ELSEVIER

Journal of Chromatography B, 696 (1997) 137–144

JOURNAL OF CHROMATOGRAPHY B

Determination of pamidronate in urine by ion-pair liquid chromatography after derivatization with 1-naphthylisothiocyanate

Rolf W. Sparidans[a], Jan den Hartigh[a,*], Jos H. Beijnen[b], Pieter Vermeij[a]

[a]*Department of Clinical Pharmacy and Toxicology, Leiden University Hospital, PO Box 9600, 2300 RC Leiden, The Netherlands*
[b]*Department of Pharmacy, Antoni van Leeuwenhoekhuis/Slotervaartziekenhuis, Louwesweg 6, 1066 EC Amsterdam, The Netherlands*

Received 5 December 1996; received in revised form 14 March 1997; accepted 27 March 1997

Abstract

A sensitive method for the determination of pamidronate disodium [(3-amino-1-hydroxypropylidene)bisphosphonate, APD] in urine has been developed and validated. The procedure involves a triple co-precipitation with calcium phosphate, solid-phase extraction on a quaternary ammonium column, derivatization with 1-naphthylisothiocyanate and ion-pair liquid–liquid extraction. From the two reaction products, naphthylthiocarbamyl-APD is converted into the other, naphthylcarbamyl-APD, by an oxidative desulphuration with hydrogen peroxide prior to analysis by ion-pair HPLC and fluorescence detection at 285/390 nm. The method has a coefficient of variation of 7% for the intra-assay precision of 99 ng ml^{-1} APD and 11% for the inter-assay precision. The lower limit of quantification is 3 ng ml^{-1} APD in 2.5 ml of human urine. © 1997 Elsevier Science B.V.

Keywords: Pamidronate; 1-Naphthylisothiocyanate; Bisphosphonates

1. Introduction

Pamidronate disodium [(3-amino-1-hydroxypropylidene)bisphosphonate, APD] is an important and well known representative of the bisphosphonates, a relatively new group of drugs, applied in the treatment of Paget's disease, hypercalcaemia and osteoporosis [1,2]. Clinical investigations into pamidronate, the first of a generation of potent aminobisphosphonates, were already started in the late 1970's. For clinical pharmacological investigations a reliable and sensitive bioanalytical assay is a prerequisite.

The first methods in bisphosphonate analysis, developed for etidronate [(1-hydroxyethylidene)bisphosphonate, EHDP] measurement in biological media, were non-chromatographic and were based on either titration with thorium diaminocyclohexanetetraacetate [3] or on detection of phosphate after decomposition of the bisphosphonate [4]. Both methods were very laborious but sensitive enough to measure EHDP concentrations in biological media during or shortly after the administration of the drug.

Bioanalytical methods, developed later for several new generation bisphosphonates, are all based on chromatography. Since the newer bisphosphonates are more potent and are therefore administered in lower doses [1,2], a more sensitive method is necessary. Chester et al. [5] applied IEC with flame photometric detection for the determination of

*Corresponding author.

PII S0378-4347(97)00226-0

clodronate [(dichloromethylene)bisphosphonate]. The same separation technique was also combined with absorbance detection after a post-column complexation. Thorium–ethylenediaminotetraacetic acid–xylenol was used as the complexing reagent for clodronate by Virtanen et al. [6]; Daley-Yates et al. [7] employed molybdenum-ascorbate for phosphate complexation after oxidation of pamidronate. A GC–MS method for clodronate was developed by Auriola et al. [8]. All other methods published are based on analysis by RPLC.

The choice of a detection method is relatively simple when the analyte possesses any specific detectable properties like the UV-absorbing chromophore in tiludronate [((4-chlorothiophenyl)-methylene)bisphosphonate] [9], the fluorescent (1-hydroxy-2-(imidazo[1,2-*a*]-pyridyl)ethylidene)bisphosphonate [10] and the oxidizable incadronate [((cycloheptylamino)methylene)bisphosphonate] [11]. However, APD and its analogue alendronate [(4-amino-1-hydroxybutylidene)bisphosphonate] lack any specific detectable characteristic. Consequently, these (amino-1-hydroxyalkylidene)bisphosphonates require derivatization for sensitive detection. Fluorescence detection after derivatization of the amino group with a fluorescent reagent seems to be the most promising approach in terms of sensitivity. Flesch et al. [12,13] used fluorescamine as a derivatization reagent for APD which resulted in a lower limit of quantification (LLQ) of 200 ng ml^{-1} in both 2 ml urine or plasma. Kline et al. [14,15] applied a derivatization with 2,3-naphtalene dicarboxyaldehyde for alendronate followed by fluorescence and electrochemical detection which resulted in a LLQ of 1 ng ml^{-1} in urine (5-ml sample) and 5 ng ml^{-1} in plasma (1-ml sample).

