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Determination of disodium 3-amino-1-hydroxypropylidene-1,1-bisphosphonate pentahydrate

M. ZELLER*, R. KESSLER, H. J. MANZ and G. SZÉKELY
Ciba-Geigy Ltd., Central Analytical Department, 4002 Basle (Switzerland)

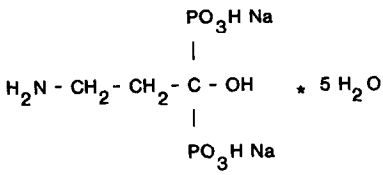
ABSTRACT

Capillary isotachophoresis was applied to the analysis of a new class of bisphosphonates. By using a commercial isotachophoretic system (Shimadzu IP-3A) with a fused-silica detection capillary in comparison with an LKB Tachophor equipped with a PTFE capillary a six-fold higher sensitivity is obtained. In addition, the time of analysis can be shortened. The developed system is applicable to the analysis of disodium 3-amino-1-hydroxypropylidene-1,1-bisphosphonate pentahydrate (Aredia), the main component of the drug formulation (dry substance for injection). Phosphite and phosphate, which are considered to be its trace level by-products, were determined simultaneously. The relative standard deviation for each compound was about 1%.

INTRODUCTION

Most applications dealing with the analysis of bisphosphonates or polyphosphonates [1–8] have been done by high-performance liquid chromatography [1], especially by ion chromatography [2–4], ion-pair chromatography and ion-exchange chromatography [5–7], and only a few by isotachophoresis (ITP) [8]. Capillary ITP is an electrophoretic method suitable for the separation of ionogenic organic and inorganic compounds which are soluble in water or water–organic and inorganic compounds which are soluble in water or water–organic solvent mixtures. For daily routine analysis of disodium 3-amino-1-hydroxypropylidene-1,1-bisphosphonate pentahydrate, $H_2NCH_2CH_2C(OH)(PO_3HNa)_2 \cdot 5H_2O$ (Aredia) (quality control and stability test), we use ITP.

Aredia is a new drug for the treatment of cancer. It has been shown that is is very effective in the treatment of tumour-induced hypercalcaemia because it inhibits



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bone resorption. In many cancer patients high blood levels of calcium occur owing to the effects of bone metastasis [9].

Hypercalcaemia can lead to disfunction of the gastrointestinal tract, of the kidneys and of the nervous system. In severe cases hypercalcaemia can even be fatal. The bisphosphonates are sympathetic chemical analogues of pyrophosphate, a natural inhibitor of crystal nucleation and bone mineralization.

Investigations have suggested that in some instances treatment with this bisphosphonate inhibits cancerous changes in the bone (metastases or secondary tumours). The drug is administered with slow intravenous infusions (between 1 and 4 h). To lower calcium levels in blood for several weeks, a single infusion is sufficient. However, the success of the therapy depends on the severity of the individual case [9,10].

Further clinical studies are necessary to determine the efficiency of Aredia in the treatment of bone metastasis without accompanying hypercalcaemia. Another very common site of metastasis is the skeleton. The disease spreads into the bone in about 60% of patients with cancer of the breast, prostate and lungs. There it causes progressive weakening and finally leads to the destruction of the bone tissue.

Therefore, it is necessary to have a highly reproducible, sensitive, accurate and simple method for the analysis of dosage forms for Aredia and potential by-products thereof.

EXPERIMENTAL

Chemicals and reagents

β-Alanine was purchased from Fluka (Buchs, Switzerland), hydroxypropylmethylcellulose (HPMC), used in the leading electrolyte system, from Prochem (Zürich, Switzerland) and Triton X-100 from Sigma (Heidelberg, Germany). All chemicals were of analytical-reagent grade and were used as received. Water was distilled twice in a quartz apparatus.

