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APPLICATION OF UV-DERIVATIVE SPECTROPHOTOMETRY FOR DETERMINATION OF SOME BISPHOSPHONATES DRUGS IN PHARMACEUTICAL FORMULATIONS

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Abstract: A method for determination of alendronate sodium salt, clodronate disodium salt and etidronate disodium salt in pharmaceutical formulation by direct UV-spectrophotometry and/or first and second derivative UV-spectrophotometry via complex formation with Cu (II) ions is described. The calibration graphs are linear in the range 25-600 mmol/L for all the investigated compounds. No interference was found from tablet excipients at the selected wavelength and assay procedure. The developed method was validated and found to be sufficiently precise and reproducible, at least for established conditions.

Keywords: bisphosphonates, alendronate sodium salt, clodronate disodium salt, etidronate disodium salt, derivative UV-spectrophotometry, pharmaceutical formulation

Bisphosphonates are pyrophosphate analogs in which the two phosphorus atoms are linked with carbon atom. This group of drug include among others: alendronate (4-amino-1-hydroxybutane-1,1-bisphosphonic acid) sodium salt, clodronate (dichloromethylene-1,1-bisphosphonic acid) disodium salt, and etidronate (1-hydroxyethane-1,1-bisphosphonic acid) disodium salt (Figure 1). Bisphosphonates inhibit the resorption of bone matrix by osteoclasts, and have been used in treatment of osteoporosis (1) and may interfere directly with some cancer mechanisms (at epidermoid carcinoma, bone tumors and other cancer cells proliferation) (2-4).

Literature data on the analysis of bisphosphonates concern mostly their determination in biological fluids, using HPLC (5-8) or CE (9-12) methods, after their derivatization indispensable for spectrophotometric or spectrofluorimetric detection. Bisphosphonates do not possess in their structure chromophoric groups giving characteristic absorption or fluorescent spectra in UV or visible region. Therefore direct spectrophotometric determination of bisphosphonates is rather not accessible. Taha and Youssef (13) proposed spectrophotometric determination of some bisphosphonates in pharmaceutical formulation based on the reaction with ninhydrin or after the oxidation with ceric (IV) sulfate. However, cited methods have some limitations: the first could be used only for bisphosphonates possessing primary amino group and the second is connected with measurement of unreacted ceric (IV) sulfate after bisphosphonates treatment. Kuljanin et al. (14) performed UV-spectrophotometric determination of alendronate in pharmaceutical formulations after complex formation with Fe (III) ions. This method based on the complexation of bisphosphonates with Fe (III) ions was also proposed for determination of aqueous solutions of bisphosphonates using ion-pair HPLC (15). Metal chelating properties of bisphosphonates with copper(II) (16, 17) or calcium (18) ions have been described with potential application as UV-spectrophotometric detection by the assay using high-performance ion-exchange chromatography (19).

In this study UV-spectrophotometric method based on complexation of bisphosphonates with Cu (II) ions (17) is applied for determination of three bisphosphonate drugs in pharmaceutical preparations.

EXPERIMENTAL

Chemicals and Reagents

Pure substances: alendronate (4-amino-1-hydroxybutane-1,1-bisphosphonic acid) sodium salt and clodronate (dichloromethylene-1,1-bisphosphonic acid) disodium salt were from Sigma-Aldrich (Poznań, Poland), etidronate (1-hydroxyethane-1,1-bisphosphonic acid) disodium salt was obtained from ICN Polfa Rzeszów S.A. (Poland). Pharma-

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ceutical preparations: OSTENIL 70 (tablets 70 mg of alendronic acid corresponding to 76.16 mg of alendronate sodium salt, from Polfa Kutno S.A., Poland), BONEFOS 800 (tablets 800 mg of clodronate disodium salt, from Schering AG S.A., Germany), OSTEDRON 400 (tablets 400 mg of etidronate disodium salt, from ICN Polfa Rzeszów S.A., Poland). $CuSO_4$ anhydrous, HNO_3 (65%) and other reagents were from POCH (Gliwice, Poland). Talk was from Amara (Kraków, Poland). Methanol was from Sigma-Aldrich (Poznań, Poland). All reagents used were at least of the analytical grade. Double distilled water was used in all experiments.