We have investigated the potential use of phenylisothiocyanate (PITC) as a reagent to obtain a UV-absorbing APD derivative suitable for HPLC analysis [16]. The usefulness of the fluorescent isothiocyanates (ITCs) fluorescein 5-ITC, 1-naphthyl-ITC (NITC) and 4-dimethylamino-NITC has also been investigated [17], which resulted in the choice of NITC for further development of a sensitive assay of APD in biological matrices, starting with urine.

The development of an analytical method for APD in urine will require, besides the optimized analytical separation and NITC derivatization, an extensive pretreatment of the biological sample. The high ionic strength in biological matrices and the presence of, for example, endogenous amine-containing compounds, can disturb both the derivatization reaction and the HPLC separation. The only selective method known, for the pretreatment in bisphosphonate analysis in urine, is co-precipitation with calcium phosphate; this was first applied by Liggett et al. [3] and developed into an adequate analytical tool by Bisaz et al. [4]. This method has been applied in most sensitive LC methods for bisphosphonates in biological media [5,6,9–15]. The addition of calcium ions, required for the co-precipitation, necessitates another extra sample treatment to remove these complexing ions from the sample before the pre-column derivatization of the bisphosphonate is performed.

In this report, a triple co-precipitation is combined with a solid-phase extraction (SPE) on a quaternary ammonium column in order to facilitate a sensitive and selective assay in combination with the NITC derivatization and an ion-pair extraction. Two reaction products, naphthylthiocarbamyl-APD (NTC-APD) and naphthylcarbamyl-APD (NC-APD) are formed [17] (Fig. 1). NTC-APD is quantitatively converted into its naphthylcarbamyl analogue by an oxidative desulphuration with hydrogen peroxide, prior to ion-pair HPLC. Optimization and validation of the methodology are reported.

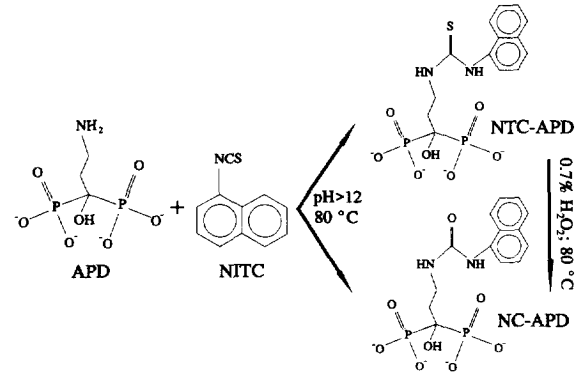

Fig. 1. Derivatization reaction of APD with NITC, including a post-treatment with hydrogen peroxide.

2. Experimental

2.1. Chemicals

APD (disodium salt) was obtained from Bufa (Uitgeest, Netherlands) and EHDP (monosodium salt) from Nogepha (Alkmaar, Netherlands). Neridronic acid [(6-amino-1-hydroxyhexylidene)bisphosphonic acid, AHD] and (3-amino-3-phenyl-1-hydroxypropylidene)bisphosphonic acid [APPD] were generously provided by Dr. C.W.G.M. Löwik (Department of Endocrinology, Leiden University Hospital, Netherlands). NITC was purchased from Janssen Chimica (Geel, Belgium). TBA-Br was obtained from Merck-Schuchardt (Hohenbrunn, Germany) and tetraoctylammonium-bromide (TOA-Br) originated from Sigma (St. Louis, MO, USA). Water was distilled at the production facility of the pharmacy, acetonitrile (HPLC-grade) was supplied by Promochem (Wesel, Germany), hydrogen peroxide (30%, w/w, pharmaceutical-grade) and nitric acid (65%, w/w, Suprapur) by Merck (Darmstadt, Germany) and triethylamine (>99% (w/w)) by Janssen Chimica. All other chemicals were of analytical-grade and originated from Merck.

