABSTRACT

A method using capillary electrophoresis (CE) for the determination of alendronate (a non-chromophoric compound) in pharmaceutical dosage forms is described. Direct UV detection is based on the on-line formation of a chromophoric complex between alendronate and $Cu^{2+}$ ions present in the electrolyte solution. The CE method has been validated to address the quantitative aspects for practical applications. Tablet dosage forms can be suitably assayed by the proposed method. Analysis of intravenous solutions encounters interference from citrate anion, a strong metal-chelating agent, present in the formulation, resulting in low and irreproducible assay results. The developed CE method obviates any derivatization procedure and involves minimum sample preparation.

## INTRODUCTION

Capillary electrophoresis (CE) has become a valuable tool in the biological sciences and pharmaceutical analysis [1–4]. It is popular owing to the recent availability of automated commercial instruments. The major advantages of CE, including high efficiency, negligible solvent consumption, flexibility and speed of methods development, prompted this investigation to explore its application to pharmaceutical analysis. An increasing number of successful reports have appeared (*e.g.*, refs. 5–8) illustrating the applications of CE to the analysis of low-molecular-mass drugs ($M_r$ < 1000). However, few publications [9,10] have addressed the quantitative aspects in pharmaceutical applications. It was the purpose of this work to investigate the reproducibility and quantification capability of this methodology for practical applications.

We have developed and validated a novel CE method for the determination of alendronate in pharmaceutical dosage formulations to evaluate the quantitative aspects of the CE technique. Alendronate (ABP; see Fig. 1 for the molecular structure) is

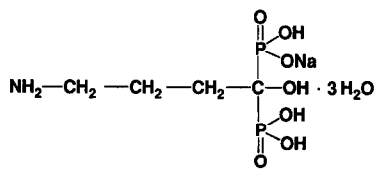


Fig. 1. Structure of alendronate, monosodium salt trihydrate of 4-amino-1-hydroxybutane-1,1-bisphosphonic acid.

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☆ Presented at the *203rd American Chemical Society National Meeting, San Francisco, CA, April 1992.*

0021-9673/92/$05.00 

categorized as a bisphosphonate drug and has important therapeutic indications in the treatment of a variety of bone diseases such as hypercalcemia of malignancy, osteoporosis and Paget's disease [11,12]. In our laboratories, methods for assaying this non-chromophoric compound have been based on reversed-phase HPLC with pre- or post-column derivatization for UV or fluorescence detection [13–15] and ion chromatography with conductivity detection [16].

The novel CE method developed in this work is based on on-line complex formation between alendronate and $CuSO_4$ (1:1 stoichiometry) present in the electrolyte solution, which permits direct UV detection via the formation of an alendronate–Cu chromophore. The chemistry of the complexation reaction will be published elsewhere [17]. This strategy of on-line complex formation, to our knowledge, has not been demonstrated in CE for the direct detection of organic compounds without a chromophore. A similar approach of on-column metal chelate formation, using 4-(2-pyridylazo) resorcinol as the chromogenic reagent, has been reported for the direct determination of some metal ions by CE [18]. The indirect UV [19,20] or indirect fluorescence [21–23] mode, which monitors the negative (decreasing) signal, had been the primary approach in CE for this purpose.

This paper reports, for the first time, the application of CE to the assay of a non-chromophoric bisphosphonate drug using on-line complexing for direct UV detection. The feasibility of applying this method to assay alendronate in tablet and i.v. dosage forms was investigated. Quantitative aspects of CE were addressed in the light of validation data including injection precision, linearity, recovery and method precision. The performance of CE was compared with previous HPLC results [13,16]. The flexibility and versatility of CE as an analytical technique for the assay of low-molecular-mass drugs are also discussed.