Apparatus

A Hitachi U-2800 double beam UV-visible spectrophotometer with PC was used. All UV spectra were recorded in 1 cm quartz cells over 190-400 nm at 25°C. Derivative spectra (first and second order) were calculated with built-in function of its dedicated software UV Solutions 2.0, using Savitzky-Golay method (order = 3, delta lambda = 6 nm) and with peak-zero (P-0) technique of measuring.

Preparation of calibration curve

From aqueous solutions of 1.5 mmol/L $CuSO_4$ and 1.5 mmol/L HNO_3 (pH 2.8), containing 1 mmol/L of each examined drug (corresponding to 250 µg/mL, 288.85 µg/mL and 271.12 µg/mL concentrations of alendronate sodium salt, clodronate disodium salt and etidronate disodium salt, respectively) were prepared solutions in the range 25-600 µmol/L (corresponding to the range concentrations of 6.78-162.72 µg/mL, 7.22-173.31 µg/mL and 6.25-150 µg/mL for alendronate sodium salt, clodronate disodium salt and etidronate disodium salt, respectively). Spectra of these solutions were recorded against blank (1.5 mmol/L $CuSO_4$, 1.5 mmol/L HNO_3, pH 2.8). The calibration curves were set up by plotting direct absorbance and derivatives absorbance against the drug concentrations. All measurements were repeated six times for each concentration. The equations of calibration line were estimated using linear regression analysis (y = ax + b), correlation coefficients (r) were calculated, and statistical evaluation of the elaborated method was performed.

Analytical procedure for the assay of elaborated bisphosphonates in pharmaceutical formulations

Ten tablets of each drug were weighted and powdered. Accurately weighted amounts of the powder, equivalent to 50 mg, 100 mg and 250 mg of alendronate sodium salt, clodronate disodium salt and etidronate disodium salt, respectively (mass tablet: 195.7 mg of OSTENIL 70, 121.2 mg of BONEFOS 800, 318.3 mg of OSTEDRON 400), were extracted with solution of 1.5 mmol/L $CuSO_4$ and 1.5 mmol/L HNO_3, pH 2.8 solution in 25 mL flasks by means of reciprocating shaker for 10 min as optimum extraction time. After filtration, the

Table 1. Calibration curves and statistical evaluation of the elaborated method.

Compound	Spectrum[a]	Wavelength [nm]	r[b]	y = ax + b		tS_a[c]	tS_b[d]	Coefficient of essentiality[e]		
				a	b			a	b	r
Alendronate sodium salt	D	233	0.9988	$5.6\ 10^{-3}$	$2.8\ 10^{-2}$	$1.3\ 10^{-5}$	$1.1\ 10^{-2}$	+	+	+
	D_1	245	0.9996	$3.0\ 10^{-5}$	$7.0\ 10^{-5}$	$1.0\ 10^{-6}$	$1.0\ 10^{-4}$	+	-	+
	D_2	254	0.9991	$2.0\ 10^{-7}$	$2.0\ 10^{-10}$	$3.6\ 10^{-9}$	$1.7\ 10^{-6}$	+	-	+
Clodronate disodium salt	D	236	0.9958	$5.6\ 10^{-3}$	$5.6\ 10^{-2}$	$4.6\ 10^{-4}$	$2.1\ 10^{-1}$	+	-	+
	D_1	261	0.9965	$2.3\ 10^{-5}$	$2.1\ 10^{-4}$	$2.2\ 10^{-6}$	$9.8\ 10^{-4}$	+	-	+
	D_2	284	0.9955	$1.3\ 10^{-7}$	$1.5\ 10^{-6}$	$2.0\ 10^{-8}$	$1.1\ 10^{-5}$	+	-	+
Etidronate disodium salt	D	232	0.9990	$6.0\ 10^{-3}$	$1.7\ 10^{-2}$	$4.9\ 10^{-5}$	$2.2\ 10^{-2}$	+	-	+
	D_1	243	0.9995	$4.0\ 10^{-5}$	$-1.0\ 10^{-5}$	$9.0\ 10^{-7}$	$3.9\ 10^{-4}$	+	-	+
	D_2	253	0.9993	$2.0\ 10^{-7}$	$-2.0\ 10^{-7}$	$9.7\ 10^{-9}$	$4.3\ 10^{-6}$	+	-	+

[a] – D (direct), D_1 (first derivative), D_2 (second derivative) spectra, respectively; [b] – correlation coefficient; [c, d] – confidence intercept (a) or of the slope (b) at 95% confidence level ($t_{a,f}$ = 2.776 for a = 0.05 and f = (n-2), where n = 6) [e] – coefficient of a (intercept), b (slope), r (correlation), respectively, tested at 95% confidence level using point hypothesis tests. „+" – essential (important), „-" – not essential (not important).