2.2. Equipment

Chromatographic analyses were performed on the following configuration: a Spectroflow 400 solvent delivery system (Applied Biosystems, Ramsey, NJ, USA), a Marathon auto-injector with a built-in column thermostat (Spark Holland, Emmen, Netherlands), equipped with a 7010-80 Rheodyne injection valve (Rheodyne, Cotati, CA, USA) and a 20- or 100-μl sample loop. The detectors were a Jasco 821-FP spectrofluorometric detector (Jasco, Hachioji City, Japan) or a Spectroflow 773 variable wavelength detector (Kratos Analytical Instruments, Westwood, NJ, USA). Data were recorded on a IPC Dynasty HE 486DX personal computer (IPC, Singapore), equipped with a Gynkosoft chromatographic data system (Softron, Gräfelfing, Germany). For SPE, Bakerbond quaternary amine (J.T. Baker, Phillipsburg, NJ, USA) and Analytichem Bond Elut diethylamine (Varian, Harbor City, CA, USA) columns (3 ml, 500 mg) were processed with a Baker spe-21* vacuum manifold (J.T. Baker). Further, a Dri-Block DB-3 heating block (Techne, Duxford, Cambridge, UK), a Vortex-2-Genie (Scientific Industries, Bohemia, NY, USA), an Eppendorf 5416 centrifuge (Eppendorf, Hamburg, Germany) and a Zymark TurboVap LV evaporator (Zymark, Hopkinton, MA, USA) were used.

2.3. Chromatographic conditions

Flushed loop injections (100 μl) were made on a Microspher C_{18} column (100×4.6 mm, d_p=3 μm, average pore diameter=13 nm, Chrompack, Middelburg, Netherlands) with a reversed-phase (R2) precolumn (10×2 mm, Chrompack). The column temperature was 30°C. The eluent (pH* 7.6–7.9) comprised 35% (v/v) of a 10 m*M* phosphate buffer, containing 10 m*M* TOA-Br and 2 m*M* EHDP as an adsorption suppressor and 65% (v/v) acetonitrile. The eluent flow was 0.8 ml min^{-1}. The excitation and emission wavelengths for fluorescence detection were 285 and 390 nm, respectively.

2.4. Sample preparation

Stock solutions of 192.7 μg ml^{-1} APD and 54.0 μg ml^{-1} APPD (I.S.) in water were stored at 4–6°C. Dilutions, if required, were prepared daily. Urine samples were stored at −20°C. A 2.5-ml sample of urine was transferred into a 10-ml conical glass tube and spiked if required with the appropriate APD solution to give final drug concentration in the range of 2 to 500 ng ml^{-1}; 100 μl of 1 mg ml^{-1} EHDP and 100 μl of 2.16 μg ml^{-1} I.S. are added. A scheme of the total procedure is shown in Fig. 2.

The procedure started with a triple co-precipitation; between all additions the sample was mixed by vortexing. First, 30 μl of 1 *M* calcium chloride was added to the sample; subsequently, portions of 25 μl of 1 *M* sodium hydroxide were added until the first amount of precipitate was clearly formed. Finally, one extra portion of sodium hydroxide was added to ensure the formation of sufficient precipitate. A pellet was formed by centrifugation for 2 min with 3.9×10^3 *g* and, after removal of the liquid phase, re-dissolved in 50 μl of 1 *M* hydrochloric

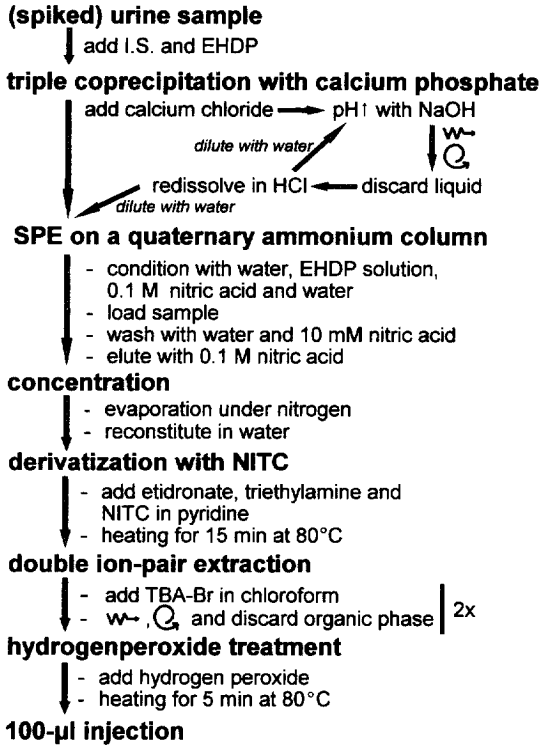

Fig. 2. Scheme of the total sample treatment procedure. ⩙: vortex mixing; ◯: centrifugation.

