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Ion exchange chromatographic determination of olpadronate, phosphate, phosphite, chloride and methanesulfonic acid

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Abstract

A method based on single column ion chromatography with UV detection was developed for purity testing and assay of monosodium olpadronate. The analyte aqueous solution is precipitated with methanol to enhance the impurities/olpadronate molar ratio, thus improving purity determination at trace levels. The resulting solution is injected into a standard chromatographic system with UV detector in indirect mode with a Waters IC Pak HR column using diluted nitric acid as the mobile phase. The method was fully validated according to ICH guidelines for the determination of phosphite, phosphate, chloride and methanesulfonic acid in olpadronate being suitable for purity testing and assay. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Olpadronate; Bisphosphonate analysis; Impurities; HPLC; Ion chromatography

1. Introduction

Monosodium olpadronate (Fig. 1) is the monosodium salt of 3-dimethylamino-1-hydroxy-propylidene-bisphosphonic acid and belongs to a group of compounds used in the treatment of several bone metabolism disorders including Paget's disease, malignant hypercalcemia, bone metastasis and osteoporosis.

One of the major drawbacks for the analysis of olpadronate and its impurities is the lack of chromophore groups, making difficult the use of UV detectors. Several approaches to the analysis of bisphosphonates have been reported in the literature. Some of them used uncommon tools in pharmaceutical routine QC laboratories like flame phosphorous selective detectors [1], capillary electrophoresis [2] or isotachophoresis [3]. Other authors used post-column derivatization methods using strong oxidants and colored phosphomolybdate generation agents [4], naphthalene dialdehyde [5–7], OPA [8], iron [9], or pre-column derivatization with FMOC [10,11] or naphthylisothiocyanate [12]. A simple chromophoric complex with Cu was also reported, though purity

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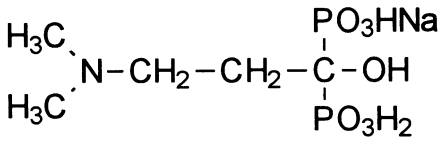

Fig. 1. Structure of monosodium olpadronate.

analysis of bisphosphonates was not the target [13,14]. Single column ion exchange chromatography was the common factor of many studies in the bisphosphonate analysis field. Refractive index [15,16] and conductivity detection [17,18] were used with nitric acid or succinic acid as mobile phase, as well as indirect UV detection using nitric acid as the mobile phase [19].

Particularly, the British Pharmacopoeia [16] recommends the use of anion exchange chromatography with a refractive index detector, both for assay and purity testing in disodium pamidronate, a closely related molecule. However, we did not succeed in using an RI detector for purity determination at the proposed level and our previous work with ion exchange chromatography (IEC) in direct mode with a conductivity detector produced a noisy baseline, which prevented further use in determination of trace levels of impurities (SAFYBI Congress, Buenos Aires, 1996, O. Quattrocchi, L. Frisardi, M. Iglesias, M. Caputto, E. Piccinni, results not published). The present work was begun based on a previous report [19] for the analysis of alendronate, etidronate and clodronate. It consisted in IEC using diluted nitric acid as mobile phase that provides the pH and optical background for the mobile phase to separate the bisphosphonate from chloride when using an UV detector with reversed polarity. However when testing purity, the olpadronate peak overlapped impurities due to the huge mass difference. The major improvement was the addition of methanol to the sample solution besides the column selection. Olpadronate is practically insoluble in methanol, thus displacing the solute balance in favor of the impurities. Then, very low detection and quantitation limits could be achieved.