Table 2. Determination of bisphosphonates in pharmaceutical formulations using elaborated method.

Compound	Spectrum[a]	Wavelenght [nm]	Declarated Mean Content[b] [mg]	Found Mean Content[c] [mg]	Recovery[d] %	SD[e]	RSD[f] %
Alendronate	D	233		73.09	95.95	2.25	3.08
sodium	D_1	245	76.16	85.59	112.37	1.88	2.19
salt	D_2	254		75.78	99.49	4.46	5.88
Clodronate	D	236		898.76	112.34	10.07	1.12
disodium	D_1	261	800.00	881.81	110.23	13.40	1.52
salt	D_2	284		810.22	101.28	24.23	2.99
Etidronate	D	232		412.51	103.13	7.78	1.89
disodium	D_1	243	400.00	361.79	90.45	9.49	2.62
salt	D_2	253		476.65	119.16	19.57	4.10

[a] – D (direct), D_1 (first derivative), D_2 (second derivative) spectra, respectively; [b, c] – declarated and found mean content of studied compounds in one tablet, respectively; [d] – the percentage of recoveries of the data obtained for studied compounds calculated for 6 independent measurements; [e, f] – standard deviations and percentage of relative standard deviations, respectively, calculated for 6 independent measurements.

extract was diluted to concentration of 50 mg/mL of each drug. Determination procedure was repeated six times. UV-spectra were recorded against reagent blank aqueous solution (1.5 mmol/L $CuSO_4$ and 1.5 mmol/L HNO_3, pH 2.8).

Precision

The precision of the elaborated method was calculated from six determinations of each drug and each pharmaceutical preparation. The percentage of relative standard deviations (%RSD) is given in Tables 2 and 3.

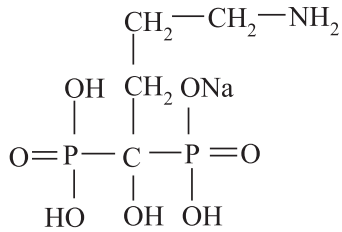
Recovery

The recovery of the method was evaluated by analyzing the model mixtures containing 12.5, 15.0 and 17.5 % of each compound, compared with declared amount of bisphosponates in tablets and the mean recoveries were obtained.

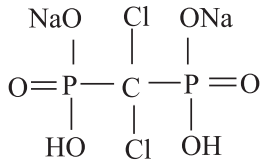
RESULTS AND DISCUSSION

Aqueous solution containing Cu^{2+} ions (1.5 mmol/L $CuSO_4$, 1.5 mmol/L HNO_3, pH 2.8) proved to be suitable for quantitative complexation of bisphosphonates studied (Figure 1), as well as for their extraction from pharmaceutical preparations. As it is known, the complexation reaction of Cu^{2+} with biphosphonates proceed in molar ratio 1:1 with best result at pH 2.8 (17). However, quantitative complexation required an excess of Cu^{2+} ions over exam-

A)



B)



C)

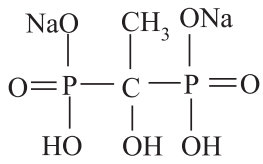


Figure 1. Structures of investigated bisphosphonates. A) Alendronate sodium salt, B) Clodronate disodium salt, C) Etidronate disodium salt.

Table 3. Recovery and precision of the elaborated method.