acid. This solution was diluted with 2.5 ml of water and again a precipitate was formed by adding 50 µl of 1 *M* sodium hydroxide. After centrifugation and removal of the liquid phase, the pellet was dissolved in a minimal amount of 1 *M* hydrochloric acid (30–50 µl) and diluted with 2.5 ml of water. Next, a precipitate was formed for the third time with 30 µl of 1 *M* sodium hydroxide. After centrifugation and removal of the liquid phase, the pellet was dissolved in a minimal amount of 1 *M* hydrochloric acid (25 or 30 µl). This was followed by dilution with 2.5 ml of water.

The resulting solution was subjected to solid-phase extraction using quaternary ammonium columns. The SPE column was preconditioned with 2.5 ml of water, 1 ml of 1 mg ml^{-1} EHDP, 2.5 ml of 0.1 *M* nitric acid and 2×2.5 ml of water. After application of the sample, the column was washed with 2×2.5 ml of water and 2.5 ml of 10 m*M* nitric acid. Next, the bisphosphonates were eluted with 2.5 ml of 0.1 *M* nitric acid and collected in a polypropene (PP) tube. The eluate was evaporated until dryness at 60°C under 0.8 bar nitrogen for 1.65–2 h. The dry residue was reconstituted in 500 µl of water by vortex mixing.

For the derivatization, 250 µl of this solution was transferred to another PP tube, to which 25 µl of 1 mg ml^{-1} EHDP, 40 µl of triethylamine and 250 µl of 20 mg ml^{-1} NITC in pyridine were added and mixed by vortexing, giving a clear, yellow solution. The tube was then sealed and heated at 80°C for 15 min. Afterwards, the reaction product was cleaned up by a two-fold ion-pair liquid–liquid extraction with 2 ml of 10 mg ml^{-1} TBA-Br in chloroform. The two phases were mixed by vortexing and the organic lower layer was discarded after centrifugation. Finally, 250 µl of the sample is transferred to a PP sample vial, 75 µl of 3% hydrogen peroxide was added and the vial was closed. The sample was mixed, heated for 5 min at 80°C and injected using the flushed loop (100 µl) of the HPLC system described.

2.5. Method optimization

For the recovery experiments, flushed loop injections (20 µl) of APD samples were made on an IC-PAK Anion HC column (150×4.6-mm, d_p=10 µm, capacity=30 µeq ml^{-1}) (Waters, Division of Millipore, Milford, MA, USA). The column temperature was 30°C. An aqueous eluent, containing 1.5 m*M* nitric acid and 0.5 m*M* copper(II)nitrate was eluted with 1 ml min^{-1}. The UV detection wavelength was 245 nm. This method was previously reported by Sparidans et al. [18].

2.6. Validation

The method was validated by analysing, intra- and inter-assay, series of six spiked samples. For the independent blanks, samples from 6 volunteers were tested; for all analyses with spiked samples the same urine sample was used. The LLD is the concentration level where the presence of APD is certain for more than 95% and was calculated from the independent blanks and the calibration line in the lower range. At the LLQ, the R.S.D. of the repeatability, as well of the deviation of the accuracy, are not more than 20% of the concentration. The LLQ was calculated from

the intra-assay data at the lowest level and the calibration line in the lower range.