## EXPERIMENTAL

### *Instrumentation*

A Spectra-Physics (San Jose, CA, USA) Model 1000 analytical capillary electrophoresis system equipped with a variable-wavelength UV–Vis detector and a data workstation was utilized in the constant-voltage mode for all experiments. A fused, uncoated silica capillary tube cartridge (Spectra-Physics) of 75 $\mu$m I.D. and 70 cm length (64 cm effective length from the anode to the detector) was used for all the studies unless stated otherwise. The applied voltage was 25 kV, the oven temperature was 25°C and the injection of sample was by vacuum for 2 s (*ca.* 0.4 ng per 4 nl). The capillary was first washed with 0.1 *M* NaOH for 1 min followed by rinsing with water for 1 min and the electrolyte solution of interest for 2 min between each injection during a series of runs. A solution of 1.6 m*M* $HNO_3$–2 m*M* $CuSO_4$ was used as an electrolyte for the determination of alendronate.

### *Chemicals and reagents*

Alendronate (MK-0217, $C_4H_{12}NO_7P_2Na \cdot 3H_2O$, $M_r$ 325.1), manufactured by Merck Research Labs. (Rahway, NJ, USA), was used as an analytical standard. All chemicals and reagents were used as received without further purification. Nitric acid (Optima grade) and copper(II) sulfate (analytical-reagent grade) were obtained from Fisher Scientific (Philadelphia, PA, USA). Deionized water with at least 18 MΩ resistance purified with a Milli-Q system (Millipore, Bedford, MA, USA) was used for electrolyte, standard and sample preparations. The electrolyte solution was filtered through a 0.45-$\mu$m microfilter (Millipore) before use.

### *Standard and sample preparations*

A method concentration of 0.1 mg/ml of alendronate was used. A standard solution was prepared by dissolving 32 mg of alendronate (equivalent to 25 mg of free acid) in 250 ml of electrolyte solution consisting of 1.6 m*M* nitric acid–2 m*M* $CuSO_4$. Tablet samples of 2.5 mg potency were dispersed and sonicated in an appropriate volume (content uniformity, one tablet in 25 ml; composite, ten tablets in 250 ml) of electrolyte solution for 5 min. The resulting solution (0.1 mg/ml) was filtered through a Millipore 0.22-$\mu$m filter unit and transferred to a sample vial for analysis. Intravenous solution (2.5 mg/ml) was diluted with the electrolyte solution to yield a concentration of 0.1 mg/ml for analysis.

## RESULTS AND DISCUSSION

### *Capillary electrophoresis*

The electrolyte solution used for the CE experiment was 1.6 m*M* $HNO_3$–2 m*M* $CuSO_4$. The addition of $CuSO_4$ to the electrolyte made possible on-line metal complex formation according to equation

$$\text{ABP} + \text{Cu}^{2+} \overset{K_{eq}}{\rightleftharpoons} \text{ABP–Cu}$$

For simplicity, ABP represents alendronate and the overall charge states shown in the above equation are not balanced. The bisphosphonate hydroxy moiety of the alendronate serves as a strong binding ligand for the transition metal with the resultant ABP–Cu complex (1:1 stoichiometry) having a UV-absorbing chromophore with an absorbance maximum at 230 nm [17]. A detection wavelength of 240 nm was selected which indicated a maximum "net" absorbance gained from the complexation reaction obtained by taking the difference between the spectrum of the ABP–Cu complex and the background [17]. Based on this on-line complexation reaction during the electrophoresis process, alendronate can be readily detected, as shown in Fig. 2, which is a typical electropherogram of a 0.1 mg/ml alendronate standard solution. Note that the absolute amount of solute analyzed here is *ca.* 0.4 ng (*ca.* 4 nl injected) and the signal shows a positive UV absorbance, unlike the negative signals from the indirect modes of detection [19–23] for non-chromophoric compounds.

