Spectrophotometric Determination of Some Drugs for Osteoporosis

Elham Anwer TAHA* and Nadia Fayek YOUSSEF

National Organisation for Drug Control and Research (NODCAR); 6 Abou Hazem st. pyramids, P.O. Box 29 Cairo, Egypt.
Received July 24, 2003; accepted August 30, 2003

Three simple, accurate and sensitive spectrophotometric methods are developed for the determination of some new drugs for the treatment of osteoporosis: risedronate sodium (I), alendronate sodium (II) and etidronate disodium (III). The first method is based on the measurement of difference in absorbance (ΔA) of risedronate sodium in 0.01 mol l^{-1} hydrochloric and 0.1 mol l^{-1} sodium hydroxide at 262 nm. Beer's law is obeyed over a concentration range of 15—150 μg ml^{-1} with mean recovery 99.75±1.22 and molar absorptivity (ε) 1.891×10^3. The second method is based on the reaction of the primary amino group of (II) with ninhydrin reagent in methanolic medium in the presence of 0.05 mol l^{-1} sodium bicarbonate. The colored product is measured at 568 nm, and the linearity range is found to be 3.75—45 μg ml^{-1} with mean recovery 99.77±0.73 and ε 9.425×10^3. The third method is based on oxidation of the three mentioned drugs with ceric (IV) sulphate in 0.5 mol l^{-1} sulphuric acid at room temperature and subsequent measurement of the excess unreacted cerium (IV) sulphate at 320 nm. The method obeyed Beer's law over a concentration range of 2—24 μg ml^{-1} for the three drugs with mean recovery 99.79±1.16, 99.73±1.38 and 99.86±1.13 and ε 14.427×10^3, 13.813×10^3 and 14.000×10^3 for drugs I, II, III respectively. The proposed methods were successfully applied for the determination of the studied drugs in bulk powder and in pharmaceutical formulations. The results were found to agree statistically with those obtained the reported methods. Furthermore, the methods were validated according to USP regulations and also assessed by applying the standard addition technique.

Key words risedronate; alendronate; etidronate; difference in absorbance (ΔA); ninhydrin; ceric (IV) sulphate

Risedronate sodium (I), is sodium trihydrogen (1-hydroxy-2-(3-pyridyl) ethylidene) diphosphonate; alendronate sodium (II), is sodium trihydrogen (4-amino-1-hydroxybutylidene) diphosphonate trihydrate and etidronate disodium (III), is disodium dihydrogen (1-hydroxy ethylidene) diphosphonate Fig. 1. They all belong to the bisphosphonate group and are used for the treatment of Paget's disease of bone and osteoporosis, they diminish bone resporption and thus reduce bone turnover.[1,2]

Few methods have been reported for their determination. (I) is a non-official drug and to our best knowledge no method has been published for its determination. For (II), spectrophotometric,[3—5] chromatographic,[6—12] capillary electrophoresis[13] and inductively coupled plasma[14] methods were reported.

For (III), USP describes a tedious titrimetric assay,[15] which requires one week for the reagent preparation. Moreover, no spectrophotometric methods have been developed to date, and few ion chromatographic methods[8,16,17] have been published.

This paper suggests a direct and simple spectrophotometric difference in absorbance (ΔA) method [1] for the determination of (I). Method [2] uses ninhydrin reagent for the determination of (II). Method [3] is based on the determination of (I), (II) and (III) through their oxidation with ceric (IV) sulphate at room temperature (25±5 °C). The advantages of the three suggested methods over already existing methods are accuracy, simplicity and low cost.

Experimental

Apparatus Shimadzu 1601 UV/vis spectrophotometer with 1 cm matched cells.

Materials and Reagents Risedronate sodium was kindly donated by Avents Co., Egypt., with a purity of 100.03%,[18] together with Actonel tablets, labeled to contain 35 mg risedronate sodium. Alendronate sodium was received from NODCAR, with a purity of 99.71%[18] and Fosamax tablets (from Global Napi Co, Egypt) were labeled to contain 10 mg of alendronic acid equivalent to 13.05 mg of alendronate sodium. Etidronate disodium was obtained from NODCAR, its purity was 99.83%.[15] Hydrochloric acid, 0.01 mol l^{-1} aqueous solution. Sodium hydroxide, 0.01 mol l^{-1} aqueous solution. Ninhydrin, 0.2% in methanol kept for 2 d at 4 °C. Sodium bicarbonate, 0.05 mol l^{-1} aqueous solution. Ceric sulphate, 0.1% in 0.5 mol l^{-1} sulphuric acid in an umber colored container.

