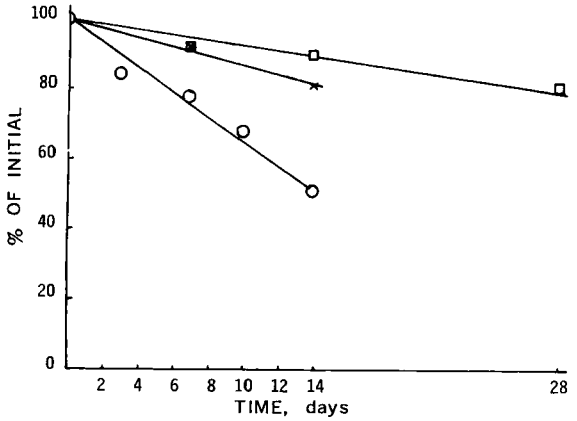

Figure 4—*Comparison of percent of initial carbachol remaining after storage at 60° in three buffer systems (0.1 M phosphate). Key: ○, pH 7.9; ×, pH 7.0; □, pH 6.0.*

use in the presence of possible hydrolysis products and in systems in which the interfering N—H groups may be excluded. The procedure is simple, rapid, and applicable to small or very dilute samples.

REFERENCES

(1) C. S. O'Brien and K. C. Swan, *Trans. Am. Ophthalmol. Soc.*, **39**, 175(1941).

(2) H. Beasley, R. A. Borgmann, T. O. McDonald, and P. R. Belluscio, *Arch. Ophthalmol.*, **80**, 39(1968).

(3) "The United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, pp. 101–102.

(4) K. Connors, in "Pharmaceutical Analysis," T. Higuchi and E. Brochmann-Hanssen, Eds., Interscience, New York, N. Y., 1961, pp. 182–263.

(5) E. H. Taylor, *Lloydia*, **27**, 96(1964).

(6) A. Heyndrickx, M. Schauvliege, and A. Blomme, *J. Pharm. Belg.*, **20**, 117(1965).

(7) L. W. Dittert and T. Higuchi, *J. Pharm. Sci.*, **52**, 852(1963).

(8) E. R. Pryde and L. G. Soper, *J. Chem. Soc.*, **1931**, 1510.

(9) G. H. Ellis and C. H. Hetzel, *Anal. Chem.*, **31**, 1090(1959).

(10) G. Schill, *Anal. Chem. Acta*, **21**, 341(1959).

(11) "The Merck Index," 8th ed., Merck and Co., Inc., Rahway, N. J., 1968, p. 204.

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Determination of Calcium in Pharmaceutical Preparations by Atomic Absorption Spectrometry

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Abstract □ Atomic absorption spectrometry is applied to the determination of calcium in pharmaceutical samples using both the direct method and the method of standard additions. Comparison with the standard chelometric method using EDTA and hydroxy naphthol blue indicates that the atomic absorption methods require less time and are equivalent to the chelometric method in precision and accuracy. The majority of interferences are eliminated by the addition of one percent lanthanum to the solutions.

Keyphrases □ Calcium determination—pharmaceuticals □ Lanthanum—assay interference elimination □ Capsule absorption—active components □ Atomic absorption spectroscopy—analysis

Pharmaceutical preparations containing calcium are usually analyzed by the USP (1) or NF (2) methods which involve chelometric titration with the disodium salt of ethylenedinitrilotetraacetic acid (EDTA) using hydroxy naphthol blue as the indicator. Many pharmaceutical preparations, however, contain phosphate and/or organic compounds which interfere with the end point of the chelometric method. The elimination of these interferences increases the time and complexity of the analysis.

This paper reports an atomic absorption spectrometric method for the analysis of calcium in many types of pharmaceutical preparations that is simple and easy to follow and is equivalent to the chelometric method in accuracy and precision. Atomic absorption spectrometry has been used to determine calcium in many types of samples (3–10) but has not been reported for pharmaceutical materials. The majority of interferences are eliminated by the addition of lanthanum to all standards and samples as shown by Yofè and Finkelstein (11) and Williams (12) for flame photometric determination of calcium and by David (3) for atomic absorption spectrometry. Lanthanum is seldom a component and thus is the element of choice for the elimination of phosphate and other interferences. Strontium (3) can also be used for the same purpose.

EXPERIMENTAL

Instrument—Atomic absorption spectrophotometer[1] equipped with a dual element (Ca-Mg) hollow cathode lamp. The photometer was operated with either a standard (single slot) or Boling (three-slot) head of 10-cm. path length at a wavelength of 4227 Å. and a slit width of 1 mm. using an air-acetylene flame with an atomizer flow rate of 2.8 ml./min.

