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Semi-automatic liquid chromatographic analysis of pamidronate in serum and citrate plasma after derivatization with 1-naphthylisothiocyanate

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Abstract

The semi-automatic method for the determination of the bisphosphonate pamidronate in serum and citrate plasma involves a manual protein precipitation with trichloroacetic acid and a manual coprecipitation of the bisphosphonate with calcium phosphate, followed by an automated solid-phase extraction on anion-exchange columns. After off-line evaporation of the extract under nitrogen and reconstitution in water, the automatic procedure is continued by automatic derivatization with 1-naphthylisothiocyanate, ion-pair liquid–liquid extraction and a treatment with hydrogen peroxide, prior to analysis by ion-pair HPLC and fluorescence detection at 285/390 nm. The intra- and inter-day precisions are 1.3 and 7%, respectively, for a standard of 100 ng ml^{-1} pamidronate in serum; the average accuracy for this standard is 107%. The lower limit of quantification is 20 ng ml^{-1} pamidronate in 1 ml of human serum. © 1998 Elsevier Science B.V.

Keywords: Pamidronate; 1-Naphthylisothiocyanate

1. Introduction

Pamidronate disodium [(3-amino-1-hydroxy-propylidene)bisphosphonate, APD] (Fig. 1) is an important representative of the bisphosphonates, a relatively new group of drugs, employed for the treatment of calcium metabolic disorders like Paget's disease of bone, hypercalcaemia and osteoporosis [1,2]. APD was the first of a generation of amino-substituted bisphosphonates. The physicochemical effects of bisphosphonates are similar to those of pyrophosphate and polyphosphates [2]; however, these phosphates are very sensitive to hydrolysis, and are therefore less suited for a long term therapeutic effect on the calcium metabolism. APD is selectively accumulated in the skeleton and the oral absorption is in the order of 1% of the administered dose [1]. To obtain more detailed clinical pharmacological data, a

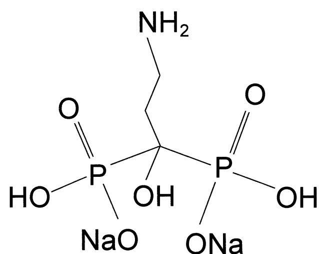

Fig. 1. Chemical structure of pamidronate disodium.

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reliable and sensitive bioanalytical assay is a prerequisite.

Especially in the last decade several analytical methods for the quantification of different types of bisphosphonates were reported. However, the number of methods dealing with serum or plasma is limited. The first method on bisphosphonate analysis in plasma, developed for etidronate [(1-hydroxyethylidene)bisphosphonate, EHDP], was based on detection of phosphate after decomposition of the bisphosphonate [3]. However, the method was very laborious and generally not sensitive enough to measure therapeutic EHDP concentrations in plasma 1 h after oral administration of the drug [3].

Bioanalytical assays, developed later for several bisphosphonates of new generations, are mostly based on high-performance liquid chromatography (HPLC). Since the newer bisphosphonates are more potent and are therefore administered in lower doses [1,2], more sensitive methods became necessary. A gas chromatographic (GC) method with flame photometric detection has been developed for APD after derivatization with isobutylchloroformate, followed by methylation with diazomethane [4]. Another GC method was based on derivatization with N,O-bis-(trimethylsilyl)trifluoroacetamide and developed for clodronate [(dichloromethylene)bisphosphonate] [5]. An approach that could be employed for all bisphosphonates is ion-exchange chromatography followed by phosphorous specific detection; flame photometric detection [6] and postcolumn complexation [7,8] have been used. The complexation was performed directly with thorium-ethylenediaminotetraacetic acid-xylenol [8] or indirectly, after oxidation of the bisphosphonate into *o*-phosphate, with molybdenum-ascorbate [7].