Two control experiments were performed in order to verify the positive signal peak assigned to the ABP–Cu complex. An electrolyte solution (1.6 m*M* $HNO_3$–2 m*M* $CuSO_4$ without alendronate sample) was run under same conditions and yielded no signal within the experimental time domain. Further, electrolyte solution without the addition of $CuSO_4$ (1.6 m*M* $HNO_3$ only) did not produce a UV signal from an alendronate sample. This evidence unequivocally identifies the positive ABP–Cu peak observed in Fig. 2. A linear correlation between the signal response and concentration (see below) additionally supports the identity of the observed positive ABP–Cu peak.

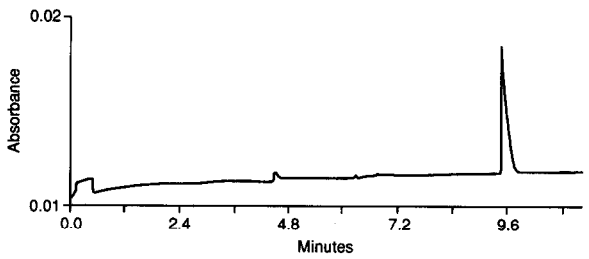


Fig. 2. Typical electropherogram of 0.1 mg/ml of alendronate standard solution dissolved in the electrolyte solution. Electrophoretic conditions as described in the Experimental section.

This novel CE procedure using the strategy of on-line complexation for determining alendronate is simpler than most of the HPLC methods available to date involving chemical derivatization [13]. It is pertinent to note that the on-line reaction in this study is facile enough (the complex formation is completed within the experimental time domain) [17] to eliminate the possible influences of the extent of reaction on the analytical results, such as migration rate, reproducibility and peak shape. The ABP–Cu complex approach is currently being applied to develop an HPLC procedure in order to provide a direct comparison between these two separation techniques. No success with the latter technique can be reported so far. The data comparison given below was made using the previously reported HPLC methods [13,16] and the proposed CE procedure. The CE method has been validated in terms of injection precision and linearity to evaluate the reproducibility and quantification capability of the CE method.

### *Validation*

Ten consecutive injections of a 0.1 mg/ml standard solution were made to test the reproducibility of migration times, peak areas and peak heights. Migration times were reproducible with a slight variation of 0.97% relative standard deviation (R.S.D.). The peak-area reproducibility was 4.3% R.S.D. and peak height showed a slightly better value of 2.4% R.S.D. The injection precision was examined without the use of an internal standard in this work to evaluate whether a quantitative precision comparable to that in state-of-the-art HPLC can be achieved on a routine basis. It is apparent that the precision found in this work is poorer than that in routine HPLC methods (generally R.S.D. < 2%). To the best of our knowledge, this relatively

TABLE I

RECOVERY OF ALENDRONATE FROM TABLET PLACEBO

Data were calculated by peak-area measurements.

| Level of potency (%) | Added (mg) | Found (mg) | Recovery (%) |
|---|---|---|---|
| 80 | 2.00 | 1.96 | 98.0 |
| 80 | 2.00 | 2.04 | 101.9 |
| 100 | 2.50 | 2.46 | 98.4 |
| 100 | 2.50 | 2.68 | 103.3 |
| 120 | 3.00 | 2.99 | 99.7 |
| 120 | 3.00 | 3.08 | 102.7 |
| | | Mean: | 100.7 |
| | | R.S.D.: | 2.3 |

high R.S.D. value is typically observed for CE techniques to date without the use of an internal standard (about 2–5% R.S.D.).

