moclavine[2] (2.0 g, 7.9 mmoles) in methanol (400 ml) was treated with 20 g of manganese dioxide (9), and the mixture was stirred at room temperature. After 12 hr, the mixture was filtered, the filtrate was evaporated to dryness, and the residue was dissolved in 10 ml of chloroform. TLC on silica gel G with an ethyl acetate–dimethylformamide–ethanol (13:1:1) solvent revealed a major product (R_f 0.77) that was nonfluorescent and gave a green color with Ehrlich's reagent, a small quantity of unreacted elymoclavine (R_f 0.23), and only traces of other Ehrlich-positive compounds.

Purification of the product was accomplished by chromatography on a silica gel (40 g) column with chloroform as the solvent. Recrystallization from chloroform–hexane provided 1.2 g (55% yield) of small white needles, mp 192–194° dec.; UV: λ_{max}(ethanol) 223 (log ϵ 4.58) and 297 (3.73) nm; IR: ν_{max} (KBr) 3170, 2930, 2800, 1680, 1400, 1180, 1070, 900, and 750 cm^{-1}; NMR: δ 2.56 (s, 3H, NCH_3), 3.13 (s, 3H, OCH_3), 6.94–7.44 (m, 4H, indole), 7.67 (s, 1H, 9=CH), 8.33 (s, 1H, NH), and 9.67 (s, 1H, CHO) ppm. The mass spectrum showed a molecular ion at *m/e* 282 (100) and a prominent ion at *m/e* 154 (48) characteristic of ergolines (10).

The product was compared with a reference sample[3] of IV and was identical in all respects (TLC; melting point; and UV, IR, NMR, and mass spectra).

DISCUSSION

Ergoline derivatives including ethers related to IV have been of interest recently as potential prolactin inhibitors (11) and α-adrenergic blocking agents (12). The manganese dioxide oxidation of elymoclavine in methanol provides a convenient one-step synthesis of IV from a readily available starting material.

It was also of interest to obtain some indication of whether manganese dioxide might be useful for the C-17 and/or C-10 oxidation of other ergolines. For this purpose, lysergol, the $\Delta^{9,10}$-isomer of elymoclavine (I), and agroclavine (VI) were treated under the same conditions used for the oxidation of elymoclavine. Lysergol was unchanged under these conditions, which was not surprising since manganese dioxide is most effective for the oxidation of allylic alcohols (13). However, it was interesting that agroclavine was also unreactive since C-10 oxidation of the $\Delta^{8,9}$-ergolenes can be accomplished with various oxidizing agents (1, 2). Additional studies are required to determine the precise utility of manganese dioxide for the C-17 and C-10 oxidation of ergolines.

[2] Isolated from ergot strain SD 58 provided by Dr. James E. Robbers, Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Ind.

[3] Provided by Dr. E. C. Kornfeld, Eli Lilly Co., Indianapolis, Ind.

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ˣ To whom inquiries should be directed.

Direct Complexometric Titration of Calcium Phosphates

MURRAY M. TUCKERMAN ˣ and M. ELEANOR SANCHEZ de RAMOS

Abstract □ Calcium was determined in calcium phosphate samples by dissolving the sample in hydrochloric acid, adding hydroxynaphthol blue indicator and triethanolamine, adjusting the pH to 12.3–12.5 with potassium hydroxide solution, and titrating with standard disodium ethylenediaminetetraacetate solution. Time can be saved and the formation of a precipitate (which dissolves readily during the titration) can be avoided by adding at least 85% of the amount of complexing agent required for titration before adjusting the pH.

Keyphrases □ Calcium—complexometric analysis in dibasic and tribasic calcium phosphates, bulk drug and tablets □ Complexometry—analysis, calcium in dibasic and tribasic calcium phosphates, bulk drug and tablets

Previously, calcium was determined in the presence of phosphate complexometrically by the addition of excess complexing agent and determination of the excess, after separation from phosphate by ion exchange (1), and also after removal of phosphate by formation and extraction of phosphomolybdate (2). All of these efforts are based on the impossibility of a direct complexometric titration of calcium in the presence of equivalent amounts of phosphate. This paper reports a simple, rapid, direct complexometric titration of calcium in the presence of phosphate.