All other published methods on liquid chromatographic bioassays of bisphosphonates are based on analysis by the more selective reversed-phase liquid chromatography (RP-LC) and are dedicated to one specific bisphosphonate or suited for the (amino-1-hydroxyalkylidene)bisphosphonates. The choice of a detection method is relatively simple if the analyte possesses any specific detectable property like UV-absorbance [9], native fluorescence [10] or electrochemical activity [11]. However, APD and its analogue alendronate [(4-amino-1-hydroxybutylidene)-bisphosphonate] lack any specific detectable characteristics. Consequently, these (amino-1-hydroxyalkylidene)bisphosphonates require derivatization prior to detection to obtain a specific detectable moiety in the analyte. Fluorescence detection after derivatization of the amino group with a fluorescent label seems to be the most promising approach in terms of sensitivity. Fluorescamine [12,13] and 2,3-naphtalenedicarboxyaldehyde [14–16] have been reported to form fluorescent derivatives of (amino-1-hydroxyalkylidene)bisphosphonates.

We previously investigated the potential use of isothiocyanates as reagents, to obtain a sensitive detectable APD derivative suitable for HPLC [17], resulting in the choice of 1-naphthylisothiocyanate (NITC) for further method development on APD analysis in biological matrices. The development of an analytical method for APD in urine using this reagent [18], resulted in a laborious method; all pretreatment steps, triple coprecipitation, solid-phase extraction (SPE), evaporation, derivatization, double liquid–liquid extraction and oxidation, were carried out manually.

In this report, we describe the development of a semi-automatic HPLC method for the determination of APD in serum or plasma based on the method, previously described for urine samples [18]. Time consuming manual pretreatment steps have been avoided as far as possible; therefore, potentialities for the automation of the SPE procedure, derivatization and liquid–liquid extraction with a robotic workstation, the ASPEC (Automatic Sample Preparation with Extraction Columns), have been fully exploited in this investigation.

2. Experimental

2.1. Chemicals

APD (disodium salt) was obtained from Bufa (Uitgeest, Netherlands) and EHDP (monosodium salt) originated 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 Acros (Geel, Belgium). Tetrabutylammonium-bromide was obtained from Merck–Schuchardt (Hohenbrunn, Germany) and tetraoctylammonium-bromide originated from Sigma (St. Louis, MO, USA). Water was laboratory-distilled, acetonitrile (HPLC-grade) was supplied by Promochem (Wesel, Germany), hydrogen peroxide [30% (w/w), pharmaceutical grade] and nitric acid (65%, Suprapur) by Merck (Darmstadt, Germany) and triethylamine [>99% (w/w)] by Acros. 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) with an ASPEC XL sample processor (Gilson, Villiers-le-Bel, France) and a Jasco 821-FP spectrofluorometric detector (Jasco, Hachioji City, Japan). The sample processor was equipped with a 402 syringe pump (Gilson), containing one 5-ml syringe, a 7010-80 Rheodyne injection valve (Rheodyne, Cotati, CA, USA) with a 100-μl sample loop and two sample vial racks (Gilson) for 32×12 and 45×15 mm vials, respectively, both thermostatted by a Thermomix BU thermostatic bath (B. Braun Biotech, Melsungen, Germany). Data were recorded on an IPC Dynasty HE 486DX personal computer (IPC, Singapore), equipped with a Gynkosoft chromatographic datasystem (Softron, Gräfelfing, Germany).

For SPE, Bakerbond 3 ml/500 mg quaternary amine SPE columns (J.T. Baker, Phillipsburg, NJ, USA) were used. Further, 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) guard column (10×2 mm, Chrompack). The column temperature was ambient. The eluent (pH*=8.0) comprised 38% (v/v) of a 10 m*M* phosphate buffer, containing 10 m*M* tetraoctylammonium-bromide and 2 m*M* EHDP as an adsorption suppressor, and 62% (v/v) acetonitrile. The eluent flow-rate was 0.8 ml min^{-1}. The fluorescence detection wavelengths were 285 nm for excitation and 390 nm for emission, respectively.