Compound	Spectrum[a]	Wavelenght [nm]	Model mixtures[b] %	Recovery[c] %	Mean Recovery[d] %	RSD[e] %	Mean RSD[f] %
Alendronate sodium salt	D	233	12.50	99.15	98.15	2.03	1.98
			15.00	97.54		1.79	
			17.50	97.75		2.12	
	D_1	245	12.50	117.86	115.86	1.17	1.53
			15.00	115.57		1.88	
			17.50	114.14		1.54	
	D_2	254	12.50	102.59	103.88	2.20	2.51
			15.00	102.57		2.56	
			17.50	103.48		2.78	
Clodronate disodium salt	D	236	12.50	113.50	111.67	2.14	2.23
			15.00	112.27		2.55	
			17.50	109.24		1.99	
	D_1	261	12.50	111.54	111.53	2.01	1.86
			15.00	110.94		1.45	
			17.50	112.12		2.12	
	D_2	284	12.50	104.36	102.53	3.12	3.04
			15.00	101,00		3.45	
			17.50	102.24		2.56	
Etidronate disodium salt	D	232	12.50	104.12	102.68	1.34	1.77
			15.00	100.74		1.79	
			17.50	103.18		2.19	
	D_1	243	12.50	88.45	87.85	3.02	2.86
			15.00	86.82		2.67	
			17.50	88.28		2.89	
	D_2	253	12.50	113.36	115.15	2.37	2.13
			15.00	119.24		1.89	
			17.50	112.86		2.12	

[a] – D (direct), D_1 (first derivative), D_2 (second derivative) spectra, respectively; [b] – the model mixtures contained 12.5, 15.0 and 17.5% of studied compounds compared to the labeled tablet amount. [c, d] – the percentage and mean percentage of recoveries of the data obtained for studied compounds calculated for 6 independent measurements, respectively; [e, f] – percentage and mean percentage of relative standard deviations calculated for 6 independent measurements, respectively.

ined 600 mmol/L concentration of alendronate sodium salt, clodronate disodium salt or etidronate disodium salt. The stability of the complex Cu^{2+}: bisphosponate was confirmed by measuring the absorbance at 232-234 nm six times within 2 h (RSD = 3.24%). It allows to obtain UV spectra: direct (Figure 2A) and to determine first and second derivative spectra (Figures 2B, 2C) of these compounds. The obtained spectra in the derivative spectrophotometric assay of bisphosphonates studied

Table 4. Limit of detection (LOD) and limit of quantitation (LOQ) of the elaborated method.

Compound	LOD [mg/mL]	LOQ [mg/mL]
Alendronate sodium salt	2.13	7.11
Clodronate disodium salt	1.55	5.17
Etidronate disodium salt	2.07	6.90