EXPERIMENTAL

All reagents and volumetric solutions were those specified in USP XIX. All experiments were done according to the following general directions, modified as indicated.

General Method—Dissolve a sample expected to contain about 225 mg of calcium, accurately weighed, in 15 ml of hydrochloric acid and 10 ml of water contained in a 100-ml volumetric flask, with the aid of gentle heat if necessary, and cool to room temperature. Dilute to volume with

Table I—Dibasic Calcium Phosphate [a] NF (Weighed Samples)

Sample	Weight, mg	0.0500 *M* Titrant, ml	Calcium, %
1	200.00	28.68	28.74
2	201.0	28.54	28.45
3	203.0	28.90	28.53
		Average	28.57
		SD	0.17
		RSD	0.6

[a] Medicinal, Mallinckrodt Chemical Works, St. Louis, Mo.

Table II—Aliquots (25 ml) of Dibasic Calcium Phosphate [a] Solution

Sample	0.0500 *M* Titrant, ml	Calcium, %
	800.0 mg in 100.0 ml	
1	28.80	28.86
2	28.50	28.56
3	28.38	28.44
	Average	28.62
	SD	0.25
	RSD	0.9
	720.0 mg in 100.0 ml	
1	25.85	28.78
2	25.90	28.83
3	25.80	28.72
	Average	28.78
	SD	0.005
	RSD	0.21
	880.0 mg in 100.0 ml	
1	31.50	28.69
2	31.46	28.66
3	31.48	28.68
	Average	28.68
	SD	0.018
	RSD	0.67

[a] Lot 761788, reagent grade, Fisher Scientific Co.

water and mix well. If the solution is not completely clear, filter through quantitative filter paper, discarding the first 10 ml of filtrate.

Transfer a 25-ml aliquot to a 250-ml vessel equipped with a magnetic stirrer and add water to make 125 ml. With constant stirring, add, in order, 0.5 ml of triethanolamine, 300 mg of hydroxynaphthol blue indicator, and, from a 50-ml buret, 97% of the calculated amount of standard 0.05 *M* disodium ethylenediaminetetraacetate solution. Add sodium hydroxide solution (45 in 100) until the initial red color changes to clear

Table III—Dibasic Calcium Phosphate [a] Solution

Initial 0.0500 *M* Titrant Added, ml	Total 0.0500 *M* Titrant Added, ml	Calcium, %
21	28.90	28.96
21	28.88	28.94
22	28.86	28.92
22	28.88	28.94
23	28.90	28.96
23	28.90	28.96
24	28.90	28.96
24	28.88	28.94
25	28.90	28.96
25	29.00	28.96
26	28.90	28.96
26	28.98	29.04
27	28.70	28.96
27	28.74	28.80
28	28.70	28.76
28	28.72	28.78
0	28.70	28.72
0	28.82	28.88
	Average	28.90
	SD	0.088
	RSD	0.3

[a] Lot 761788, reagent grade, Fisher Scientific Co.

Table IV—Tolerance for Added Phosphate

0.8% Na_2HPO_4 Solution Added, ml	Phosphorus to Calcium Molar Ratio	0.0500 *M* Titrant, ml	Calcium, %
5	1.07	28.84	28.90
5	1.07	28.82	28.88
10	1.15	28.82	28.88
10	1.15	28.80	28.86
25	1.38	28.80	28.86
25	1.38	28.80	28.86
50	1.77	28.80	28.86
50	1.77	28.78	28.84
100	2.53	28.70	28.76

blue, continue the addition dropwise until the color changes to violet, and then add an additional 0.5 ml (7–8 ml total is usually required). The pH should be between 12.3 and 12.5.