The lower precision of CE is probably due to the design of the automated injector and the extremely small injection volume in the nanoliter range. This conclusion was drawn from the following observations based on the literature [24–28], which suggests electrophoretic mobility of the solute, solute–capillary wall interaction (or adsorption) and the sample introduction systems as the major factors affecting precision. It has been recommended that the measured peak area is better normalized routinely by dividing the area by the migration time to correct for the electrophoretic variation [9]. In this study, peak-area normalization yielded a 4.6% R.S.D. (*vs.* 4.3% unnormalized) and did not improve the final reproducibility data because the migration time precision was satisfactory. Typical procedures for capillary pretreatment between each experimental run (see Experimental) were conducted to minimize the cumulative effect of solute–capillary interaction during the consecutive runs which might lead to sample loss and peak distortion, resulting in precision problems. The results of a set of experimental runs with omission of capillary pretreatment yielded approximately the same precision, although the peak revealed a slight tailing. This indicates that the solute–capillary interaction affects the analytical precision to a negligible extent under the CE conditions used in this study. We therefore consider that the sample injection device is the major factor responsible for the relatively high R.S.D. value as exemplified in the literature [29]. The use of an internal standard to eliminate the injection variation [9,10,30] has been reported to yield R.S.D. $<2\%$ in some instances. In this study, a propyl analogue of alendronate was employed as an internal standard without a satisfactory resolution for quantification under the described conditions. The search for a suitable internal standard is currently under investigation.

Linearity of the detector response over the concentration range between 50% and 150% of the method concentration (0.05–0.15 mg/ml) was examined. Linear plots of both peak area and peak height *vs.* absolute amount of alendronate yielded correlation coefficients ($R^2$) that were $\geqslant 0.999$ in both instances. A non-zero intercept ($y = 7.8 \cdot 10^3 x - 3.1 \cdot 10^2$) occurred in the plot of peak area *vs.* amount of alendronate (less pronounced in the peak-height plot) in some instances. This phenomenon was found to be insignificant with respect to the accuracy of the measurement at the method concentration (0.1 mg/ml).

TABLE II

CONTENT UNIFORMITY DATA FOR 2.5-mg TABLETS ASSAYED BY VARIOUS METHODS

Data are averages for ten tablets based on peak-area measurements.

| CE method | | HPLC–FMOC method[a] | | IC–conductivity method[a] | |
|---|---|---|---|---|---|
| Found (mg) | % of label | Found (mg) | % of label | Found (mg) | % of label |
| 2.50 (R.S.D. 2.1%) | 100.2 | 2.45 (R.S.D. 0.7%) | 97.8 | 2.47 (R.S.D. 0.8%) | 98.9 |

[a] Data cited from Ref. 16.

*Analysis of formulations*

The CE method for the assay of alendronate in 2.5-mg tablet was validated in terms of recovery, content uniformity and composite assay. Data for recovery obtained by spiking aliquots of a stock solution of alendronate drug (0.5 mg/ml) on placebo in duplicate at 80%, 100% and 120% levels of potency are listed in Table I. Good recovery data (average 100.7%) were obtained with 2.3% R.S.D. Content uniformity tests on ten replicate tablets and two composite assays ($N$ = 10 tablets each) were performed to establish the method precision. Acceptable percentages of the label claims were obtained for uniformity (100.2% as listed in Table II) and two composite assays (102.1% and 101.7%). For comparison purposes, the uniformity data obtained previously by HPLC with 9-fluorenylmethyl chloroformate derivatization for UV detection (HPLC–FMOC) and ion chromatography with conductivity detection (IC–conductivity) methods [13,16] are also listed in Table II. Note that the CE method produces about a 1–2% difference from the HPLC results. This is probably due to the assay variation, which is not statistically significant. The R.S.D. for establishing the CE method precision was 2.1%, which represents the sum of the variations from the assay and the manufacture of the tablet formulation. It is pertinent to note that the R.S.D.s without an internal standard for CE are slightly higher than the HPLC results (< 1%, Table II), probably because of present instrumental limitations discussed previously. Regardless of the poorer precision, the present CE method can be used for the direct assay of alendronate tablets. The electrolyte solution, sample and standard solutions were stable for at least 72 h. Most important, the time required for capillary equilibration with the electrolyte solution before generating reproducible data was generally about 1–2 h.

