Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, Nos 10–12, pp. 881–887, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00

Determination of disodium clodronate in bulk material and pharmaceuticals by ion chromatography with post-column derivatization*

JUSSI P. KOSONEN

Leiras Oy, Analytical Development, P.O. Box 415, SF-20101, Turku, Finland

Abstract: A validated ion chromatographic method for the determination of disodium clodronate in bulk material and pharmaceuticals is described. Separations are performed on a poly(styrene-divinylbenzene) copolymer column using 40 mM nitric acid as mobile phase at a flow rate of 0.5 ml min^{-1}. The analyte is detected by UV absorption at 300 nm after post-column derivatization with acidic iron(III) solution (0.25 ml min^{-1}). The proposed ion chromatographic method is validated in terms of selectivity, precision, linearity, accuracy and ruggedness.

Keywords: *Bisphosphonate; clodronate disodium; ion chromatography; pharmaceutical analysis.*

Introduction

Clodronic acid in the disodium form (BONEFOS®) [disodium clodronate tetrahydrate; disodium (dichloromethylene)-bisphosphonic acid tetrahydrate)], see Fig. 1, has been investigated as an inhibitor of enhanced osteoclastic bone resorption and bone mineral dissolution and as a drug for Paget's disease and malignant hypercalcaemia [1, 2]. Disodium clodronate has not been described in any Pharmacopoeia and for this reason the development of a method for the assay of the drug was undertaken. An accurate and well characterized method for the determination of disodium clodronate is necessary for the evaluation of the bulk drug and the measurement of the stability of pharmaceutical formulations. The assay method was required to be free from interference by related substances and degradation products.

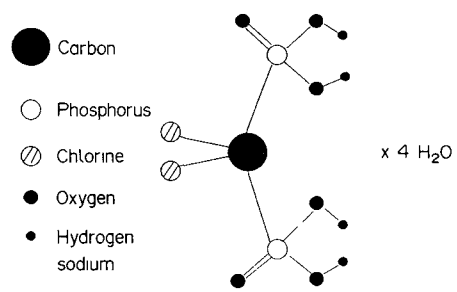

Figure 1
Disodium clodronate tetrahydrate.

A limited number of analytical procedures are available in the literature for the determination of clodronic acid. These methods involve isotachophoresis with detection of non-absorbing zones [3], high-performance liquid chromatography (HPLC) using an ion-exchange column with a flame photometric detector to measure phosphorous emission [4] or with post-column indirect fluorescence detection using the alumin–morin complex [5] and capillary gas chromatography–mass spectrometry with pre-column trimethylsilylation [6]. These procedures, however, require specialized equipment and detectors and, furthermore, involve derivatization processes that have inherent limitations because of lengthy, cumbersome procedures and in some cases, poor yields, which are inconvenient for routine applications.

Generally, bisphosphonic acids are non-volatile and strongly polar compounds which dissociate completely at neutral pH. As a consequence it is convenient to analyse these compounds as such by HPLC. The determination of disodium clodronate represents a distinct challenge because of the lack of a UV chromophore in the molecule, the tendency of the compound to strongly chelate metals and a propensity to produce tailing peaks. Although the already published HPLC methods [4, 5]

*Presented at the "Fourth International Symposium on Drug Analysis", May 1992, Liège, Belgium.

describe elegant applications for the determination of disodium clodronate there is still a need for a more generally applicable method. The detection modes used previously are not as established in quality control laboratories as UV detection. Therefore, a more general method has been developed and validated. This paper describes a method for dealing with the above problems to provide a suitable assay procedure for the determination of disodium clodronate.

Disodium clodronate is one of many compounds that cannot be analysed directly by liquid chromatography with UV detection because of its lack of a UV chromophore. This problem can be overcome by labelling with a chromophore. The chromatographic system described here is based upon the ion chromatography using post-column derivatization with acidic iron(III) ion developed for the determination of inorganic anions [7] and other polyvalent anions [8]. The new procedure is simple enough for a large number of samples, and also it enables the monitoring of the stability of the bulk material and pharmaceuticals with reasonable selectivity and simplicity.

Experimental

Equipment

A metal-free Dionex model 4500i liquid chromatograph was used consisting of a variable wavelength detector, operated at 300 nm, a gradient pump and a pneumatic post-column reagent delivery system. Samples (25 μl) were injected by means of an automated sampler system onto a guard column (IonPac AG7, 50 × 4 mm i.d.) in series with a separation column (IonPac AS7, 250 × 4 mm i.d.) packed with poly(styrene-divinylbenzene) copolymer (10 μm particles). Analyte peak areas were measured using AI-450 Chromatography Software (Dionex) running on a personal computer (IBM).