Instrumentation

Analytical capillary ITP was performed on two different instruments: an LKB 2127 Tachophor (Pharmacia, Bromma, Sweden) equipped with UV (254 nm) and conductivity detectors, using a PTFE capillary (43 cm × 0.5 mm I.D.), and an IP-3A semi-automatic ITP system from Shimadzu (Tokyo, Japan), using a PTFE separation capillary (16 cm × 0.7 mm I.D.) and a fused-silica detection capillary (17 cm × 0.2 mm I.D.).

Conditions

LKB Tachophor. The driving current was maintained for 16 min at 150 μA (starting voltage 3 kV) and then reduced to 50 μA for reasons of detection (end voltage 5 kV). The signals were recorded on an ITP-5A two-channel integrator (Shimadzu).

Shimadzu IP3-A. The current gradient applied was (1) 300 μA until 5.5 kV was reached, (2) 200 μA until 2261 μA min was reached, (3) 40 μA for about 4 min and (4) 15 μA for detection. The signals were recorded on an ITP-5A two-channel integrator (Shimadzu).

Electrolyte systems

LKB Tachophor. The leading electolyte was 25 ml of 0.1 *M* hydrochloric acid and 445.51 mg of β-alanine dissolved in 250 ml of 0.2% (w/v) nitrogen-saturated HPMC solution (pH 3.6) and the terminating electrolyte was 10 ml of 0.1 *M* sodium hydroxide and 20 ml of 0.1 *M* acetic acid diluted in water to 200 ml with 125 μl of Triton X-100 being added (pH 4.7).

Shimadzu IP3-A. The leading electrolyte was 50 ml of 0.1 *M* hydrochloric acid and 891.2 mg of β-alanine diluted in water to 500 ml with 500 μl of Triton X-100 being added (pH 3.6) and the terminating electrolyte was the same as for the LKB Tachophor.

Standard solution

Approximately 100.0 mg of Aredia working standard were accurately transferred into a 50-ml volumetric flask, 5 ml of phosphite solution (1 mg/ml HPO_3^{2-}) and 10 ml of phosphate solution (1 mg/ml PO_4^{3-}) were added and the mixture was diluted to volume with distilled water. A 6-μl volume of this solution containing *ca.* 9.1 μg of bisphosphonate, 0.6 μg of phosphite and 1.2 μg of phosphate was applied.

Sample solution

The contents of five vials of the drug formulation were dissolved in water and further water was added to give 100 ml.