Reagents—Lanthanum oxide, code 528.[2] All other reagents were A.C.S., USP, or NF grade. Deionized water was used for all solutions.

[1] Perkin-Elmer model 303.

[2] American Potash and Chemical Co.

Solutions—5% (w/v) Lanthanum in 25% (v/v) HCl: dampen 58.65 g. La_2O_3 with water and dissolve by slow addition of 250 ml. of concentrated HCl. Dilute to 1,000 ml. with deionized water.

Calcium Stock Standard—1.000 mg./ml. Dissolve 1.249 g. of $CaCO_3$ in 75 ml. of 1 *N* HCl and dilute to 500.0 ml.

Calcium Dilute Stock Standard—0.0500 mg./ml. Dilute 5.00 ml. of the stock standard to 100.0 ml.

Calcium Working Standards—(1 to 10 mcg./ml.). Pipet 10.00 ml. of the dilute stock standard and 10.0 ml. of the 5% La solution into a 50 ml. volumetric flask and dilute to volume to prepare the 10 mcg./ml. standard. Repeat using 8.00, 6.00, 4.00, 2.00, and 1.00 ml. of the dilute stock standard to obtain 8, 6, 4, 2, and 1 mcg./ml. standards, respectively.

Blank Solution—Dilute 10.0 ml. of the 5% La solution to 50.0 ml.

EDTA—0.0500 *M* as in USP XVII, page 1083.

Hydroxy Naphthol Blue—As in USP XVII, page 1068.

PROCEDURES

EDTA Titration

The USP XVII(1) and NF XII(2) procedures were utilized with phosphate interference eliminated by ion-exchange separation (11) or by extraction of the molybdophosphate into a chloroform–*n*-butanol mixture (12). Triethanolamine was added to complex iron and aluminum when present (13). Samples containing organic materials which would interfere with the indicator action were dry-ashed before titration.

Atomic Absorption Methods

Sample Preparation—*Tablets*—Accurately weigh a portion of the tablet composite equivalent to about 100 mg. of Ca into a 100-ml. volumetric flask, dissolve in 15.0 ml. of 1 *N* HCl, and dilute to volume. Dilute 5.00 ml. of this solution to 100.0 ml. Pipet 5.00 ml. of the diluted solution into a 50.0-ml. volumetric flask, add 10.0 ml. of the 5% lanthanum solution, and dilute to volume.

Syrups, Suspensions, and Injections—Accurately measure an aliquot equivalent to approximately 100 mg. of Ca into a 100-ml. volumetric flask, add 15.0 ml. of 1 *N* HCl, and dilute to volume. Dilute 5.00 ml. of this solution to 100.0 ml. Pipet 5.00 ml. of the diluted solution into a 50.0-ml. volumetric flask, add 10.0 ml. of the 5% lanthanum solution, and dilute to volume.

Elixirs—Evaporate the alcohol from an accurately measured aliquot containing about 100 mg. of Ca under a current of air on the steam bath. Add 15 ml. of 1 *N* HCl, transfer to a 100-ml. volumetric flask and dilute to volume. Dilute stepwise as directed under syrups, suspensions, and injections.

Determination—Direct Method—Allow the burner to equilibrate using a fuel-rich (reducing) flame for 5 to 10 min. Zero the instrument with the blank solution and then optimize the spectrometer response using the most concentrated standard. Determine the percent absorption or absorbance of the working standards and the sample solutions. If the readings are in percent absorption, convert to absorbance using a conversion table or calculate by $A = -\log(100 - \%A)$. Plot the absorbance *versus* concentration in mcg./ml. for the standards and read the concentrations of the samples from this graph as mcg./ml. of calcium in the final sample dilutions. Calculate the milligrams of calcium in the original sample as follows:

$$\text{mcg./ml.} \times \frac{1\text{ mg.}}{1{,}000\text{ mcg.}} \times \frac{50\text{ ml.}}{5\text{ ml.}} \times \frac{100\text{ ml.}}{5\text{ ml.}} \times 100\text{ ml.} = \text{mg. of Ca in aliquot or sample}$$

If the concentration in mcg./ml. as read from the standard graph is designated as *C*, this calculation becomes:

$$\text{mg. of Ca in aliquot or sample} = 20\,C$$

This value is then converted by the usual calculation to the basis of calcium declared in the sample.