2.4. Sample preparation

Stock solutions of 495 μg ml^{-1} APD and 51.3 μg ml^{-1} AHD (I.S.) in water were stored at 4–6°C. The pamidronate stock solution was stored for maximum of six months. Aqueous dilutions, if required, were prepared daily. Serum and citrate plasma samples were stored at −20°C.

An 1-ml sample of serum or citrate plasma was transferred into an 1.5-ml polypropylene micro test tube and spiked, if required, with the appropriate APD solution to give final drug concentrations in the range of 10 to 989 ng ml^{-1}; next, 20 μl of 5 mg ml^{-1} EHDP, 35 μl of 0.1 g ml^{-1} citric acid (to serum only) and 30 μl of 2.05 μg ml^{-1} AHD were added. A scheme of the total procedure is shown in Fig. 2. . The procedure started with a protein precipitation by adding 200 μl of 20% trichloroacetic acid, closing the tube, vortex-mixing vigorously and centrifuging for 5 min at $14\cdot10^3$ *g*. The clear supernatant was transferred into a 10-ml conical glass tube and the bisphosphonates were precipitated with calcium phosphate by adding 30 μl of 1 *M* calcium chloride, 40 μl of 0.1 *M* sodium dihydrogenphosphate and 200 μl of 1 *M* sodium hydroxide, with vortex-mixing after each addition. A pellet was formed by centrifugation for 2 min at $3.9\cdot10^3$ *g* and, after removal of the liquid phase, redissolved in 25 μl of 1 *M* hydrochloric acid.

The procedure was continued by automatic SPE on a quaternary ammonium column using the ASPEC system; water was applied in the syringe for the automatic liquid displacement. The SPE column was preconditioned (5 ml min^{-1}) with 2.5 ml water, 1 ml of 1 mg ml^{-1} EHDP, 2.5 ml of 0.1 *M* nitric acid and 5 ml water. After dilution with 2.5 ml water and mixing by aspiration and dispensing, the sample was loaded on the column and the column was then washed with 1 ml water (both at 2 ml min^{-1}). The

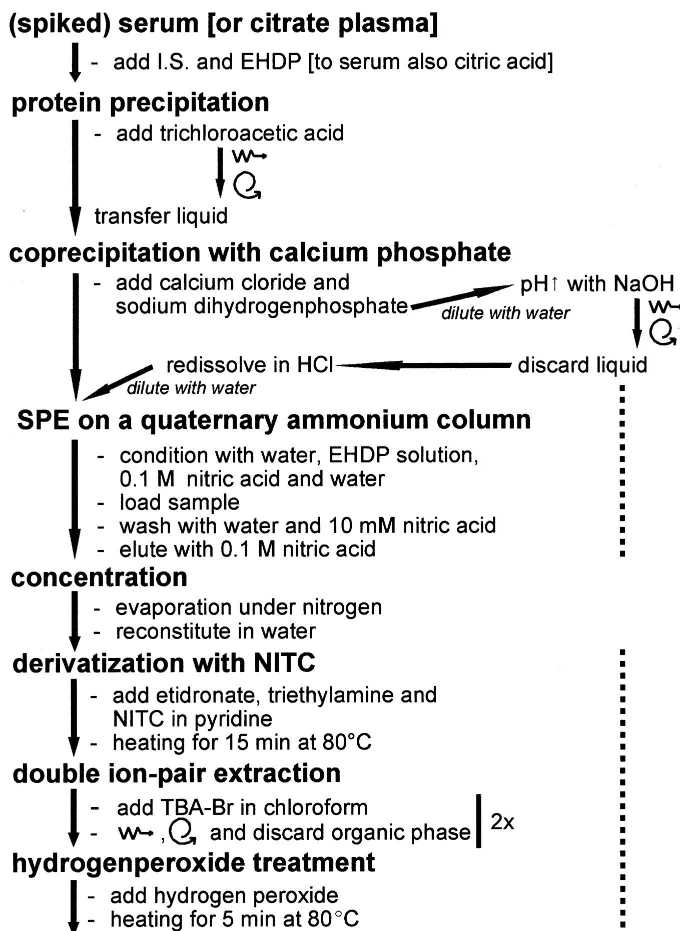

Fig. 2. Scheme of the total sample treatment procedure. ⤳: vortex mixing; ↻: centrifugation; · · ·: automatic parts of the total procedure.