Standard Stock Solutions For method [1] (ΔA): Risedronate sodium standard solution (0.6 mg ml^{-1}) was prepared in distilled water.

For method [2] using ninhydrin: Alendronate sodium standard solution, (0.15 mg ml^{-1}) was prepared in distilled water.

For method [3] using ceric (IV) sulphate: Risedronate sodium, alendronate sodium and etidronate disodium standard solutions, (80 μg ml^{-1}) were prepared in distilled water.

All standard solutions could be used within one week and were stored at 4 °C.

Sample Preparations Four tablets for drug I, or ten tablets for drug II were accurately weighed and powdered. A synthetic tablet mixture was prepared for etidronate disodium III. A definite amount of the powdered tablets equivalent to 60 mg of I for method [1], 15 mg of II for method [2] and 60 mg of the drugs I, II, III for method [3] was transferred into a 100 ml volumetric flask, then extracted with 50 ml water by shaking for 10 min, filtered and the volume was completed with distilled water Solutions were prepared with final concentrations 0.6 mg ml^{-1} for method [1] 0.15 mg ml^{-1} for method [2] and 80 μg ml^{-1} for method 3.

Procedures. Method [1] ΔA Different aliquots of standard solution equivalent to 0.15—1.5 mg (I) were transferred into two series of 10 ml volumetric flasks. The first series was completed with 0.01 mol l^{-1} hydrochloric acid and the second series with 0.01 mol l^{-1} sodium hydroxide. The absorbance difference (ΔA) was measured at 262 nm against the drug in sodium hydroxide as a blank.

Method [2] Using Ninhydrin Reagent Different aliquots of standard stock solution equivalent to 37.5—450 μg (II) were transferred into a series of 10 ml volumetric flasks 0.5 ml sodium bicarbonate, 2.5 ml of ninhydrin was added and the mixture was heated in a water bath at 90±5 °C for 20 min. The flasks were cooled and the volume was made up to the mark with distilled water. The absorbance was measured at 568 nm against a reagent blank.

Method [3] Using Ceric (IV) Sulphate Different aliquots of standard stock solutions equivalent to 20—240 μg for the three cited drugs (I, II, III) were transferred into a series of 10 ml volumetric flasks. 1.5 ml of ceric sulphate was added and allowed to stand for one hour at ambient temperature

* To whom correspondence should be addressed. e-mail: dr_elhamtaha@hotmail.com

(25±5 °C). The volume was completed with 0.5 mol l^{-1} sulphuric acid and the absorbance of the blank solution (1.5 ml of ceric sulphate was completed with 0.5 mol l^{-1} sulphuric acid) was measured against each experiment. The difference in absorbance is proportional to the amount of ceric sulphate consumed by the three mentioned drugs.

Results and Discussion

Alendronate sodium II and etidronate disodium III shown in Fig. 1 do not have a chromophore and no absorbance throughout the UV/vis bands of the spectrum has been detected. Therefore, neither drug can be determined by ordinary direct spectrophotometric methods.

Method [1] [ΔA] Risedronate sodium I possesses pyridyl group as an appreciable chromophore (Fig. 1), which is responsible for the protonation as aniline in an acidic medium, whereas in the alkaline medium no change was observed. It exhibits a UV absorption at 262 nm in aqueous acid higher than in aqueous alkali. The ΔA method depends on measuring the difference in absorbance of equimolar portions of risedronate sodium solution in 0.01 mol l^{-1} hydrochloric acid as a test and the drug in 0.01 mol l^{-1} sodium hydroxide as a blank as shown in Fig. 2. The difference in the absorbance ΔA is proportional to the concentration of drug I. Different molarities (0.01—0.1 mol l^{-1}) of hydrochloric acid and sodium hydroxide were studied and no difference in absorbance was observed, so 0.01 mol l^{-1} solutions were preferred for safety of the environment . A (1%, 1 cm) of risedronate sodium was calculated at 262 nm and found to be 152 in aqueous acid and 91 in aqueous alkali which can be used for its direct estimation.