Identical CE conditions were applied to the assay of i.v. solutions; however, erratic and irreproducible results were obtained. The assay results for ten aliquots of i.v. solutions analyzed by CE yielded an average of 38.4% of the label claim and an R.S.D. of 34.1%. It is pertinent to note that a control i.v. sample containing no citrate anion (an excipient present in the i.v. formulation) yielded nearly a 100% drug recovery. It is therefore believed that the presence of citrate anion, which is also a strong metal-chelating agent, tends to distort the equilibrium between alendronate and $Cu^{2+}$ and results in the irreproducible formation of the alendronate–Cu complex. Attempt to increase the $Cu^{2+}$ concentration for complexing with both components could result in an excessive background absorbance and hence a detector over-range. Consequently, the strategy of on-line copper complex formation is not applicable to formulations containing a competitive metal-chelating agent.

*Comparison of CE and HPLC methods*

Table III summarized several parameters of alendronate assays obtained using the proposed CE method and the previous derivatized HPLC–FMOC method [13] and IC–conductivity method [16] for a general overview. As indicated, CE demonstrates the most efficient separation power. CE also shows advantages over HPLC in terms of the speed of method development and ease of assay procedure, including simple sample preparation. Additionally, CE requires and extremely small amount of organic solvent-free electrolyte, which

TABLE III

SUMMARY OF CHROMATOGRAPHIC PARAMETERS FROM VARIOUS ASSAY METHODS FOR ALENDRONATE

| Method | Theoretical plates ($N$) | Tailing factor (5% peak height) | Sample preparation (time needed per tablet) | Mobile phase consumption (per run) | Precision [R.S.D. (%)] |
|---|---|---|---|---|---|
| CE (this work) | *ca.* 47 000 | 1.07 | 8 min | *ca.* 0.5 μl | 2–5 |
| HPLC–FMOC[a] | *ca.* 400 | 0.87 | *ca.* 4 h | 10 ml | <2 |
| IC–conductivity[b] | *ca.* 1500 | 0.86 | 8 min | 6 ml | <2 |

[a] Data cited from ref. 13 for Hamilton PRP-1 column.

[b] Data cited from ref. 16 for Waters IC-Pak HR anion-exchange column.

avoids the problem of solvent disposal. Nevertheless, the drawback of this method is the poorer precision. Although the CE method demonstrates feasibility for alendronate assay, it might be necessary to improve the precision by adding an internal standard.

## CONCLUSIONS

CE in conjunction with on-line complexation with $Cu^{2+}$ is applicable to the direct assay of alendronate. This has simplified the analytical procedure for such a non-chromophoric bisphosphonate drug. This on-line generation of a chromophore for direct detection has demonstrated a new avenue of UV detection for compounds that have no significant chromophore, other than the indirect approach, which monitors the decreasing (negative) signal of the electrolyte background. Quantitative aspects of CE established by the validation of this method show that CE is suitable for the assay of alendronate in tablet dosage forms. Assay of i.v. solutions by the same approach was hindered by the competitive complex formation with citrate anion present in the formulation. The precision of the CE technique is poorer than that of routine HPLC, probably owing to the limitations of sample injection with the instrumentation currently available. Perhaps a better precision could be achieved by adding a suitable internal standard to circumvent the injection variation. Several advantages can be claimed for CE, such as high efficiency, small sample amount, low solvent consumption and ease of analysis. Currently, CE is utilized as an alternative chromatographic method for the verification of specificity for the analysis of low-molecular-mass drugs and is considered to be an excellent complementary separation technique to HPLC. From our perspective, the flexibility and versatility of CE, which permit rapid operation for method development and various separation modes on a single separation capillary, should make it particularly attractive in pharmaceutical analysis and should be increasingly pursued in the pharmaceutical industry.

## ACKNOWLEDGEMENTS

The authors thank D. Hollinshead of Spectra-Physics for the loan of a capillary electrophoresis instrument. Technical support from F. Tinter and P. Simko of Spectra-Physics is also appreciated.

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