column was washed further (3 ml min^{-1}) with 4 ml water and 2.5 ml of 10 m*M* nitric acid. After elution (2.5 ml min^{-1}) of the bisphosphonates with 2.5 ml of 0.1 *M* nitric acid and collection in a PP tube, the eluate was evaporated off-line until dryness at 80°C under 0.6 bar nitrogen for 50–75 min. The residue was reconstituted in 500 μl water by vortex-mixing.

Next, the automatic procedure was continued with methanol in the syringe for the liquid displacement. For the derivatization 50 μl of 1 mg ml^{-1} EHDP, 75 μl triethylamine and 500 μl of 20 mg ml^{-1} NITC in pyridine were added and mixed with the sample by air bubbling to form a clear yellow solution. Subsequently, the sample was transferred into an open 2.5-ml polypropylene sample vial (45×15 mm) and heated at ca. 80°C for 15 min. The reaction mixture was transferred into a 10- mm I.D. glass tube and treated with a twofold ion-pair liquid–liquid extraction with 2 ml of 10 mg ml^{-1} tetrabytylammonium-bromide in chloroform to clean-up the sample. The two phases were mixed by bubbling 3 ml air (5 ml min^{-1}) through the liquid and were separated after waiting for 1 min; the lower organic layer was subsequently removed and rejected. Of the resulting sample 300 μl was transferred together with 90 μl of 3% hydrogen peroxide into an open 750-μl polypropylene sample vial (32×12 mm), heated at ca. 80°C. After 5 min, 100 μl was transferred directly onto the HPLC column using flushed-loop injection.

2.5. Validation

The method was validated analysing series of six samples. Serum samples from six individual patients were tested as independent blanks and spiked samples of a volunteer were applied for calibration and intra-day validation. Pooled serum of about seven patients was used for the inter-day validation after spiking. The lower limit of detection (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 lower lower limit of quantification (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-day data at the lowest level and the calibration line in the lower range.

3. Results and discussion

For the development of a method for the determination of APD in serum, the manual method for the determination of APD in urine [18] was taken as the starting-point. The consecutive pretreatment steps in that assay prior to injection were a triple calcium phosphate precipitation, SPE, evaporation, derivatization, a double ion-pair liquid–liquid extraction and an oxidative desulphuration. The last step was required to convert the analyte completely into the naphthylcarbamyl derivative; this structure was previously confirmed by fast atom bombardment-mass spectrometry [17]. Instability of the derivative at ambient temperature was not observed after overnight storage [18]. The citric acid, present in citrate plasma or added to serum, played an essential role during liquid–liquid extraction, this is discussed in Section 3.2.3.

3.1. Protein precipitation

It was expected that for serum or plasma a protein removal step would be useful or even necessary and that the number of coprecipitation steps with calcium phosphate could be reduced. Precipitation with trichloroacetic acid was used to remove proteins from citrate plasma and tested in combination with a different number of calcium precipitation steps: 0, 1 or 2. A double coprecipitation without previous protein removal was also investigated and all alternatives were succeeded by the treatment according to the manual method for urine [18]. Finally, protein precipitation combined with a single coprecipitation step with calcium phosphate was selected as the optimal method because of the least interference in the blank chromatogram and the highest bisphosphonate recovery.