Reagents

Analytical grade nitric acid (65%, Baker), sulphuric acid (95–97%, Baker), perchloric acid (70–72%, Merck) and iron(III) nitrate nonahydrate (minimum purity 99%, Merck) were used.

References materials

Disodium hydrogen orthophosphate dihydrate (Baker; A), phosphonoformate trisodium (Astra; B), dichloromethylphosphonate (C), hydroxymethylenebisphosphonate (D), monochloromethylenebisphosphonate (E), and disodium clodronate tetrahydrate synthesized by Leiras were used.

Analytical procedure

Internal standard solution. 2.0 ml of sulphuric acid was pipetted into a 1000 ml volumetric flask and diluted with water to volume.

Post-column reagent. 0.5 g of $Fe(NO_3)\cdot 9H_2O$ was dissolved in water, 20 ml of 70% $HClO_4$ added and the solution made up to 1000 ml with water.

Sample preparation. About 125 mg of bulk drug was weighed accurately, dissolved in water and diluted with water to 50 ml.

Capsule and tablet samples were weighed to obtain the equivalent of about 200 mg of disodium clodronate, dissolved in water and sonicated for 10 min at room temperature. The solution was diluted with water to 100 ml and a portion of solution was centrifuged at 2000 rpm for 10 min.

Samples of the injectable preparation were pipetted to obtain the equivalent of 120 mg of disodium clodronate and diluted with water to 100 ml.

Two millilitres of the above solutions of bulk material, capsule and tablet, and 3.0 ml of the solution of injectable preparation were pipetted into 100 ml volumetric flasks, and 5.0 ml of internal standard solution added. The flasks were made up to volume with water. A typical chromatogram is shown in Fig. 2.

Results and Discussion

Method development

In order to develop a stability-indicating assay method which may be used in pharmaceutical product development it is necessary to ensure the separation of the analyte from its degradation products. Disodium clodronate and most of its degradation products are tetraprotic acids so that the mobile phase pH can be used to control their anionic charge. In the separation described here, consistent with anion-exchange behaviour, aqueous 40 mM nitric acid was used as the eluent for the retention and separation of disodium clodronate and internal standard from the impurities. The pH of the mobile phase (40 mM) is about

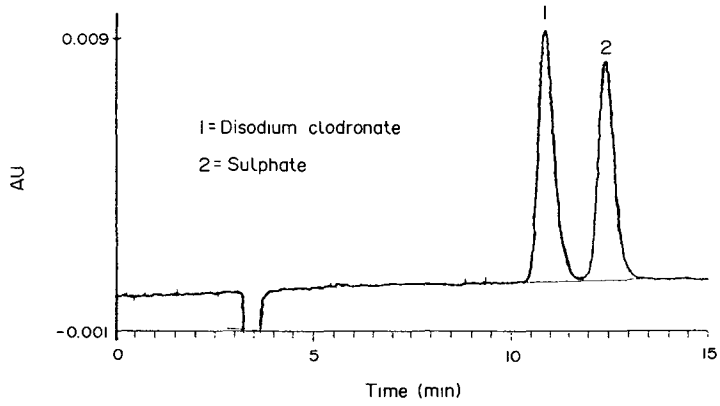

Figure 2
Typical chromatogram of disodium clodronate and sulphate (internal standard). Experimental conditions as given in the text.

1.4 so that the molecules are predominantly in the monovalent (−1) form. If the pH of the mobile phase is increased the overall charge of the molecules increases so that they elute later due to their higher affinity for the anion-exchange resin. Initial experiments to optimize the eluent showed that disodium clodronate behaves as would be expected in an anion-exchange system. Thus as the amount of acid decreased (pH increased) the retention time was found to increase. For instance, with a nitric acid concentration of 40 mM the retention time of disodium clodronate was found to be about 11 min but for 20 mM nitric acid it was 50 min. The degradation products behaved similarly to disodium clodronate.