Under constant stirring, continue the titration of the solution dropwise with the standard 0.05 *M* disodium ethylenediaminetetraacetate solution (titrant) to a clear blue end-point that persists for at least 60 sec.

Dibasic Calcium Phosphate NF—Use a 200-mg sample of material as is (Table I).

Influence of Sample Size on Results—Use sample sizes of 180, 200, and 220 mg of dibasic calcium phosphate NF (Table II).

Influence of Volume of Standard 0.05 *M* Disodium Ethylenediaminetetraacetate—Use various volumes for initial addition to 25-ml aliquots of a solution containing 4.000 g of anhydrous dibasic calcium phosphate NF in 500 ml in place of the 97% of the calculated amount called for in the general directions (Table III).

Tolerance for Added Phosphate—Add various amounts of 0.8% Na_2HPO_4 to 25-ml aliquots of a solution containing 800.0 mg of anhydrous dibasic calcium phosphate NF in 100 ml (200.0 mg/sample) (Table IV).

Tribasic Calcium Phosphate NF—Use 135-, 150-, and 165-mg samples to show the influence of sample size (Table V).

Comparison with Extraction Method of Kleinman and Schriftman (2)—Use 5-ml aliquots of a solution containing 1.000 g of anhydrous dibasic calcium phosphate NF in 100 ml (50 mg/sample) (Tables VI and VII) or 5-ml aliquots of a solution containing 760.0 mg of tribasic calcium phosphate NF in 100 ml (38 mg/sample) (Table VIII).

Assay of Dibasic Calcium Phosphate Tablets—Weigh about 2.4 g of finely powdered tablets, dissolve in 30 ml of hydrochloric acid and 20 ml of water, and dilute to 200 ml. Take 25-ml aliquots for assay (Table IX).

Table V—Tribasic Calcium Phosphate [a] (25-ml Aliquots)

Sample	0.0500 *M* Titrant, ml	Calcium, %
	1.200 g in 200 ml	
1	27.78	37.11
2	27.80	37.14
3	27.76	27.09
4	27.78	37.11
5	28.00	37.41
6	28.00	37.41
7	27.72	37.03
	Average	37.19
	SD	0.14
	RSD	0.4
	540.0 mg in 100 ml	
1	25.20	37.41
2	25.22	37.44
3	25.00	37.11
	Average	37.32
	SD	0.22
	RSD	0.3
	660.0 mg in 100 ml	
1	30.20	36.68
2	30.24	36.72
3	30.22	36.70
	Average	36.70
	SD	0.029
	RSD	0.08

[a] Lot 25872, Baker Analyzed.

Table VI—Dibasic Calcium Phosphate [a] Solution (Extraction Method)

Sample	0.0500 *M* Titrant, ml	Calcium, %
1	7.10	28.46
2	7.08	28.38
3	7.10	28.46
4	7.10	28.46
	Average	28.44
	SD	0.039
	RSD	0.14

[a] Lot 761788, reagent grade, Fisher Scientific Co.

Table VII—Dibasic Calcium Phosphate [a] (Aliquots of Same Solution by Extraction and Direct Titration)

Sample	0.0500 *M* Titrant, ml	Calcium, %
	By Extraction	
1	7.04	28.22
2	7.04	28.22
3	7.04	28.22
	Average	28.22
	SD	0.00
	RSD	0
	By Direct Titration	
1	7.16	28.70
2	7.16	28.70
3	7.18	28.78
	Average	28.73
	SD	0.017
	RSD	0.2

[a] Lot 761788, reagent grade, Fisher Scientific Co.