3.2. Method automation

The ASPEC is a robotic workstation, especially designed for SPE with disposable extraction columns but has also the potency to perform other sample preparation techniques [19]. Handling like diluting, pipetting or injection are relatively simple; however, it is a challenge to perform a derivatization or a liquid–liquid extraction with the ASPEC. Advantages of automation with the ASPEC can be: time saving, higher sample throughput, safety, better reproducibility [20] and a higher recovery. The last effect may be achieved by optimizing the SPE flowrate [21].

In the presented method only both precipitation steps and the evaporation of the aqueous SPE eluate offered no possibilities for automation with the ASPEC. Automation of all other sample treatment steps results in two automated parts of the total analytical procedure: the first part is the SPE and the second part is the derivatization with all the following steps. In the first part, water is employed in the syringe for automatic liquid displacement by the syringe pump. However, in the second automated part of the procedure water could not be used as the dispensing liquid because of its incompatibility with both chloroform and NITC. Therefore, methanol was used in the syringe during the second automated part of the analysis. Acetone was also tested as the dispensing liquid but decreased the derivatization yield significantly compared to methanol.

The investigations that resulted in the automation of the different analytical procedures are described below.

3.2.1. Solid-phase extraction

In contradiction to pressure-controlled manual SPE methods on a vacuum manifold, the ASPEC can directly control the solvent flow through the SPE column. The influence of the variation of the solvent flow-rate on the recovery of the bisphosphonates was investigated. Parameters limiting the flow-rate are the permeability of the column and the time consumption of the total procedure. The solvent flow-rate in the SPE procedure was varied from 2 to 10 ml min^{-1} for the conditioning steps, from 0.7 to 4 ml min^{-1} for the sample loading and the first washing step and from 1 to 5 ml min^{-1} for the other washing steps and the elution step. The (reproducibility of) the recovery of APD did not vary significantly for the different flow-rates and was between 60 and 65% for a solution of 0.3 μg ml^{-1} APD in water. However, the recovery of AHD showed a larger variation compared to APD. The use of this I.S. was preferred because APPD, applied as the I.S. in the manual assay for APD in urine [14], showed even a higher variation of the recovery. To obtain sufficient resolution between the naphthylcarbamyl derivatives of APD and AHD in the chromatogram, the eluent was slightly changed: a higher pH (pH*=8.0) and a lower acetonitrile percentage in the eluent (62%) were used. Three batches of SPE columns were used of which the third showed a significantly higher back pressure compared to the other two. In the automated SPE procedure the "air push volume" after each elution step was approximately doubled for the third batch to correct this difference in permeability. This "air push volume" is a volume of air employed after dispensing a subsequent solvent in order to push the remaining solvent into the column through the solid-phase.

3.2.2. Derivatization and oxidative desulphuration

The derivatization and the hydrogen peroxide treatment were performed in polypropylene vials to avoid adsorption on the wall of glass vials [18]. Unfortunately, when these investigations were started, only polypropylene vials with a double wall were available in the dimensions (32×12 or 45×15 mm) and volume (>1 ml) required for derivatization. A double-wall vial will result in a decreased heat exchange between the heating block and the derivatization mixture. For the post treatment with hydrogen peroxide 750-μl polypropylene vials (32×12 mm) with a single wall could be applied. Direct injection from a heated (ca. 80°C), sealed vial after the hydrogen peroxide treatment did not result in a reproducible injection, in despite of the use of a double needle. Probably, a very-high underpressure was formed in the vial during aspiration of the sample. Therefore, derivatization and hydrogen peroxide treatment were performed in open vials. Typically, there was no significant difference between the derivatization in a capped or an open vial.

In Fig. 3, the yield of the naphthylcarbamyl derivatives of APD, AHD and APPD is shown as a function of the reaction time in the vial racks at ca. 80°C. This figure shows a higher reaction rate of AHD compared to the other two aminobisphosphonates; the APPD derivative appears to be less stable compared to the other two. The optimum reaction time for the derivatization is between 15 and 30 min. Since all samples had to be derivatized sequentially, 15 min was chosen to keep the total sample pretreatment time as short as possible.