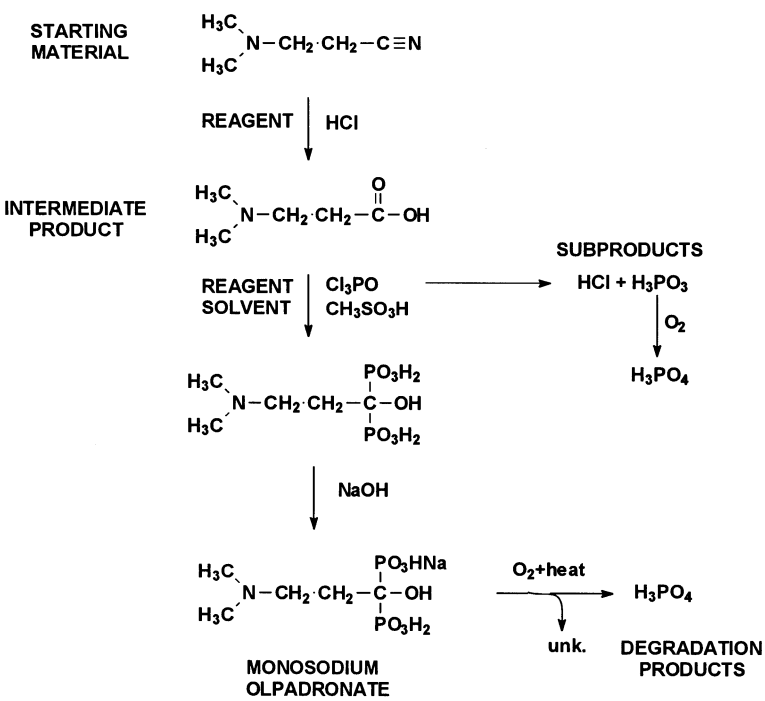

Fig. 2. Olpadronate impurity profile: impurities coming from the synthesis and forced degradation.

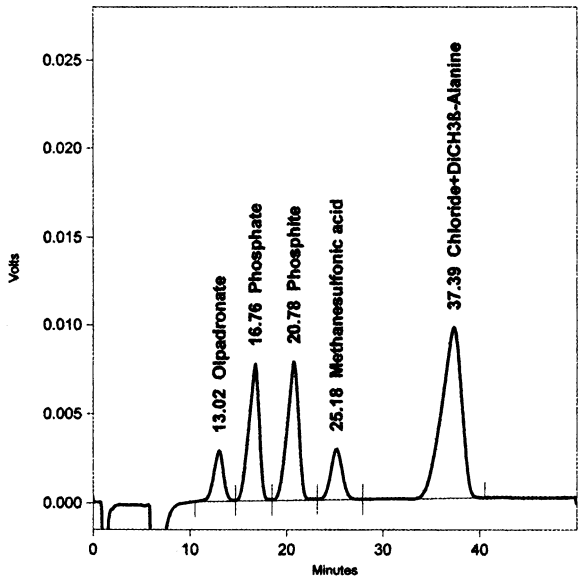

Fig. 3. Chromatogram of a mixture containing 50 μg/ml of each of the following: monosodium olpadronate, phosphate, phosphite, chloride and dimethyl-β-alanine and 20 μg/ml methanesulfonic acid. The solution (100 μl) was injected into a Waters IC Pak HR, 75 × 4.6 mm ID column with 6 μm particles. The mobile phase was 18 mΩ water adjusted to pH 2.9 with 10% HNO_3 running at 0.7 ml/min. UV detection at 235 nm with reversed polarity.

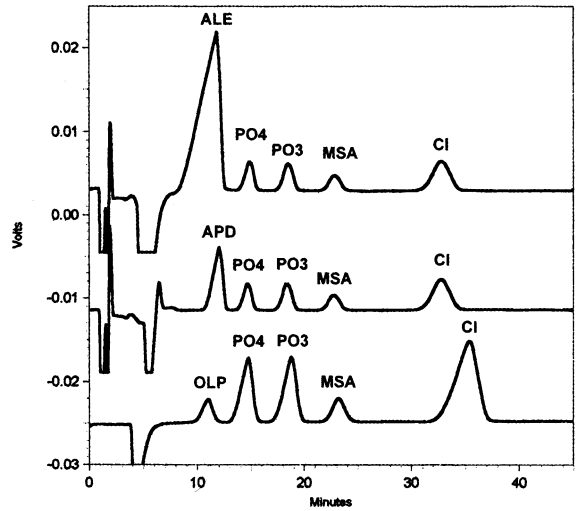

Fig. 4. Purity analysis of alendronate (ALE), pamidronate (APD) and olpadronate (OLP). Each bisphosphonate (50 mg) spiked with 0.5% phosphate, phosphite and chloride and 0.2% methanesulfonic acid was dissolved with water (1 ml for OLP, 2 ml for ALE and APD), diluted to 10 ml with methanol and filtered prior to injection. Chromatographic conditions as in Fig. 3.