3. Results and discussion

3.1. Method optimization

The derivatization of APD with PITC [16,17] did not lead to a method sensitive enough to fulfil bioanalytical requirements, the low ng ml^{-1} range. Therefore, an investigation into the potential use of four different UV-absorbing and fluorescent isothiocyanates [17] was started in order to select the most promising reagent for further development of an assay of APD in biological media. Eventually, NITC was chosen, particularly because of its high fluorescence yield and efficiency of its ion-pair liquid–liquid extraction as a sample clean-up procedure after derivatization. To achieve a more efficient separation, a Microspher C_{18} column (d_p=3 μm) was applied instead of the Chromspher C_{18} column (d_p=5 μm) used in the previous work [17]. The endogenous urine compounds and by-products of the derivatization reaction could be effectively separated from the APD and I.S. derivatives as early eluting peaks. The separation could be improved by a strong ion-pairing agent in the eluent and a high pH, a low modifier content and a low ionic strength of the eluent. On the other hand, NC-APD and NC-APPD have to elute within a reasonable time. The application of TOA as an ion pairing agent is effective in separating endogenous compounds from (amino-1-hydroxyalkylidene)bisphosphonate derivatives as fast eluting peaks. Unfortunately, it also decreases the retention differences between the different bisphosphonate derivatives. AHD, applied by Flesch et al. [12] as an internal standard for the analysis of APD, elutes too near to NC-APD in our analytical system, so we were forced to apply APPD (extra phenyl ring) that differs chemically more from APD than AHD (extra propylidene chain). The optimization of the individual steps of the sample preparation is discussed below.

3.1.1. Co-precipitation

Co-precipitation with calcium phosphate is a method, specific for bisphosphonates, for which recoveries of up to 90% are reported by several authors [9,10,14,15] for several bisphosphonates. Therefore, this treatment is the method of first choice for the isolation of bisphosphonates from a biological matrix. If this precipitation is used as a part of this complete analytical procedure, it has to meet an extra requirement besides specificity and a high recovery: the ionic strength of the resulting sample solution should not exceed a critical strength in order to retain APD on the SPE column. The corresponding critical amount of 1 *M* hydrochloric acid, to be applied for the dissolution of the precipitate and diluted with 2.5 ml of water before SPE, is 25 to 50 μl and thus the amount of precipitate has to be kept to a minimum. On the other hand, very small amounts of precipitate can cause extra sample losses, which apparently is in contrast to the work of Bisaz et al. [4] and Kline et al. [14]. To illustrate this: if the addition of the extra (third) portion of 25 μl of 1 *M* sodium hydroxide was omitted during the first precipitation step for two typical urine samples (spiked with 20 μg ml^{-1} APD), an extra sample loss of approximately 20% was found. To avoid this problem, the extra addition of 25 μl 1 *M* sodium hydroxide, added after the first appearance of the precipitate, was introduced in the procedure. A second reason to keep the amount of precipitate to a minimum is the influence of phosphate, which is not removed by SPE, on the derivatization reaction. After attempts with a double co-precipitation, the third step was added as an extra clean-up and to obtain a smaller amount of precipitate. The recovery of APD from urine (20 μg ml^{-1}) after the triple co-precipitation method was 80.2%±3.5 (n=5) and 76.4%±4.2 (n=5) for two different spiked urine samples of one individual.

3.1.2. Solid-phase extraction

The SPE procedure is the most important source of potential sample loss. For example, without the application of EHDP as an adsorption suppressor in SPE column pretreatment and as an addition to the sample, the APD recovery decreased from almost 100% for 1 ml of an 1 mg ml^{-1} APD solution in water to 0% for 10 μg ml^{-1}. For the SPE procedure as described, including the EHDP addition to the sample, the recovery decreased only to approximately 50% for 1 μg ml^{-1} and lower concentrations of APD. In order to increase the SPE recovery, we

investigated diethylamine (DEA) columns as an alternative (also applied by Kline et al. [14,15], who claimed an overall recovery of over 90%, using a citrate–phosphate buffer as the eluting solvent for SPE). Applying this solvent was not considered here because the amount of phosphate applied severely hinders the derivatization reaction (see next paragraph); for citrate similar effects are to be expected. To achieve the requirements of the NITC derivatization, volatile eluting ions are highly preferred. Therefore, the original SPE procedure was not changed for the DEA columns, except that the strength of the eluting nitric acid solution was increased from 0.1 to 0.25 *M*. With these DEA columns we found a recovery of approximately 70% (against 50% for the quaternary ammonium columns) for both 2.5 ml of a 2 μg ml^{-1} APD solution in water and a 8 μg ml^{-1} solution in blank urine. However, for a 30 ng ml^{-1} urine sample the overall recovery prior to the derivatization, was only ≈13% (compared to 45% with the quaternary ammonium column), as calculated by comparison with the direct derivatization of APD in water. In conclusion, the DEA columns were not a useful alternative here.