3.2.3. Liquid–liquid extraction

The liquid–liquid extraction was expected to be the most difficult part of the procedure planned to be

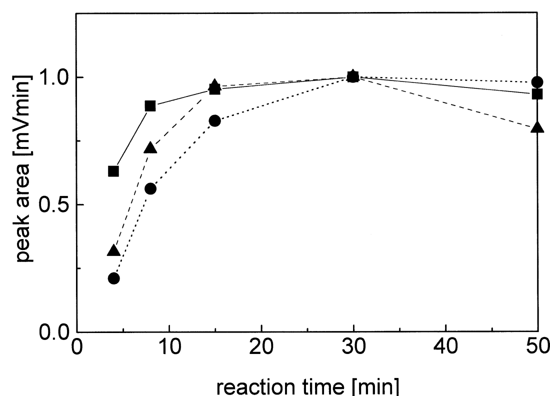

Fig. 3. Formation of the naphthylcarbamyl derivatives of three aminobisphosphonates as a function of the reaction time. 500 μl of an aqueous standard solution with 198 ng ml^{-1} APD (●), 123 ng ml^{-1} AHD (■) and 369 ng ml^{-1} APPD (▲) was derivatized for the indicated time in duplicate. The indicated result is the average peak area of the two experiments, relative to the maximum peak area measured for the specific component in these experiments.

automated. Vortex-mixing and centrifugation, applied originally in the liquid–liquid extraction [18], could both not be automated with the ASPEC. Mass transfer between the two phases can only be accelerated by two simple mixing methods: air bubbling or aspirating/dispensing the liquid; phase separation can only be achieved by waiting. The aspirating/dispensing method was not chosen for automatic mixing, because, with the needle at the bottom of the tube, the two phases will hardly be mixed. The needle had to be at the bottom of the tube to facilitate rejection of the lower organic layer; the software of the ASPEC does, unfortunately, not allow different needle positions during one program. In the first experiments, using APD standards diluted with water, mixing by bubbling 3 ml air resulted in a satisfying clean-up, although the manual procedure with vortex-mixing resulted in cleaner chromatograms. When citrate plasma, or especially serum samples, were processed, the liquid–liquid extraction did lead to dramatically worse blank chromatograms compared to those of the standards in water. Therefore, the extraction was improved by mixing in a more narrow tube (I.D.=10 instead of 14 mm). Extraction of a processed serum sample was more difficult, compared to citrate plasma, because of the occurrence of a white suspended solid during the liquid–liquid extraction, hindering the phase separation. The formation of this white precipitate was simply avoided by adding citric acid to the initial serum sample. The amount of citric acid to be added to serum samples was determined by measuring the citrate concentration in citrate plasma with an ion-exchange chromatographic assay, based on the method of den Hartigh et al. [22].

3.3. Validation

Examples of chromatograms after complete processing of serum samples are shown in Fig. 4. The components of interest are well separated from other peaks, there is only a small disturbing peak at the NC-APD retention time; therefore, the LLD was higher than expected based on a $S/N=3$ level (10 ng ml^{-1} instead of 1 ng ml^{-1}). The LLQ was estimated at 20 ng ml^{-1}. The intra- and inter-day validation parameters at four concentration levels are tabulated in Tables 1–3. Calibration lines in two concentration