Method [2] Using Ninhydrin Alendronate contains a primary aliphatic amino group which is known to react with ninhydrin reagent. This reagent is used for the determination of primary amines and amino acids.[19,20] Drug II reacts with ninhydrin in the presence of sodium bicarbonate *via* oxidation deamination of the primary amino group followed by condensation of the reduced ninhydrin to form the colored reaction product, Ruhemenn's purple, with maximum absorbance at 568 nm as shown in Fig. 3.

To optimize the reaction conditions, different parameters have been investigated such as reagent and sodium bicarbonate concentrations, temperature, time and solvents. 2.5 ml of 0.2% ninhydrin reagent was adequate for maximum color intensity. The concentration of sodium bicarbonate was also studied as the reaction proceeds only in an alkaline medium. Different molarities (0.01—0.1 mol l^{-1}) were studied, 0.5 ml of 0.01 mol l^{-1} sodium bicarbonate gave maximum absorption. Drug (II) was capable of reaction with ninhydrin only at higher temperatures by heating in a water bath at (90±5 °C) for 20 min. Different diluting solvents have been tried: water, methanol and ethanol. Water gave the best results and the reaction product was stable for at least 1 h.

Method [3] Using Ceric (IV) Sulphate Cerium (IV) is a strong oxidizing agent that has been used in the determination of several drugs including paracetamol[21] and aztreonam.[22] Spectrophotometric determination of drugs I, II and III was achieved through their oxidation using excess ceric (IV) sulphate in the presence of 0.5 mol l^{-1} sulphuric acid at room temperature. The amount of the consumed ceric equivalent to the concentration of these drugs was determined by measuring the absorbance of ceric solution (as blank) against

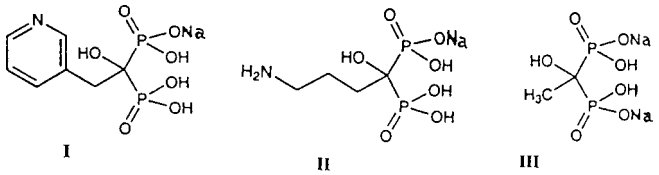

Fig. 1. Structure of the Studied Drugs

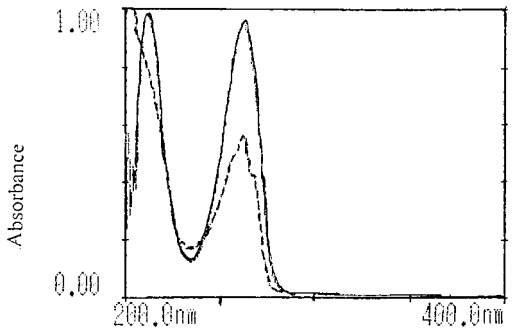

Fig. 2. Absorption Spectra of Risedronate Sodium (60 μg ml^{-1}) in 0.01 N Hydrochloric (—) and 0.01 N Hydroxide (- - -)

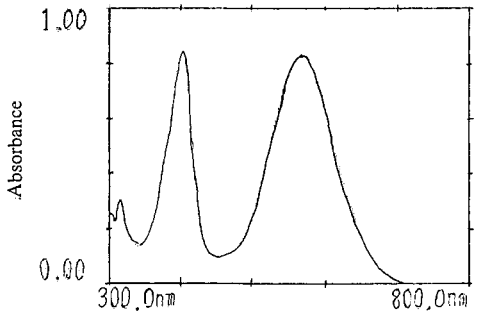

Fig. 3. Absorption Spectrum of the Reaction Product of Alendronate Sodium (30 μg ml^{-1}) with Ninhydrin