RESULTS AND DISCUSSION

As shown in Fig. 1, we applied equal amounts to the two different detection

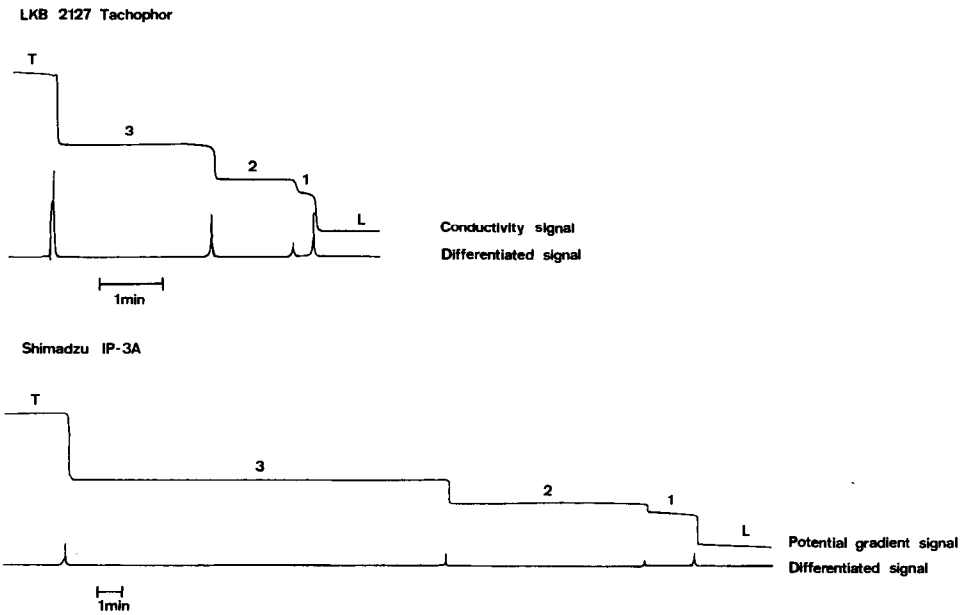

Fig. 1. Comparison of the LKB and Shimadzu systems. Equal amounts of standard solution (10 μl) were injected. (1) = 2 μg of phosphite; (2) = 0.5 μg of phosphate; (3) = 13 μg of bisphosphonate. L = leading ion (chloride); T = terminating ion (acetate).

TABLE I

ACCURACY OF THE ASSAY

Weight taken (mg)	Weight found from zone length (mg)	Recovery (%)
105.96	110.66	104.44
103.79	106.81	102.91
104.03	107.71	103.53
108.20	111.05	102.63
111.30	112.72	101.27
118.14	118.88	100.63
Mean		102.57
95% confidence limits		101.09–104.05

TABLE II

PRECISION (REPEATABILITY) OF THE ASSAY

Weight taken (mg)	Zone length relative to 30.0 mg (mm)
105.96	22.6
103.79	22.2
104.03	22.3
108.20	22.2
111.30	21.9
118.14	21.9
110.87	21.8
102.67	21.9
107.66	22.3
104.70	22.3
Mean	22.1
Standard deviation	0.25
Relative standard deviation	1.15%

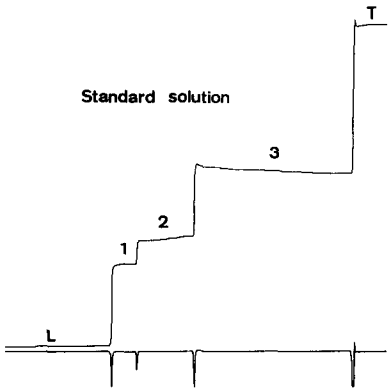

Fig. 2. Standard solution containing (1) 0.6 μg of phosphite, (2) 1.2 μg of phosphate, (3) 9.1 μg of bisphosphonate. L = leading ion (chloride); T = terminating ion (acetate); 6 μl of standard solution were injected; apparatus, LKB 2127 Tachophor with conductivity detector.

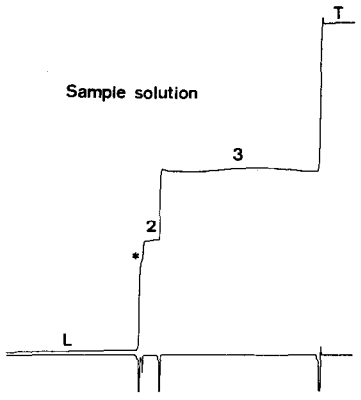

Fig. 3. Sample solution containing 150 mg of bisphosphonate per 100 ml. (* = Impurity from the electrolyte system; 2 = phosphate; 3 = bisphosphonate. L = leading ion (chloride); T = termination ion (acetate); 6 μl of sample solution were injected; apparatus, LKB 2127 Tachophor with conductivity detector.

capillaries. The increase in the zone length was derived from the ratio of the inside diameters of the two capillaries. With the fused-silica detection capillary, the zone lengths were six times longer. Obviously, the same volume needs a much longer capillary distance during detection because of the reduced diameter. Further, there is an increase in accuracy. ITP can be applied easily to this problem and it has the advantage of excellent accuracy and precision (see Tables I and II); further, UV-invisible components could be detected at trace levels.

By using the electrolyte systems mentioned above, routine analysis can be done in 35 min with good selectivity (Figs. 2 and 3). The total analysis time was *ca.* 10 min shorter by using the Shimadzu system than with the LKB system. One of the reasons is the semi-automatic rinsing and filling with the electroytes.

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