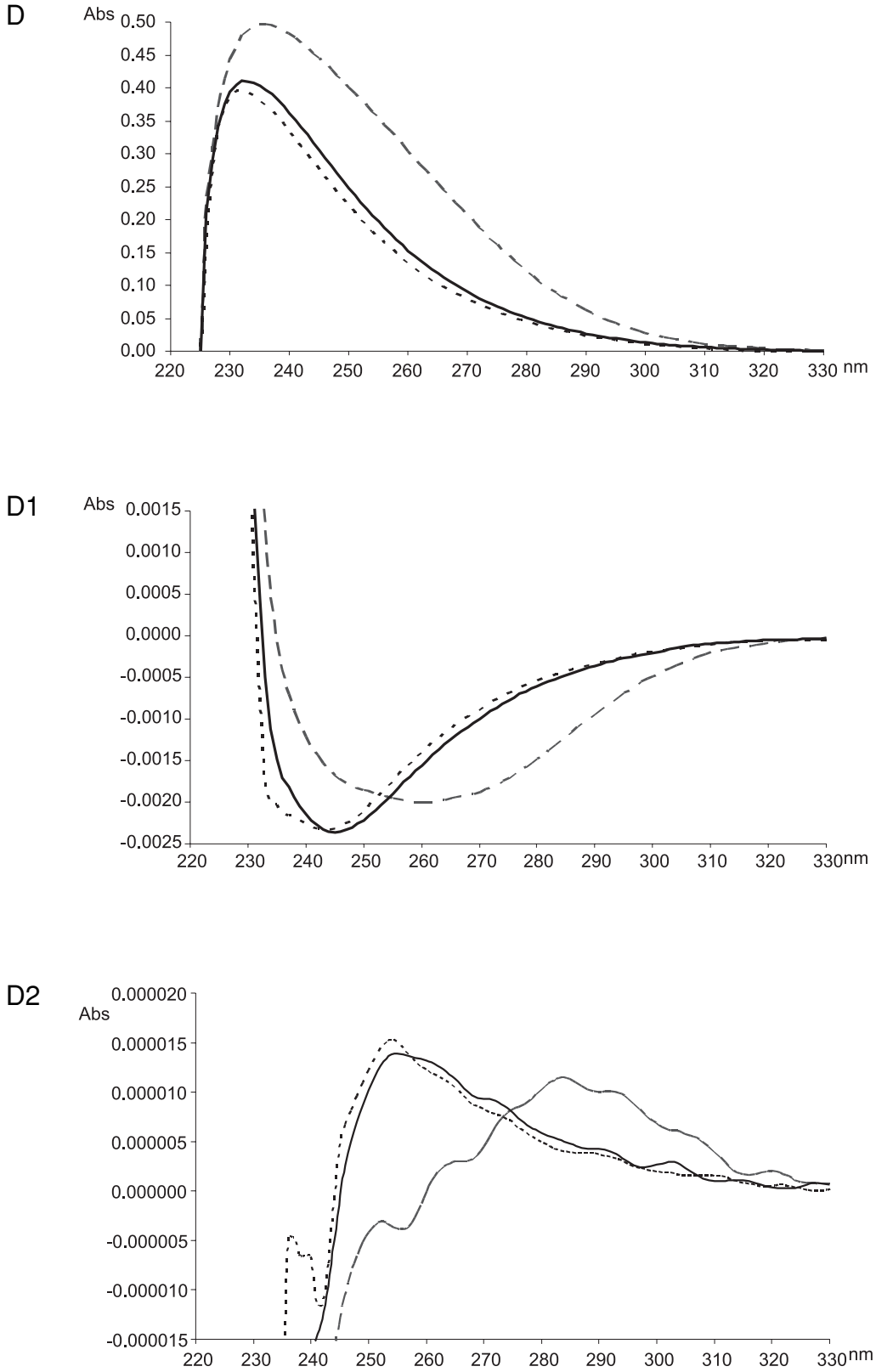


Figure 2. Direct (D), first (D1) and second (D2) derivative spectra of alendronate (solid line), clodronate (dashed line), etidronate (dotted line) at concentration 250 mmol/L in 1.5 mol/L $CuSO_4$, 1.5 mol/L HNO_3, pH 2.8 solution.

were measured by "peak-zero" technique. Spectra of higher order of derivatives were not obtained. Calculated calibration curves (for direct absorbance or derivative absorbance vs. drug concentration) were characterized by a very good linearity ($r > 0.999$ in most cases) and statistical parameters (Table 1) in concentration range 6.78 – 162.72 μg/mL, 7.22 – 173.31 μg/mL and 6.25 – 150 μg/mL for alendronate sodium salt, clodronate disodium salt and etidronate disodium salt, respectively. After statistical evaluation value of slope (b) it turned out not important (in most cases) and obtained from extraction data were calculated from equation $y = ax$.

It was found that extraction procedure of bisphosphonates from powdered tablets using aqueous solution 1.5 mmol/L $CuSO_4$ and 1.5 mmol/L HNO_3 at pH 2.8 is simple and effective. The excipients in tablets did not interfere with spectrophotometric measurement of absorbance over 190-400 nm wavelength region.

The results of the determination of alendronate sodium salt, clodronate disodium salt and etidronate disodium salt in pharmaceutical formulations (Table 2) showed good precision (RSD = 1.12 – 5.88%). However, the highest accuracy conditions of elaborated method described as mean recovery value was found for direct spectra for alendronate sodium salt (95.95%) and etidronate disodium salt (103.13%), and for second derivative spectra in the assay of alendronate sodium salt (99.49%) and clodronate disodium salt (101.28%) (Table 2) Moreover, the precision of elaborated method verified by analysis of model mixtures is given in Table 3 and, as shown, it is characterized by good intermediate precision for all derivatives and wavelengths studied (RSD = 1.53 – 3.04%) with better one than obtained for powdered tablets (Table 2). Analysis of the model mixtures confirmed that the highest accuracy conditions of elaborated method described as mean recovery value is for direct spectra of alendronate sodium salt (98.15%) and etidronate disodium salt (102.68%), and for second derivative spectra in the case of alendronate sodium salt (103.88%) and clodronate disodium salt (102.53%) (Table 3). The calculated values of the limit of detection (LOD) and limit of quantitation (LOQ) give rise to a signal that is three and ten times, respectively, the noise of the method (Table 4).

The three elaborated methods (direct, first and second order derivative spectrophotometry) for the determination of three bisphosphonates in tablets are rapid and simple, but only some derivatives and wavelengths proved the accurate linearity, precision and recovery and may be recommended for routine quality control of these drugs.

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