3.1.3. Evaporation and derivatization

Evaporation and derivatization needs to be executed in PP tubes to avoid adsorption on the wall of the glass tubes. When, for instance, 2.5 ml of an 0.8 μg ml^{-1} APD solution in water with 100 μl of 1 mg ml^{-1} EHDP added, is evaporated, 80–90% of the bisphosphonate was recovered in a PP tube against 30% in a glass tube. After derivatization, double extraction and hydrogen peroxide treatment of 100 μl of 0.2 mg ml^{-1} APD in water, 15–20% of the bisphosphonate appeared to be underivatized; the relative amount of NC-APD formed did not differ significantly when lower concentrations were derivatized. The derivatization reaction can, theoretically, be influenced by components remaining from the urine sample or introduced in the previous pretreatment steps. Three of these components were specifically investigated with the following results: (1) EHDP, applied as an adsorption suppressor, prevents excessive sample losses at low concentrations (≤1 μg ml^{-1} APD in the derivatized sample). (2) The nitric acid residue after evaporation decreases the derivatization yield only a few percentages. (3) A phosphate concentration of 10–30 m*M* in an aqueous APD sample to be derivatized provides the optimal yield of NC-APD, which is 5–10% higher than in pure water. However, with higher phosphate concentrations the yield decreases strongly (≈25% for 100 m*M* phosphate). Since the average amount of phosphate in the evaporation residue, recovered from blank urine, is ≈10 μmol (determined with the IEC method from den Hartigh et al. [19]), this residue is redissolved in 500 μl of water to obtain approximately the optimal 20 m*M* phosphate concentration. Because 250 μl is sufficient to fill the 100-μl injection loop, the other half of the sample could be kept in reserve.

3.2. Validation

Examples of chromatograms after complete processing of urine samples are shown in Fig. 3. The components of interest were well separated from other peaks, there is only a small disturbing peak at

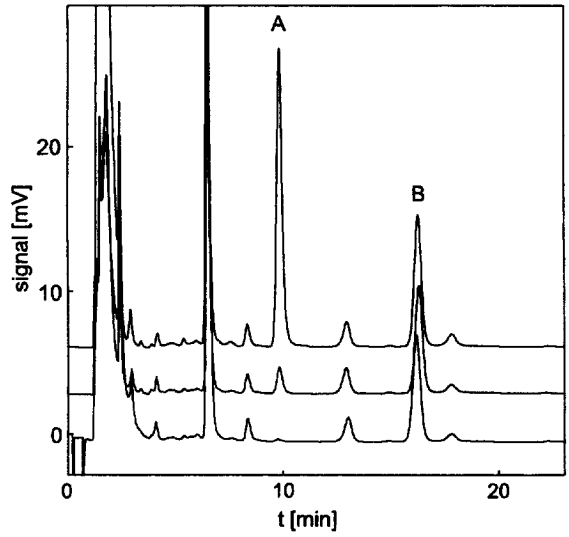

Fig. 3. Chromatograms of urine spiked with respectively 0, 8 and 99 ng ml^{-1} APD. A. NC-APD; B. NC-APPD (I.S.). Chromatographic conditions: injection volume: 100 μl; column: Microspher C_{18} (100×4.6 mm, d_p=3 μm, average pore diameter=13 nm); precolumn: reversed-phase (10×2 mm); column temperature: 30°C; eluent: 35% (v/v) 10 m*M* phosphate buffer, containing 10 m*M* TOA-Br and 2 m*M* EHDP, and 65% (v/v) acetonitrile, pH* 7.6–7.9; eluent flow: 0.8 ml min^{-1}. Detection: fluorescence at λ_{ex}=285 nm and λ_{em}=390 nm.

Table 1
Intra-assay precision (repeatability) at different concentrations of APD spiked to urine

Concentration (APD) (ng ml^{-1})	Relative peak area	R.S.D. (%)	n
0	0.018	16	6
9.88	0.240	5	6
98.8	2.20	7	6
494	11.1	7	6

Table 2
Inter-assay precision (reproducibility) and accuracy at different concentrations of APD spiked to urine

Concentration (APD) (ng ml^{-1})	Relative peak area	R.S.D. (%)	Accuracy (%)	n
0	0.031	29		6
9.88	0.254	11	113	5
98.8	2.25	12	103	6
494	12.3	11	115	6

the NC-APD retention time; therefore, the lower limit of detection (LLD) was higher than judged at the $S/N=3$ level (1 ng ml^{-1} instead of 0.2 ng ml^{-1}). Intra- and inter-assay validation parameters at three concentration levels are tabulated in Table 1 and Table 2. The LLD and the LLQ were estimated at 1 ng ml^{-1} and 3 ng ml^{-1} respectively. Calibrations lines in three concentration ranges, calculated by least squares regression analysis were:

$y=0.0149(\pm 0.0021)+0.0208(\pm 0.0004)\cdot x$
(range=0–8 ng ml^{-1}, $n=6$, $r^2=0.998$)

$y=0.022(\pm 0.023)+0.0207(\pm 0.0008)\cdot x$
(range=0–50 ng ml^{-1}, $n=5$, $r^2=0.995$)

$y=0.05(\pm 0.38)+0.0216(\pm 0.0013)\cdot x$
(range=0–500 ng ml^{-1}, $n=5$, $r^2=0.990$).

The concentration of APD (99 ng ml^{-1}) in spiked urine samples was not influenced by 10 extra freeze–thaw cycles when compared to the inter-assay validation results. The recovery, for 99 ng ml^{-1} of APD in urine (inter-assay data) undergoing the sample treatment steps prior to the derivatization was ≈45%. This was calculated by comparison with an aqueous sample with an identical amount of APD that was only submitted to derivatization and the subsequent analytical procedures. The SPE was the main cause for this sample loss.

4. Conclusions

A selective method for the analysis of APD in urine is presented with only one drawback: the laboriousness of the sample pretreatment. However, for parts of the analytical procedure, automation should be possible which will be investigated in the future. The validation data indicate that this method is appropriate for the bioanalysis of APD in human urine; the application of this method in clinical pharmacological research will be started after automation, as far as possible, of the analytical procedure. The method is two decades more sensitive compared to bioanalytical methods reported before on this specific bisphosphonate in urine [7,11,12]. The development of a semi-automatic bioanalytical method for the determination of APD in serum is started, based on this method for urine; results will be published later [20].

References

[1] A. Fitton, D. McTavish, Drugs 41 (1991) 289.
[2] H. Fleisch, Drugs 42 (1991) 919.
[3] S.J. Liggett, Biochem. Med. 7 (1973) 68.
[4] S. Bisaz, R. Felix, H. Fleisch, Clin. Chim. Acta 65 (1975) 299.
[5] T.L. Chester, E.C. Lewis, J.J. Benedict, R.J. Sunberg, W.C. Tettenhorst, J. Chromatogr. 225 (1981) 17.
[6] V. Virtanen, L.H.J. Lajunen, J. Chromatogr. 617 (1993) 291.

[7] P.T. Daley-Yates, L.A. Gifford, C.R. Hoggarth, J. Chromatogr. 490 (1989) 329.
[8] S. Auriola, R. Kostiainen, M. Ylinen, M. Mönkkönen, P. Ylitalo, J. Pharm. Biomed. Anal. 7 (1989) 1623.
[9] J.-P. Fels, J. Guyonnet, Y. Berger, W. Cautreels, J. Chromatogr. 430 (1988) 73.
[10] T. Usui, R. Kawakami, T. Watanabe, S. Higuchi, J. Chromatogr. B 652 (1994) 67.
[11] T. Usui, T. Watanabe, S. Higuchi, J. Chromatogr. 584 (1992) 213.
[12] G. Flesch, S.A. Hauffe, J. Chromatogr. 489 (1989) 446.
[13] G. Flesch, N. Tominaga, P. Degen, J. Chromatogr. 568 (1991) 261.
[14] W.F. Kline, B.K. Matuszewski, W.F. Bayne, J. Chromatogr. 534 (1990) 139.
[15] W.F. Kline, B.K. Matuszewski, J. Chromatogr. 583 (1992) 183.
[16] R.W. Sparidans, J. den Hartigh, W.M. Ramp-Koopmanschap, R.H. Langebroek, P. Vermeij, J. Pharm. Biomed. Anal., accepted.
[17] R.W. Sparidans, J. den Hartigh, J.H. Beijnen, P. Vermeij, J. Chromatogr. A, accepted.
[18] R.W. Sparidans, J. den Hartigh, P. Vermeij, J. Pharm. Biomed. Anal. 13 (1995) 1545.
[19] J. den Hartigh, R. Langebroek, P. Vermeij, J. Pharm. Biomed. Anal. 11 (1993) 977.
[20] R.W. Sparidans, J. den Hartigh, J.H. Beijnen, P. Vermeij, J. Chromatogr. B, submitted.