RESULTS AND DISCUSSION

For the 30 direct titrations of a single sample of dibasic calcium phosphate NF[2] (Tables II, III, and VII), the average calcium content was 28.82%. The standard deviation was 0.16%, which gives a relative standard deviation of 0.6%. All standard deviations were calculated from range values using literature values of d_2 (3). When tested against the average for the 30 determinations of dibasic calcium phosphate, some subgroups of Table II, the duplicate groups of Table II, and the subgroup of Table VII did not meet the *F*-test for homogeneity of variance at the 95% confidence level. Therefore, it was not quite correct to test the significance of the difference between the individual subgroup average and the general average by the Student *t* test. However, when the test was applied, the differences between the subgroup average and the general average were not significant at the 95% confidence level. Therefore, neither a variation of up to 10% in the sample size nor the amount of complexing agent initially added in the titration affects the assay result, and the extraction method gives the same results as the general method.

Table VIII—Tribasic Calcium Phosphate [a] (Aliquots of Same Sample by Extraction and Direct Titration)

Sample	0.0500 *M* Titrant, ml	Calcium, %
	By Extraction	
1	6.92	36.49
2	6.92	36.49
3	6.92	36.49
	Average	36.49
	SD	0.00
	RSD	0
	By Direct Titration	
1[b]	6.98	
2	6.98	
3	6.96	
4[b]	6.92	
	Average	36.70
	SD	0.16
	RSD	0.4

[a] Lot 25872, Baker Analyzed. [b] Sample 1 had no ethylenediaminetetraacetate added initially; Sample 4 had 75% of the calculated amount of ethylenediaminetetraacetate added initially.

Table IX—Assay of Dibasic Calcium Phosphate Tablets

Aliquot	0.0500 *M* Titrant, ml	Dibasic Calcium Phosphate Dihydrate, mg per Average Tablet	Percent of Claim
		Lot A[a]	
1	28.16	476.08	97.96
2	28.12	475.40	97.82
3	28.08	474.72	97.68
4	28.10	475.06	97.75
		Average	97.80
		SD	0.14
		RSD	0.1
		Lot B[b]	
1	28.10	480.2	98.81
2	28.02	478.9	98.53
3	27.90	476.8	98.11
4	28.00	478.5	98.46
		Average	98.48
		SD	0.34
		RSD	0.3
		Lot C[c]	
1	28.20	479.7	98.70
2	28.54	485.5	99.89
3	28.38	482.7	99.33
4	28.52	485.1	99.82
		Average	99.44
		SD	0.58
		RSD	0.6

[a] For Lot A, the weight of 20 tablets was 11.7534 g, average tablet weight was 0.588 g, the label claim was 7.5 grains (486.0 mg)/tablet, sample weight was 2.3925 g, and a 25-ml aliquot contained 0.2991 g. [b] For Lot B, the weight of 20 tablets was 11.6618 g, average tablet weight was 0.583 g, the label claim was 7.5 grains (486.0 mg)/tablet, sample weight was 2.3483 g, and a 25-ml aliquot contained 0.2935 g. [c] For Lot C, the weight of 20 tablets was 12.0077 g, average tablet weight was 0.60039 g, the label claim was 7.5 grains (486.0 mg)/tablet, sample weight was 2.4278 g (in 200 ml), and a 25-ml aliquot contained 0.3035 g.

Comparison of the extraction method with the direct titration (Tables VII and VIII) shows that the difference is not significant at the 99% confidence level but is significant at the 95% confidence level. This result is attributed to the zero standard deviation of one set of determinations. The assumption of any reasonable standard deviation to replace the zero actually observed yields the result that the difference in averages is not significant at either confidence level.

The general method is applicable to solutions containing added phosphate until the molar ratio of phosphorus to calcium approaches 2:1 (Table IV). When this ratio was at 2.5:1, the precipitate formed dissolved so slowly that the end-point was unduly prolonged, even though the result was acceptable. These data indicate that the direct method for dibasic calcium phosphate (phosphorus to calcium ratio = 1:1) and tribasic calcium phosphate (phosphorus to calcium ratio = 2:3) should show no phosphate interference and that the general method should work in solutions containing a considerable molar excess of phosphate over calcium.