Method of Standard Additions—Prepare samples as directed above through "dilute 5.00 ml. of this solution to 100.0 ml." In a series of three 50-ml. volumetric flasks, pipet 0, 1.00, and 2.00 ml. of the

Table I—Analysis of Typical Pharmaceutical Preparations

Type of Sample	Amount Declared	Amount Found as Percentage of Amount Declared		
		By EDTA Titration	By Atomic Absorption—Direct	By Atomic Absorption—Std. Additions
Hypocalcemics				
Syrup	92 mg. Ca/4 ml.	98.3	97.0	97.0
Injection	89.6 mg. Ca/10 ml.	96.6	96.4	97.4
Antacids				
Suspensions	1.00 g. $CaCO_3$/5 ml.	101.9	101.5	97.9
Tablets	370 mg. $CaCO_3$/tablet	101.6	105.4	102.8
Hematinics				
Tonics	130 mg. calcium glycerophosphate/2 fluid drams	103.0[a]	104.5	108.5
	130 mg. calcium glycerophosphate/2 fluid drams	100.5[a]	100.5	103.5
Tablets	37.4 mg. Ca/tablet	128.4[b]	126.5	127.0
	53 mg. Ca/tablet	104.0[b]	109.1	109.4
Laxatives				
Capsules Soft gelatin (clear)	240 mg. calcium bis (dioctyl) sulfosuccinate	109.7	111.1	113.9
Soft gelatin (red)	60 mg. calcium bis (dioctyl) sulfosuccinate	109.0[b]	107.7[b, c]	112.3[d]
Analgesics				
Tablets	60 mg. calcium gluconate/tablet	103.8	104.5	107.2
Vitamin-mineral preparations				
Elixirs	100 mg. calcium glycerophosphate/45 ml.	98.9[e]	101.7	96.0
Tablets	270 mg. calcium lactate/tablet	159.6[b]	157.0	157.0
	100 mg. Ca/tablet	140.4[b]	138.1	138.0
	243 mg. Ca/tablet	94.7[b]	91.9	92.7
	250 mg. Ca/tablet	108.2[b]	109.1	112.0
	350 mg. Ca/tablet	103.6[b]	104.2	103.0
Capsules	40.1 mg. total Ca/capsule	116.4[b]	115.2	116.2
Syrup	40 mg. Ca/5 ml.	105.5[e]	108.8	108.2
SD[f]		±0.7	±0.7	±2.2

[a] Phosphate interference removed by extraction. [b] Dry-ashed before analysis. [c] Solution containing dissolved capsules gives 116.8% due to interference of gelatin. [d] Contains dissolved gelatin. [e] Phosphate removed by ion exchange. [f] Calculated from differences in duplicates (W. J. Youden, "Statistical Methods for Chemists," Wiley, New York, N. Y., 1961, p. 16).

Table II—Ratio of Variance[a]

	Critical (5%)	Calculated Value
EDTA *vs.* atomic absorption direct	2.12	1.12
EDTA *vs.* atomic absorption additions	2.12	1.06
Atomic Absorption direct *vs.* atomic absorption additions	2.12	1.05

[a] Calculated as difference between sum of squares and square of sum divided by degrees of freedom using the data in Table I directly.

Table III—Recovery Studies

Type of Sample	Amt. Ca Added, mg.	—Amount Ca Recovered, %— EDTA	Atomic Absorption Direct	Atomic Absorption Additions
Antacid tablet	25	102.4	101.4	102.4
Tonic phosphate	25	98.4[a]	97.2	100.4
Vitamin-mineral tablet	25	99.6[b]	103.2	104.0

[a] Phosphate interference removed by extraction. [b] Tablet plus added calcium dry-ashed before analysis.

dilute stock standard. Add 5.00 ml. of the diluted sample solution and 10.0 ml. of the lanthanum solution to each and dilute to volume. These solutions now contain the calcium in the sample plus 0, 1.00, and 2.00 mcg./ml. of added calcium. With the same conditions listed above for the direct method, zero the spectrometer with the blank and measure the percent absorption or absorbance of the three sample solutions. If the values are in percent absorption, convert to absorbance and plot the absorbance of the three solutions against concentration as 0, 1.00, and 2.00 mcg./ml. Ca. The concentration base line should be set up with zero as the third division from the right with increasing concentration in either direction from zero. Draw the best straight line through the three absorbance values and extrapolate through zero concentration to zero absorbance. The intersection of the extrapolated line with the concentration base line gives the concentration of calcium in the sample as mcg. of Ca/ml. in the diluted sample solution. Calculate the milligrams of Ca in the sample or aliquot as in the direct method and convert to the declared basis.