2. Experimental

2.1. Instrumentation

The chromatographic system was a Shimadzu LC-10 A with a LC-10AS pump, SPD-M10AV spectrophotometric detector, SCL-10 system controller, SIL-10 A automatic injector, DGU-14 A degasser and Class vp 4.2 software running on a personal computer. The column was a Waters IC Pak Anion HR, 75 mm long filled with 6 μm particles of polymethacrylate resin with quaternary ammonium functional groups. The mobile phase was a diluted solution of nitric acid prepared adjusting 18 mΩ water to pH 2.9 with 10% HNO_3. The flow rate was 0.7 ml/min and the detection wavelength was adjusted to 235 nm with reversed polarity.

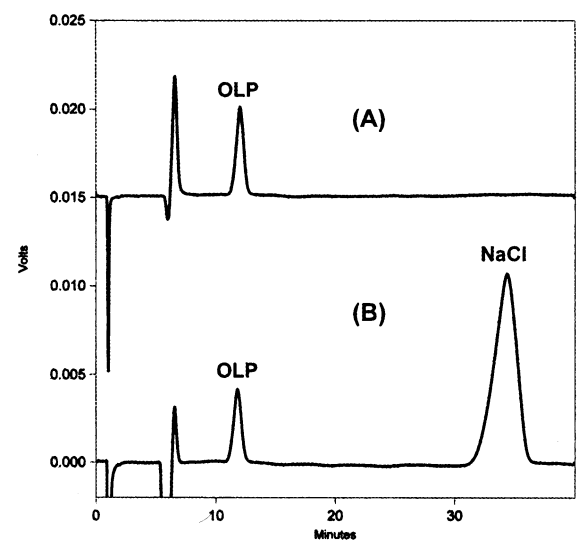

Fig. 5. Assay of (A) olpadronate tablets (50 mg/tablet) and (B) solution for injection (1 mg olpadronate and 8.5 mg NaCl/ml). The powder of one milled tablet was extracted with 100 ml water, filtered and injected. The solution for injection (1 ml) was diluted to 50 ml with water and injected directly. Chromatographic conditions as in Fig. 3.

Table 1
Linearity testing, range, LOD and LOQ[a]

Specie	Line equation	R^2	Range	LOD	LOQ
NaH_2PO_4	$Y=791600X-11711$	0.9968	0.02–1.0[b]	0.005	0.015
NaH_2PO_3	$Y=1000000X-3778$	0.9996	0.02–1.0[b]	0.004	0.013
MSA	$Y=1000000X-3931$	0.9994	0.02–1.0[b]	0.004	0.015
NaCl	$Y=2000000X+22733$	0.9991	0.006–1.0[b]	0.002	0.006
OLP (assay)	$Y=1342X-13036$	0.9999	5–1000[c]	–	–

[a] The equation of the curve were obtained with 12 points by quadruplicate. LOD and LOQ were calculated as 3 S/N and 10 S/N ratios, respectively. MSA, methanesulfonic acid.
[b] In percent.
[c] In μg/ml.

2.2. Chemicals, reagents and formulations

Olpadronate, pamidronate and alendronate were synthesized by Gador S.A. Other chemicals were of analytical grade. Olpadronate tablets contained 50 mg monosodium olpadronate in a 200-mg tablet, which also combined lactose, starch, PVP, croscarmellose, sodium laurylsulfate, microcrystalline cellulose and magnesium stearate as excipients. Olpadronate solution for injection contained 5 mg monosodium olpadronate and 42.5 mg sodium chloride in 5 ml water for injection in a colorless ampoule. Both products were manufactured by Gador S.A.

2.3. Procedure

2.3.1. Purity testing

Olpadronate (50 mg) was dissolved in 1 ml water, diluted to 10 ml with methanol, mixed, centrifuged at 3000 rpm for 10 min and filtrated through 0.45 μm pore Nylon membrane and 100 μl of the resulting solution was injected into the system. A solution containing in 10 ml about 50 mg olpadronate reference standard (matrix), 0.1 mg methanesulfonic acid (0.2%) and 0.25 mg sodium phosphate, sodium phosphite and sodium chloride (0.5% of each) was processed in the same way. A matrix blank may be prepared, precipitated with methanol and injected to discount any impurity peak present in the reference standard.

2.3.2. Assay (both for raw material and pharmaceutical product)

An aqueous sample solution (20 μl) containing about 0.2 mg olpadronate per ml was directly injected into the system and compared with an olpadronate reference standard solution.