The major obstacles in the development of a separation method for disodium clodronate are the lack of a UV chromophore in the molecule and its strong chelating ability. Direct conductivity detection is not practical because of the high background conductance of the nitric acid mobile phase. Due to its strong chelating ability, disodium clodronate yields asymmetric peaks due to interactions with metal ions leached from the HPLC system or metal ions as impurities in the stationary phase. To overcome these problems the use of a metal-free ion chromatograph coupled with a post-column derivatization system was studied. Post-column derivatization is performed by introducing a suitable reagent into the column effluent. Iron(III) was chosen as the post-column reagent because (a) it gives a chemical reaction with disodium clodronate leading to UV absorption; (b) the reaction between iron(III) and disodium clodronate is rapid; and (c) iron(III) can be used in acidic medium, which also is more favourable for disodium clodronate than an alkaline medium.

The oxygen atoms are the potential ligand atoms in the disodium clodronate molecule. When these atoms bind to a metal ion, the ligand atoms lose their protons. The complexation reaction of disodium clodronate with iron(III) was followed at pH 0.5–6.0 and the absorption spectra were recorded between 230–400 nm. It was found that disodium clodronate and iron(III) form a chelate with a maximum absorbance between 240 and 285 nm. The absorptivity of the chelate increases and the wavelength of the maximum absorbance shifts to the lower wavelengths as pH decreases. If the pH is increased to 4.0 the absorbance of the chelate decreases probably because of the concurrent hydrolysis of iron(III). The stoichiometry of the reaction between disodium clodronate and iron(III) ion is 1:1 determined by the application of Job's method.

The examination of the effects of the length (50–140 cm) and diameter (0.3–0.5 mm) of post-column tubing indicated that the diameter and the length of the tube have significant effects on peak shape. A post-column tube with an internal diameter of 0.3 mm and a length of 140 cm gave peak shapes suitable for quantitative analysis.

The detector wavelength was optimized by determining the average peak heights for duplicate 25 μl injections of a 4 μg ml^{-1} disodium clodronate solution for various wavelengths between 290 and 310 nm. The peak heights were normalized for noise by dividing the average heights by the corresponding baseline noise. The noise-normalized peak height

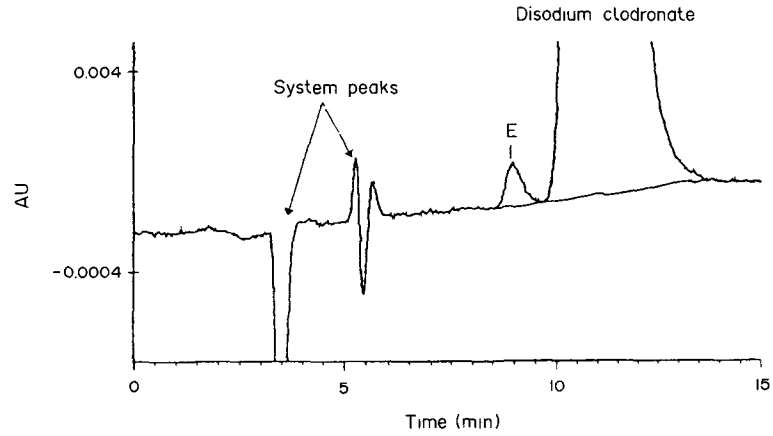

Figure 3(a)
Chromatogram of intact disodium clodronate. Experimental conditions as given in the text. E = monochloromethylene-bisphosphonate.

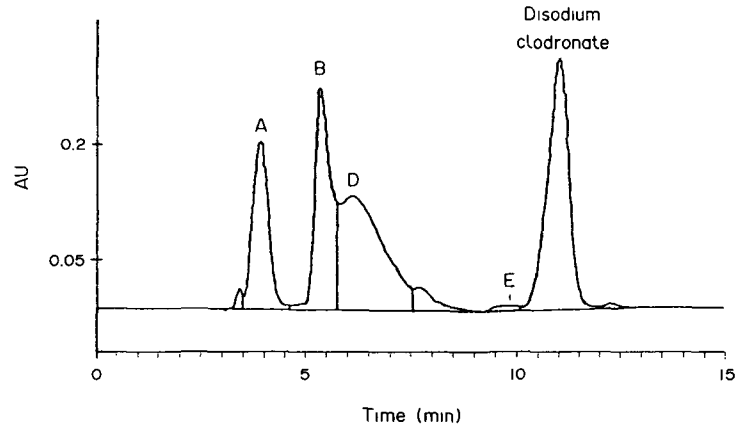

Figure 3(b)
Chromatogram of forcibly degraded disodium clodronate. Experimental conditions as given in the text. A, hydrogen orthophosphate; B, phosphonoformate; D, hydroxymethylenebisphosphonate; E, monochloromethylenebisphonate.