Sodium Enhancement Study—A series of standards containing 5.0 p.p.m. Ca and 5, 10, 100, 200, and 1,000 p.p.m. of Na, respectively, were analyzed by the direct method with and without the addition of the lanthanum solution. Both the standard single-slot and the Boling three-slot burners were used in these studies.

Recovery Study—Known amounts of calcium were added to samples of three different types of preparation and determined by all three methods.

RESULTS AND DISCUSSION

The results of the determination of typical pharmaceutical preparations by all three methods are shown in Table I. The standard deviations were calculated from the differences of duplicate samples since the wide variation of the results expressed as percent of the amount declared gives an unduly large estimate of the standard deviation calculated from the sum-of-squares method. Even though the standard deviation of the method of additions is larger than the other two, the analysis of variance shown in Table II indicates no significant difference in precision between any of the three methods. Data for the recovery study is presented in Table III and shows that recoveries by all three methods are satisfactory. However, the recoveries by the method of standard addition are approximately 2% higher than the direct atomic absorption method and the values by the method of additions shown in Table I are also approximately 2% higher than those by the direct method. For this reason, it is recommended that the method of additions be used only as a check for those samples which may contain interfering substances which are not removed by the proposed direct procedures. Such interferences include the presence of large organic molecules such as gelatin or large amounts of dissolved solids which may refract or absorb light. If divergent results are obtained from the two methods the sample should be ashed before analysis.

An example of interference due to large molecules is the laxative sample shown in Table I which was packaged in soft red gelatin capsules. In this particular sample, a part of the calcium had migrated into or been adsorbed on the capsule itself, so that the contents gave low values for calcium. Dissolution of the entire sample including the capsule was necessary to recover all the calcium. Direct determination of the solution containing the capsules gave a value of 116.8% of the declared amount of Ca while determination on a sample which was ashed before dissolution gave a value of 107.7% of the declared amount of calcium. The contents alone showed 65.2% of the declared amount of calcium. This sample also indicates that capsules should be checked separately for the components being determined in any suspect sample.

Since many pharmaceutical preparations contain sodium in amounts equal to or more than the calcium, sodium enhancement studies were made. For samples containing 5 p.p.m. of Ca and 1% lanthanum, the addition of up to 1,000 p.p.m. of Na had no effect on the amount of Ca determined when compared to standards which did not contain sodium. In the absence of lanthanum the presence of sodium caused significant enhancement of the absorption signal. This confirms the results of David (10) on calcium in plant materials.

The addition of 1% lanthanum to each solution offsets the interference of anions such as phosphate, sulfate, and silicate, and the enhancement due to sodium; therefore, lanthanum should be added to all samples whether or not interference or enhancement is expected.

REFERENCES

(1) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, pp. 89–94.

(2) "National Formulary," 12th ed., Mack Publishing Co., Easton, Pa., 1965, pp. 67–68.

(3) D. T. David, *Analyst*, **85**, 495(1960).

(4) J. B. Willis, *Anal. Chem.*, **33**, 556(1961).

(5) E. G. Gimblet, A. F. Marney, and R. W. Bonsnes, *Clin. Chem.*, **13**, 204(1967).

(6) D. T. Trudeau and E. F. Freier, *ibid.*, **13**, 101(1967).

(7) J. B. Willis, *Spectrochem. Acta*, **16**, 259(1960).

(8) G. K. Billings and J. A. S. Adams, *At. Absorption Newsletter*, **3**, 65(1964).

(9) E. L. Obermiller and R. W. Freedman, *Fuel*, **44**, 199(1965).

(10) D. T. David, *Analyst*, **84**, 536(1959).

(11) J. Yofè and R. Finkelstein, *Anal. Chim. Acta*, **19**, 166(1958); through *Chem. Abstr.*, **54**, 1168(1960).

(12) C. H. Williams, *ibid.*, **22**, 163(1960); through *Chem. Abstr.*, **54**, 7953(1960).

(13) J. S. Kim, and M. M. Tuckerman, *J. Pharm. Sci.*, **56**, 1309(1967).

(14) L. Kleinman and H. Schriftman, *ibid.*, **56**, 516(1967).

(15) F. J. Welcher, "*The Analytical Uses of Ethylenediaminetetraacetic Acid*," D. Van Nostrand, Princeton, N. J., 1961, p. 116.

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