Table 2
Accuracy testing for olpadronate purity testing (n:4)[a]

Species	Level (%)	R.S.D. (%)	Error (%)
NaH_2PO_4	0.3	0.70	−9.8
	0.5	1.37	−2.1
	0.8	0.59	+1.3
NaH_2PO_3	0.3	0.50	−2.4
	0.5	1.70	+0.6
	0.8	0.50	−0.3
NaCl	0.3	0.98	−2.3
	0.5	1.87	+1.1
	0.8	0.46	+0.6
Methanesulfonic acid	0.1	1.22	−0.5
	0.2	0.76	0.0
	0.5	0.61	+0.7

[a] Standard solution contained olpadronate reference standard spiked with 0.5% of sodium phosphate, sodium phosphite, sodium chloride and 0.2% of methanesulfonic acid.

Table 3
Precision testing: repeatability (intra-day, n:7) and intermediate precision as inter-day variation and reproducibility (n:4)[a]

Impurity		Intra-day	Inter-day		Reproducibility			
					Analyst 1 — equipment 1		Analyst 2 — equipment 2	
Specie	Level (%)	R.S.D. (%)	Found (range)	R.S.D. (%)	Found (range)	R.S.D. (%)	Found (range)	R.S.D. (%)
PO4	0.5	0.9	98.7 (95.9–100.9)	2.2	97.9 (96.2–99.1)	1.3	95.9 (95.9–96.2)	0.1
PO3	0.5	0.6	98.8 (97.1–101.4)	1.2	100.6 (98.9–102.5)	1.7	102.1 (101.2–103.5)	1.1
MSA	0.2	1.1	102.0 (99.2–103.8)	1.9	100.0 (99.3–101.0)	0.8	101.2 (99.4–101.4)	1.3
Cl	0.5	0.8	99.4 (96.3–102.0)	2.7	101.1 (99.1–103.1)	1.9	102.8 (102.3–103.0)	0.3

[a] OLP, olpadronate; PO4, phosphate; PO3, phosphite, Cl, chloride.

Table 4
Robustness in olpadronate purity testing[a]

	Resolution				Efficiency (N, theoretical plates/column)					Last peak (min)
	OLP PO4	PO4 PO3	PO3 MSA	MSA Cl	OLP	PO4	PO3	MSA	Cl	
Flow rate										
0.6	1.8	2.1	2.5	4.8	10300	15100	24100	48300	28400	42.2
0.7	1.8	2.2	2.5	4.7	10500	15300	24300	48100	28900	36.1
0.8	1.8	2.0	2.4	4.8	10200	14300	22200	46700	28300	31.4
pH										
2.8	1.9	2.3	2.6	4.9	10500	14200	24000	47300	31200	26.0
2.9	1.8	2.2	2.5	4.7	10500	15300	24300	48100	28900	36.1
3.0	1.8	2.1	2.4	4.6	9000	15400	23700	46300	27600	40.8
Column aging										
Old column (500 inj.)	1.5	1.8	1.9	3.8	9000	10900	15900	26600	19600	34.9
New column	1.8	2.2	2.5	4.7	10500	15300	24300	48100	28900	36.1
Column brand										
Merck	–	1.6	1.3	3.7	–	5100	6800	13000	10600	21.6
Waters	1.8	2.2	2.5	4.7	10500	15300	24300	48100	28900	36.1
Hamilton	0.6	1.8	4.7	4.3	7600	6500	10600	–	14800	75.6

[a] Resolution, efficiency and analysis time were considered for method optimization. Tabulated data is the mean of duplicate injections. See discussion in Section 3.2.6.

3. Results and discussion

3.1. Method optimization

No standard algorithm was used for method optimization, though every chromatographic parameter was considered to enhance precision and accuracy as well as to reduce the detection and quantitation limit for impurities with appropriate analysis time. In earlier studies we separated olpadronate from phosphate and phosphite using direct IEC with conductivity detection (O.