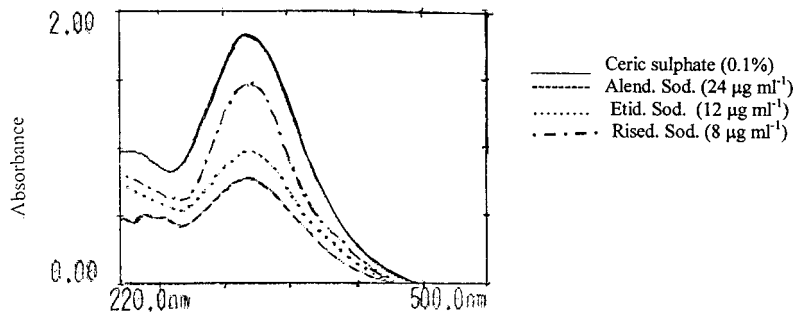

Fig. 4. Absorption Soectra of the Reaction Products of the Studied Drugs with Ceric (IV) Sulphate

the test solution at 320 nm (Fig. 4).

All factors affecting the reactions were thoroughly studied namely, ceric sulphate concentration, solvents, temperature and time. Optimum conditions were achieved when the volume of Ce^{4+} added was at least double that consumed at the end of the reaction. Different solvents such as methanol, ethanol, water and sulphuric acid with different normalities were tried and 0.5 mol l^{-1} sulphuric acid gave the highest absorbance difference. Cooling and heating at different temperatures were tisted and room temperature was suitable for complete reaction. It was found that 60 min in contact with acidified solution of Ce^{4+} is sufficient for complete oxidation of the cited drugs, as indicated by the highest absorbance difference at 320 nm. The reaction products were found to be stable for at least 1 h.

Stoichiometry of the reactions for methods [2] and [3] was studied by Job's method of continuous variation. The molar ratio of the drug to reagent was found to be (1 : 2) as in Fig. 5. The mechanism of the reactions is suggested in Charts 1 and 2.

The performance of the current methods was assessed by calculation of the *t*- and *F*-values compared with the reported acid-base titrimetric method using 0.1 mol l^{-1} sodium hydroxide for drugs I and II[18] and the official method for drug III.[15] The results obtained showed that the calculated *t*- and *F*-values did not exceed the theoretical values (95% confidence limits for the five degrees of freedom) (Table 1).

Method Validation The methods were tested for linearity, accuracy and precision. Using the above spectrophotometric procedures, linear regression equations were obtained. The regression plots showed a linear dependence of the absorbance over the Beer's law range given in Table 2. The table also summarizes the results of the statistical analysis of the experimental data, such as slopes, intercepts, correlation coefficients, relative standard deviation (R.S.D.), detection limit and quantitation limit. In order to determine the accuracy and precision of the methods, solutions containing three different concentrations of the studied drugs were prepared and analyzed in three replicates within 3 d (Table 2).

The proposed methods were used for determination of the three drugs in their pharmaceutical formulations and the standard addition technique was applied to assess validation of the methods. Comparison of the results obtained by the proposed methods with those obtained by ion-exchange chromatographic methods[18] for drugs I & II and USP method[15] for drug III showed that the recommended procedures are more economical as regards solvent and reagent consumption without any loss of accuracy or precision (Table 3).

Interference The specificity of the methods was checked by observing whether there was any interference of the tablet excipients. Spectrophotometric measurements showed that placebo samples did not have any absorption under the experimental conditions. The diluents and additives such as magnesium stearte, lactose, microcrystalline cellulose and croscarmellose sodium did not interfere with the analysis, even when present in high concentration.

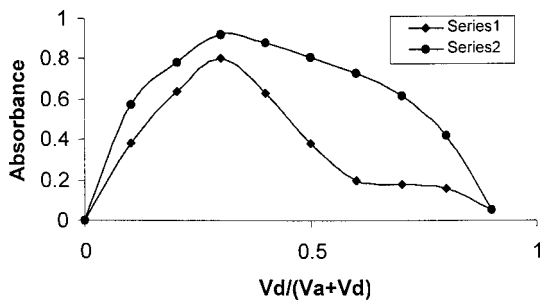

Fig. 5. Determination of the Stoichiometry of the Reaction Using Method [2], (0.125×10^{-4} mol l^{-1} Solutions) at 568 nm, Series 2 and Method [3], (3×10^{-4} mol l^{-1} Solutions) at 320 nm, Series 1