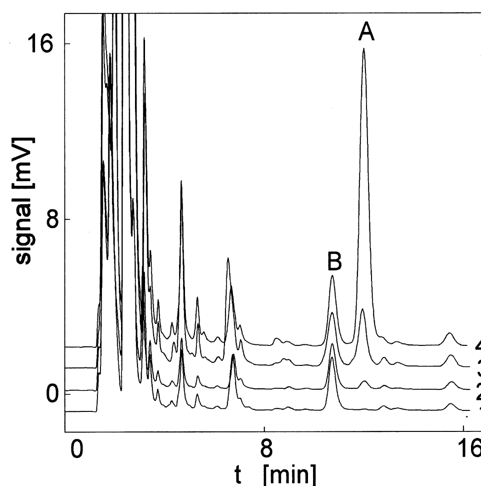

Fig. 4. Chromatograms of serum spiked with APD. The concentration of APD is respectively, 0 (1), 20 (2), 89 (3) and 492 ng ml^{-1} (4). (A) Naphthylcarbamyl-APD, (B) naphthylcarbamyl-APPD (I.S.). Chromatographic conditions: injection volume: 100 μl; column: Microspher C_{18} (100×4.6 mm, d_p=3 μm, average pore diameter=13 nm); guard column: reversed-phase (10×2 mm); column temperature: ambient; eluent: 38% (v/v) 10 m*M* phosphate buffer, containing 10 m*M* tetraoctylammonium-bromide and 2 m*M* EHDP, and 62% (v/v) acetonitrile, pH*=8.0; eluent flow: 0.8 ml min^{-1}. Detection: fluorescence at λ_{ex}=285 nm and λ_{em}=390 nm.

Table 1
Intra-day precision (repeatability) at different concentrations of APD (*c*) in serum and citrate plasma[a]

c (ng ml^{-1})	Relative area	R.S.D. (%)	*n*
0	0.027	32	6
19.6	0.174	3.4	6
97.9	0.844	1.3	5
490	4.89	2.7	6
49.1[a]	0.621	5.1	6

Table 2
Inter-day precision (reproducibility) at different concentrations of APD (*c*) in serum

c (ng ml^{-1})	Relative area	R.S.D. (%)	*n*
19.7	0.195	16	6
98.4	0.984	7	5
492	4.79	9	5

Table 3
Inter-day accuracy at different concentrations of APD (c) in serum and citrate plasma[a]

c (ng ml^{-1})	Accuracy (%)	n
19.7	113	2
98.4	107	2
492	97	2
49.1[a]	101	1

ranges, calculated by least squares regression analysis were:

$$y = -0.040(\pm 0.021) + 0.01059(\pm 0.00034)x$$
$$(\text{range} = 10 - 99 \text{ ng ml}^{-1}, n = 6, r^2 = 0.996)$$

$$y = -0.10(\pm 0.08) + 0.01005(\pm 0.00014)x$$
$$(\text{range} = 99 - 989 \text{ ng ml}^{-1}, n = 6, r^2 = 0.9992)$$

The concentration of APD (492 ng ml^{-1}) in spiked serum samples was not influenced by six extra freeze–thaw cycles, nor by storage during 10 months (in both, a 98 and a 492 ng ml^{-1} sample), when compared to the inter-day results. The recovery, for 98 ng ml^{-1} APD in serum (inter-assay data) undergoing the sample treatment steps prior to the derivatization, was approximately 50%. This was calculated by comparison with an aqueous sample with an identical amount of APD that was submitted only to derivatization and the following analytical procedures. The SPE was the main source of this sample loss.

Compared to previously published results of bioanalytical bisphosphonate assays for pamidronate in serum or plasma, the described method for analysis of serum samples is 10-fold more [13] or at least equally [4,7] sensitive. Only the method for alendronate in plasma, based on derivatization with 2,3-naphthalene dicarboxyaldehyde [14,15], that should also be suited for its analogue pamidronate, is slightly more sensitive than the method described in this paper. For the repeatability of the bioanalytical assay reported in this paper, R.S.D. values for serum samples are reported in a lower range (1–3%) than for all known validated bioanalytical bisphosphonate assays [23]. This may possibly be contributed to the automation of the major part of the pretreatment procedure. The time consumption of the described automated method per sample is approximately one half compared to the previously developed manual method for pamidronate in urine [18].