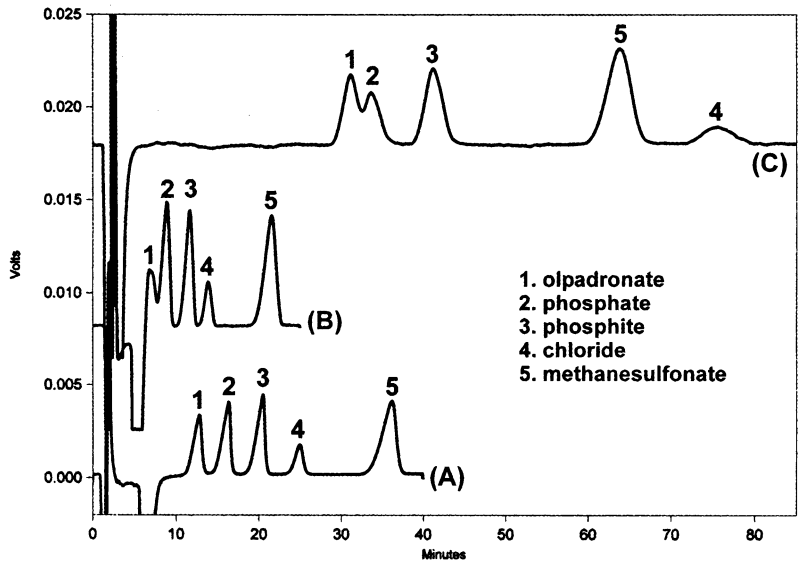

Fig. 6. Selectivity change from different column sources. Olpadronate (50 mg) was spiked with phosphate, phosphite and chloride (0.5% each) plus 0.2% methanesulfonic acid was dissolved with 1 ml water, diluted to 10 ml with methanol, filtered and injected into the system. (A) Waters IC Pak, 75 × 4.6 mm ID with 6 μm particles of quaternary ammonium functional groups bonded to polymethacrylate support, (B) Merck IC AN-1, 100 × 4.6 mm ID with 10 μm particles of quaternary ammonium functional groups bonded to polymethacrylate, (C) Hamilton PRP-X100, 150 × 4.1 mm ID with 10 μm particles of quaternary ammonium functional groups bonded to styrene divinylbenzene.

Quattrocchi, G. Bianco, R. Servant, E. Piccinni, International Ion Chromatography Symposium, September 1994, Turin, results not published). However, sensitivity was poor and not adequate for impurity testing. UV detectors, besides widespread use in pharmaceutical laboratories give better signal to noise ratio and stability, improving accuracy and sensitivity. Detection wavelength was optimized as described below in Section 3.2.6.

Mobile phase composition and pH was adjusted to get appropriate resolution while minimizing analysis time. As expected, retention and resolution increased with pH though greater analysis time made this greater resolution worthless. Flow rate was the maximum recommended by the column manufacturer with no noticeable decrease in column life.

Three column brands using two different types of support were used for anion exchange, styrene–divinylbenzene and polymethacrylate polymers bonded to the functional quaternary ammonium group. A polymethacrylate-support material was selected based on retention and resolution characteristics for this particular separation (see Section 3.2.6.4).

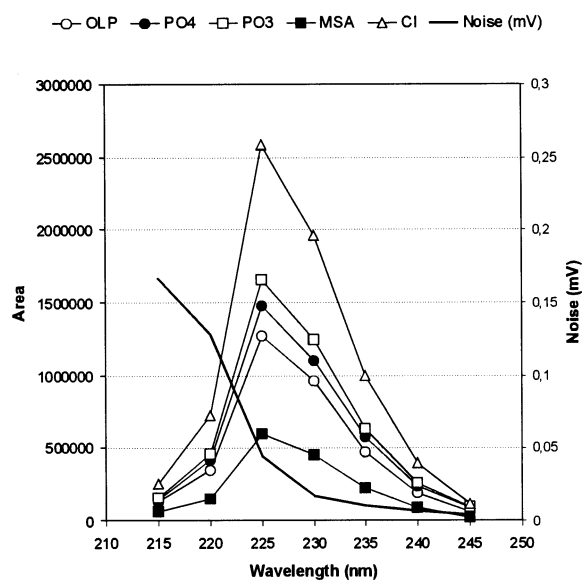

Fig. 7. Wavelength selection using a UV detector with reversed polarity for olpadronate analysis.

3.2. Validation

3.2.1. Specificity

Fig. 2 illustrates the impurity profile of monosodium olpadronate coming from the synthesis (starting material, reagents, by-products) and forced degradation products, all of them considered as potential contaminants in the final product. They include dimethylamino propionitrile, dimethyl-β-alanine, phosphate, phosphite, chloride and methanesulfonic acid used as a solvent. Forced degradation studies in strong acid and alkaline media, photolysis, thermal and oxidative conditions were carried out. Olpadronate could be degraded only by strong oxidative conditions (boiling in hydrogen peroxide solution), releasing phosphate. The organic fragment of the oxidative cleavage could not be identified up to the moment, though we are presently working on its elucidation. Yet separated from olpadronate, the method cannot quantify *N*,*N*-dimethylamino propionitrile which elutes with the system peak and dimethyl-β-alanine, which co-elutes with chloride peak. However, these compounds can be easily determined by TLC using silica gel, methanol–ammonia (90:3) as the mobile phase and a iodine chamber for detection. 0.05% LOD could be achieved for each compound though these impurities were never found in regular production batches. As shown in Fig. 3, olpadronate is well separated from all its potential impurities.