Table 1
Normalization area reports for the intact and degraded disodium clodronate samples shown in Fig. 3(a) and (b)

Normalization (area)			
Retention time (min)	Area	Area (%)	Component name
	Intact disodium clodronate		
8.33	2624	0.09	E
11.53	2984800	99.91	Disodium clodronate
	Degraded disodium clodronate		
3.40	17650	0.58	Unknown
3.87	482121	15.97	A
5.33	611604	20.25	B
6.07	828212	27.43	D
7.70	89691	2.97	Unknown
9.03	16430	0.54	E
11.53	965854	31.99	Disodium clodronate
12.24	8103	0.27	Unknown

A, B, D and E; see reference materials. Experimental conditions as given in the text.

versus wavelength curve indicated that the maximum sensitivity is achieved under these conditions at about 300 nm instead of at the absorbance maximum of the chelate (285 nm).

Method validation

Selectivity. In order to demonstrate that the assay method is stability indicating it is necessary to show that disodium clodronate and the internal standard can be separated from potential degradation products which could form during the shelf-life of the product. This was demonstrated by exposing a sample containing a 4 mg ml^{-1} of disodium clodronate to extreme conditions (treated with acid, base, peroxide, heat and UV light). Only the degradation caused by hydrolysis in basic solution was remarkable. Figure 3(a) and (b) show the comparative chromatograms and Table 1 the results for bulk samples of intact and degraded disodium clodronate. The degraded sample was obtained by refluxing the sample in 0.1 M sodium hydroxide solution for 1 h. The chromatograms and tabulated data clearly indicate higher levels of impurities in the degraded sample. Note that there are differences between chromatograms 2 and 3(a) because the sample in Fig. 3(a) does not contain sulphate and disodium clodronate concentration is hundred-fold compared with Fig. 2, and the recorder is at higher sensitivity.

A chromatogram of disodium clodronate spiked with four major possible impurities is presented in Fig. 4. The relative retention times and capacity factors of these impurities are given in Table 2.

Precision. In order to determine the precision of the method, six samples of bulk material and pharmaceutical formulations were assayed. The precision for the determination of disodium clodronate in bulk material, tablet, capsule and injectable formulations determined on six replicate assays are represented by RSD values of 0.9, 1.3, 0.7 and 0.8% respectively, see Table 3.

Linearity. The method is linear in concentration range of 50–175% of the normal sample concentration (0.02–0.07 mg ml^{-1} of disodium clodronate) with a correlation coefficient of 0.9999 ($n = 6$). The equation corresponding to the graph can be expressed as $y = 294x - (9.9 \times 10^{-3})$, where x is the concentration of

Table 2
Selectivity of the assay. Relative retention times (R_t) and capacity factors (k') of the possible impurities and sulphate

Compound	R_t	k'
A	0.36	0.1
B	0.50	0.5
C	0.38	0.2
D	0.58	0.9
E	0.84	1.7
Disodium clodronate	1.00	2.1
Sulphate	1.19	2.5

A, B, C, D and E; see reference materials. Experimental conditions as given in the text.

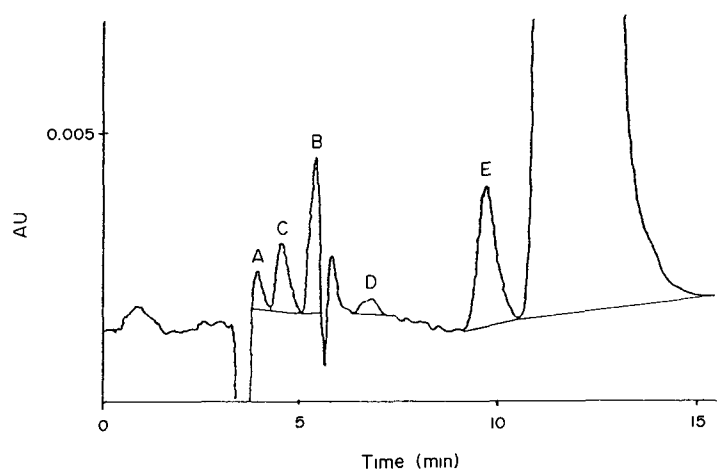

Figure 4
Chromatogram obtained by injecting disodium clodronate and a mixture of probable impurities. Experimental conditions as given in the text. A, hydrogen orthophosphate; B, phosphonoformate; C, dichloromethylphosphonate; D, hydroxymethylenebisphosphonate; E, monochloromethylenebisphonate.