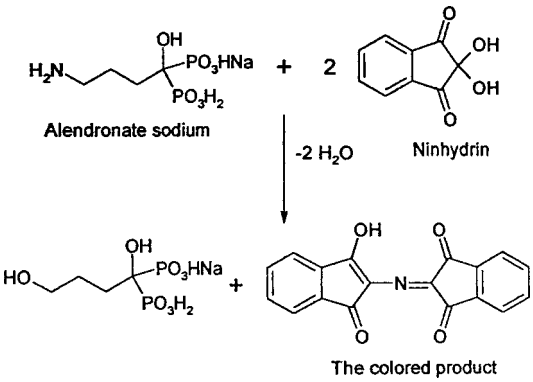

Chart 1. The Suggested Reaction Mechanism between Alendronate and Ninhydrin

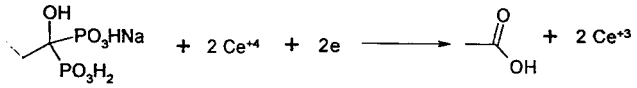

Chart 2. The Suggested Reaction Mechanism between the Three Drugs and Ceric (IV) Sulphate.

Table 1. Comparison between the Proposed Methods and Reported Methods for the Determination of the Cited Drugs in Bulk Powder

Items	Method 1 ΔA	Method 2 Using ninhydrin	Method 3 Using ceric (IV) sulphate			Reported method[15,18]		
	Risedronate	Alendronate	Risedronate	Alendronate	Etidronate	Risedsonate	Alendronate	Etidronate
n	5	6	6	6	6	5	5	5
Mean[a]	99.75	99.77	99.79	99.73	99.86	100.03	99.71	99.83
S.D.	1.22	0.73	1.16	1.38	1.13	0.94	0.80	1.12
Variance (V)	1.49	0.53	1.35	1.90	1.28	0.88	0.64	1.25
Standard error (S.E.)	0.55	0.30	0.48	0.56	0.46	0.42	0.36	0.50
t-test	0.36 (2.306)[b]	0.13 (2.262)[b]	0.33 (2.262)[b]	0.03 (2.262)[b]	0.4 (2.262)[b]			
F-test	1.69 (5.05)[c]	1.66 (6.26)[c]	1.53 (6.26)[c]	2.97 (6.26)[c]	1.02 (6.26)[c]			

a) Mean of *n* experiments. *b*) Theoretical *t*-value. *c*) Theoretical *F*-value.

Table 2. Validation Report on Spectrophotometric Methods for the Determination of the Studied Drugs

Parameter	Method 1 ΔA	Method 2 Using ninhydrin	Method 3 Using ceric (IV) sulphate		
	Risedronate	Alendronate	Risedronate	Alendronate	Etidronate
Linearity range $\mu g\,ml^{-1}$	15—150	3.75—45	2—24	2—24	2—24
Regression equation					
Slope	0.0062	0.029	0.0473	0.0425	0.0560
Intercept	0.0034	0.0027	0.0385	0.0103	0.0385
Correlation coeff.	0.9997	0.9997	0.9984	0.9990	0.9980
Accuracy (mean±R.S.D.)	99.75±1.22	99.77±0.73	99.79±1.16	99.73±1.38	99.86±1.13
Precision (S.D.) ($\mu g\,ml^{-1}$)	1.22	0.73	1.16	1.38	1.13
Detection limit ($\mu g\,ml^{-1}$)	4.8	1.2	0.66	0.66	0.66
Quantitation limit ($\mu g\,ml^{-1}$)	16	4.1	2.05	2	2

Table 3. Comparison between the Proposed Methods and Reported Methods for the Determination of the Cited Drugs in Their Pharmaceutical Dosage Forms

Item	Method 1 ΔA		Method 2 Using ninhydrin		Method 3 Using ceric (IV) sulphate					Reported method[15,18]		
	Risedronate		Alendronate		Risedronate		Alendronate		Etidronate	Rised-	Alen-	Etidronate
	Tablet	Added	Tablet	Added	Tablet	Added	Tablet	Added	Synthetic tablet	sonate	dronate	
n	5	5	5	5	5	5	5	5	5	5	5	5
Mean[a]	105.0	99.68	104.61	100.02	104.80	99.85	104.62	99.53	100.77	104.20	103.5	101.06
S.D.	0.61	0.91	0.71	0.62	0.45	0.64	0.87	0.79	0.42	0.84	1.12	0.56
V	0.37	0.83	0.50	0.38	0.20	0.41	0.76	0.62	0.18	0.71	1.25	0.31
S.E.	0.25	0.41	0.32	0.28	0.20	0.29	0.39	0.35	0.19	0.37	0.50	0.25
t-test	1.75		1.87		1.43		1.77		0.92			
F-test	1.92		2.5		3.55		1.64		1.72			

a) Mean of *n* experiments.