The method proved to be similarly useful for the analysis of other related molecules as pamidronate and alendronate, both for purity and potency testing (Fig. 4) and can be easily adapted for the analysis of pharmaceutical formulations (Fig. 5).

3.2.2. Linearity and range

Linearity was evaluated on a monosodium olpadronate solution spiked with monosodium phosphite, monosodium phosphate, sodium chloride and methanesulfonic acid ranging from 0.005 to 2.0% impurity level. Solutions containing 2% impurity level gave distorted peaks and were not considered. The system was linear both for purity determination and for olpadronate assay as shown in Table 1.

3.2.3. Accuracy

Accuracy was estimated from quadruplicate sets of samples as the percent error in the recovery test using the proposed method on olpadronate spiked with monosodium phosphate, phosphite, chloride at the 0.3, 0.5 and 0.8% level and methanesulfonic acid at the 0.1, 0.2 and 0.5% level.

The larger difference between added and found value was seen for phosphate at the lower concentration level (see Table 2). This was attributed to co-precipitation, as phosphate seems to be more sensitive to that factor. Co-precipitation becomes more important as phosphate concentration increases and, higher concentration in the standard solution compared to the sample solution lead to false lower results. Even then, results are adequate for analytical purposes though can be fine tuned preparing a standard with closer impurity levels.

3.2.4. Precision

Precision was assessed as the R.S.D. repeatability as an intra-day test (same day, analyst and chromatograph) and intermediate precision, tested as inter-day variation (different day, same analyst and chromatograph) and reproducibility (different day, analyst and chromatograph). The results are shown in Table 3.

3.2.5. Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined graphically as 3 S/N ratio and 10 S/N ratio, respectively, by quadruplicate measurements on solutions containing olpadronate spiked with 0.02% of each impurity (see Table 1).

3.2.6. Robustness

Resolution, efficiency and analysis time were evaluated after variation of the following parameters to check robustness (Table 4).

3.2.6.1. Flow rate. Though varying analysis time, flow rate did not affect chromatographic performance significantly.

3.2.6.2. pH of the mobile phase. Change in ± 0.1 pH units did not change chromatographic performance significantly though, as predicted, higher pH increased analysis time.

3.2.6.3. Column aging. Column aging was an important parameter since the column manufacturer did not recommend the use of methanol, here used as the sample solvent. Yet, a column used for several months with the same type of application showed lower efficiency but still a good performance.

3.2.6.4. Column brand. Three column brands were tested: Merck IC AN-1 and Waters IC Pak both with quaternary ammonium functional groups on polymethacrylate based material and a Hamilton PRP-X100 with the same functional group on styrene divinylbenzene support (see Fig. 6).

Both Merck and Waters separated each component of the mixture efficiently. However, olpadronate eluted near the void volume when using the Merck column, making its quantitation for assay purposes difficult.

The Hamilton column did not separate olpadronate from phosphate efficiently. On the other hand, it showed different selectivity as methanesulfonic acid eluted after the chloride peak. This seems to be produced by a reversed phase mechanism between the non-polar part of the molecule with the styrene–divinylbenzene moiety additionally to the proposed ion exchange mechanism. Analysis time was also doubled when using the Hamilton column.

3.2.6.5. Wavelength. Detection wavelength was varied between 215 and 245 nm in 5 nm increments using reversed polarity (see Fig. 7). It was found that the lamp energy (or the energy reaching the photodetector) falls below 225 nm with noise increase. On the contrary, the peak area decreases when wavelength increases. A balanced wavelength was chosen to improve peak area/noise ratio. Wavelength can be varied between 225 and 240 nm to fine-tune noise and sensitivity.

4. Conclusions

A new method for the analysis of monosodium olpadronate was developed. The method is stability indicating, simple, specific, precise, accurate, robust and can be used for purity testing and potency assay of olpadronate raw material. It can also be easily transferred to olpadronate pharmaceutical formulations as well as structural related bisphosphonates like pamidronate and alendronate.

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