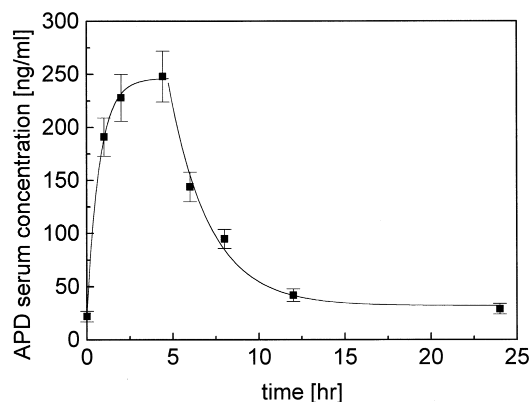

Fig. 5. APD concentration in serum of a 79-year-old osteoporotic female during and after a 4.75-h infusion with a daily dose of 15 mg APD in 0.5 l of 0.9% sodium chloride on the fifth day of a five day treatment. Error bars indicate the 95% confidence limits, calculated by weighted linear regression analysis.

Finally, an example of the APD concentration in serum during and after the infusion of 15 mg APD in an osteoporotic patient is shown in Fig. 5, demonstrating the applicability of the assay for clinical samples.

4. Conclusions

A selective semi-automatic method for the analysis of APD in serum or plasma is presented. The validation data indicate that this method is appropriate for the bioanalysis of APD with increased speed in human serum or citrate plasma; the application of this method in clinical research has been started.

The ASPEC is an instrument with more analytical possibilities than only SPE and injection: the derivatization and liquid–liquid extraction as described were performed adequately.

References

[1] A. Fitton, D. McTavish, Drugs 41 (1991) 289.
[2] H. Fleisch, Drugs 42 (1991) 919.

[3] S. Bisaz, R. Felix, H. Fleisch, Clin. Chim. Acta 65 (1975) 299.
[4] N. Sakayima, H. Kataoka, M. Makita, Biomed. Chromatogr. 9 (1995) 243.
[5] S. Auriola, R. Kostiainen, M. Ylinen, M. Mönkkönen, P. Ylitalo, J. Pharm. Biomed. Anal. 7 (1989) 1623.
[6] T.L. Chester, E.C. Lewis, J.J. Benedict, R.J. Sunberg, W.C. Tettenhorst, J. Chromatogr. 225 (1981) 17.
[7] P.T. Daley-Yates, L.A. Gifford, C.R. Hoggarth, J. Chromatogr. 490 (1989) 329.
[8] V. Virtanen, L.H.J. Lajunen, J. Chromatogr. 617 (1993) 291.
[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] L.E. King, R. Vieth, J. Chromatogr. B 678 (1996) 325.
[17] R.W. Sparidans, J. den Hartigh, J.H. Beijnen, P. Vermeij, J. Chromatogr. A 782 (1997) 211.
[18] R.W. Sparidans, J. den Hartigh, J.H. Beijnen, P. Vermeij, J. Chromatogr. B 696 (1997) 137.
[19] P. Beals, in: W.J. Hurst (Ed.), Automation in the Laboratory, VCH, New York, 1995, Ch. 5, p. 109.
[20] G. Hotter, G. Gómez, I. Ramis, G. Bioque, J. Rosselló-Catafau, E. Gelpi, J. Chromatogr. 607 (1992) 239.
[21] Ph. Hubert, P. Chiap, B. Evrard, L. Delattre, J. Crommen, J. Chromatogr. 622 (1993) 53.
[22] J. den Hartigh, R. Langebroek, P. Vermeij, J. Pharm. Biomed. Anal. 11 (1993) 977.
[23] R.W. Sparidans, J. den Hartigh, in: R.W. Sparidans (Ed.), Bisphosphonates: a Chromatographic Approach as a Contribution to the Clinical Evaluation of Pamidronate and Olpadronate, Syntax Publishers, Tilburg, 1997, Ch. 2, p. 25.