Conclusion

The spectrophotometric methods described were found to be simple, sensitive and accurate compared with the official or reported methods. Therefore, they could be applied for the determination of drugs I, II, and III in the bulk powders and in pharmaceutical preparations. The results obtained confirm the suitability of the proposed methods for the precise analysis of these drugs. Since the suggested methods are simple, reliable and no expensive laboratory technique is needed, they can be used for routine analysis in quality control laboratories.

References

1) Seam C. S., "The Complete Drug Reference," Pharmaceutical Press, U.K., 2002, p. 744.
2) Edwin K. J., "Goodman and Gilman's the Pharmacological Basis of Therapeutics," 10th ed., McGraw-Hill Inc., 2001, p. 1733.
3) Zhang Y. K., Fan S. Y., He L. E., Ma J. L., Qi C. Y., *Fenxi Huaxu*, **28**, 1181 (2000).
4) Meyya Nathan S. N., Rama Sharma G. V. S., Bhanuprakash Reddy V., Suresh B., *Indian Drugs*, **38**, 462—463 (2001).
5) Kuljanin J., Jankovic I., Nedeljkovic J., Prstojevic D., Marinkovic V., *J. Pharm. Biomed. Anal.*, **28**, 1215—1220 (2002).
6) Kwong E., Chiu A. M. Y., Mc Clintock S. A., Cotton M. L., *J. Chromatogr. Sci.*, **28**, 563—566 (1990).
7) Tsai E. W., Chamberlin S. D., Forsyth R. J., Bell C., Ip D. P., Brooks M. A., *J. Pharm. Biomed. Anal.*, **12**, 983—991 (1994).
8) Han Y. H. R., Qin X. Z., *J. Chromatogr.*, **719**, 345—352 (1996).
9) De Marco J. D., Biffar S. E., Reed D. G., Brooks M. A., *J. Pharm. Biomed. Anal.*, **12**, 1719 (1989).
10) Ptacek P., Klima J., Macek J., *J. Chromatogr. B*, **767**, 111—116 (2002).
11) Kline W. F., Matuszowski B. K., *J. Chromatogr. Biomed. Appl.*, **583**, 183—193 (1992).
12) "European Pharmacopoeia," 3rd ed., Council of Europe, Strasbourg, 2001, pp. 1405—1406.
13) Tsai E. W., Singh M. M., Lu H. H., Ip D. P., Brooks M. A., *J. Chromatogr.*, **626**, 345—250 (1992).
14) Reed D. G., Martin G. P., Konieczny J. M., Brooks M. A., *J. Pharm. Biomed. Anal.*, **13**, 1055—1058 (1995).
15) "U.S. Pharmacopeia 26," United States Pharmacopeial Convention Inc., Rockville, 2003, p. 767.
16) Shon E. M., Pietrzyk D. J., *Anal. Chem.*, **60**, 1397—1400 (1988).
17) Eric W. T., Dominic P. I. P., Marvin A. B., *J. Pharm. Biomed. Anal.*, **11**, 513—516 (1993).
18) Private communication with Avents Co., Egypt and Global Napi Co., Egypt, 2000.
19) Fritz F., "Spot Tests in Organic Analysis," 7th ed., Elsevier Publishing Company, 1975, p. 493.
20) Hisham E. Abdellatef, Hawa M. Khalil, *J. Pharm. Biomed. Anal.*, **31**, 209—214 (2003).
21) Sultan S. M., Alzamil I. Z., Alrahman A. M. A., Altamrah S. A., Asha Y., *Analyst* (London), **111**, 919—921 (1986).
22) Hoda M., *J. Pharm. Biomed. Anal.*, **31**, 767—774 (2003).