Table 3
Precision of the assay method for disodium clodronate

Formulation	Number of determinations	Assay	RSD (%)
Bulk material	6	99.1%	0.9
400 mg Capsule	6	402.8 mg	0.7
800 mg Tablet	6	811.7 mg	1.3
60 mg ml^{-1} Inf. conc.	6	57.4 mg ml^{-1}	0.8

Experimental conditions as given in the text.

Table 4
Recovery of disodium clodronate added to experimental formulations

Formulation	Added amount (mg)	Number of determinations	Recovery (%)	RSD (%)
400 mg Capsule	323–503	6	99.5	0.5
800 mg Tablet	644–968	6	100.7	0.6

Experimental conditions as given in the text.

disodium clodronate injected (μg ml^{-1}) and y is the corresponding peak response.

Accuracy. The accuracy of the method was determined by assaying spiked placebo samples of capsules and tablets and comparing the results obtained with the expected values. The spiked samples were obtained by adding disodium clodronate to the tablet and capsule ingredients in duplicate at 80, 100 and 120% level of the normal sample concentration. The spiked samples went through an entire sample preparation procedure. The recovery of disodium clodronate was satisfactory with a mean recovery of 99.5 and 100.7% for capsule and tablet, respectively, see Table 4.

Ruggedness. The ruggedness of the method was evaluated by determining the stability of the sample solutions and batch-to-batch variability of the column.

The analytical solutions were found to remain stable over a period of 60 h in glass bottles at room temperature.

The parameters calculated from the chromatograms of two different batches of columns showed satisfactorily small batch-to-batch variability in column resolving power, see Table 5. The difference between the two columns in terms of number of theoretical plates is mainly due to the fact that the first column (1255) has been used over a year and the second column (3052) is new.

Table 5
System suitability tests performed using two different columns supplied by one column manufacturer

Batch of AS7 column	k'_1	k'_2	R	n
1255 (Old column)	2.1	2.6	2.0	2500
3052 (New column)	2.3	2.6	2.0	5100

k'_1 = Capacity factor for disodium clodronate. k'_2 = Capacity factor for sulphate. R = Peak resolution. n = Number of theoretical plates. Experimental conditions as given in the text.

Conclusions

A specific, stability-indicating high-performance ion chromatographic method for the determination of disodium clodronate has been developed using post-column derivatization with iron(III) ion and UV detection. The method demonstrates an advantage in ease of sample preparation, and is shown in this study to have acceptable precision and accuracy for routine analysis of disodium clodronate in bulk materials and pharmaceuticals. The post-column derivatization also offers an advantage in more selective detection compared with indirect detection mode where all of the non-absorbing species respond. Based upon the results described here the method enables the determination of disodium clodronate in an accurate and easy manner.

Acknowledgement — The excellent technical support of Ms M. Jokinen is acknowledged.

References

[1] A.J.P. Yates, R.C. Percival, R.E.S. Gray, R.M. Atkins, G.H. Urwin, N.A.T. Hamdy, C.J. Preston,

M.N.C. Beneton, R.G.G. Russell and J.A. Kanis, *Lancet* **1**, 1474–1477 (1985).
[2] I. Elomaa, C. Blomqvist, P.Gröhn, L. Porkka, A.-L. Kairento, K. Selander, C. Lamberg-Allardt and T. Holmström, *Lancet* **1**, 146–148 (1983).
[3] Ph. Gysen and G. Heynen, *Biochemical and Biological Applications of Isotachophoresis* (A. Adam and C. Schots, Eds), pp. 81–84. Elsevier, Amsterdam (1980).
[4] T.L. Chester, E.C. Lewis, J.J. Benedict, R.J. Sunberg and W.C. Tettenhorst, *J. Chromatogr.* **225**, 17–25 (1981).
[5] S.E. Meek and D.J. Pietrzyk, *Anal. Chem.* **60**, 1397–1400 (1988).
[6] S. Auriola, R. Kostiainen, M. Ylinen, J. Mönkkönen and P. Ylitalo, *J. Pharm. Biomed. Anal.* **7**, 1623–1629 (1989).
[7] T. Imanari, S. Tanabe, T. Toida and T. Kawanishi, *J. Chromatogr.* **250**, 55–61 (1982).
[8] A.W. Fitchett and A. Woodruff, *Liq. Chromatogr. HPLC Mag.* **1**, 48–49 (1983).

[Received for review 29 April 1992; revised manuscript received 26 June 1992]