When three different lots of dibasic calcium phosphate tablets were analyzed, recoveries appeared to be acceptable and the standard deviations were in the range expected from the determinations of the parent compound (Table IX).

It was not possible to compare the proposed method with the currently official methods because the official methods determine the phosphate rather than the calcium content. However, the proposed method is much more rapid than the official methods for dibasic calcium phosphate NF and tribasic calcium phosphate NF, has acceptable reproducibility, and is comparable in results to the last published method for calcium phosphate (2). Moreover, it determines the physiologically active portion of the molecule (both official calcium phosphates are designated as "calcium supplement").

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[x] To whom inquiries should be directed.

Mass Fragmentographic Determination of Plasma Etomidate Concentrations

MICHAEL J. VAN HAMME [*], JOHN J. AMBRE [‡x], and MOHAMED M. GHONEIM [*]

Abstract □ The intravenous anesthetic etomidate was measured in human plasma by mass fragmentography. The method is accurate, sensitive, and specific. Results of the analyses indicate that after a single 0.3-mg/kg iv dose of etomidate, there are at least three phases in its disappearance from human plasma. Detectable plasma concentrations exist for more than 6 hr after injection.

Keyphrases □ Etomidate—mass fragmentographic analysis, human plasma □ Mass fragmentography—analysis, etomidate in human plasma □ Hypnotics—etomidate, mass fragmentographic analysis, human plasma

Etomidate is a new imidazole hypnotic used intravenously for induction of anesthesia. Compared to thiopental, the standard intravenous anesthetic, etomidate produces less cardiovascular and respiratory depression (1, 2). Like thiopental, etomidate induces sleep rapidly (10–15 sec) and has a short duration of action (2). Unlike the short-acting barbiturates, however, it does not produce a "hangover" effect (3) due to its rapid disappearance from nervous tissue and plasma and the dependence of this process upon rapid metabolism rather than redistribution (4). However, etomidate disposition has not been studied extensively.

Because etomidate has promise as an anesthetic induction agent (5), a sensitive, specific assay for use in future clinical pharmacological studies was needed. The developed method is based on combined GLC–mass spectrometry and single ion monitoring, a technique called mass fragmentography.

EXPERIMENTAL

Reference Compounds—Authentic etomidate sulfate [(*R*)-(+)-ethyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate sulfate] and propoxate hydrochloride [(+)-propyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate hydrochloride] were used as received[1]. Aqueous solutions of propoxate, 1 and 2 μg/ml, were used as internal standards. All etomidate concentrations are expressed in terms of free base.

GLC–Mass Spectrometry—A quadrupole gas chromatograph was interfaced to a mass spectrometer[2] with a glass jet separator and glass transfer lines. A 1.5-m (5-ft) × 2-mm i.d. glass column was silanized and packed with 5% OV-225 on 100–120-mesh Supelcoport[3]. Helium was the carrier gas at a flow rate of 20 ml/min. The temperature of the injection port was 250°; the column, separator, and transfer line temperatures were 230, 260, and 230°, respectively. Operating conditions for the mass spectrometer were: electron energy, 70 ev; emission ion current, 200 amp; and power supply, 2.5 kv.

Complete mass spectra of authentic etomidate and propoxate were first obtained by using the solid probe inlet to the mass spectrometer. The identities of these compounds as they eluted from the chromatograph were confirmed by comparison of their complete mass spectra to those of the authentic compounds.

These operating conditions also were used for mass fragmentography. The intensity of the *m/e* 105 and 77 ions was monitored using a programmable multiple-ion monitor[4] (at a sensitivity of 10^{-9}) and a dual-pen strip-chart recorder. The *m/e* 105 ion was monitored for the detection and quantitation of both etomidate and propoxate since it was the most abundant. The mass marker was used to center the ion window with a window width of less than 1 amu. The mass marker was not in operation during sample analyses.

Procedure—Blood samples were obtained from patients who underwent anesthetic induction with etomidate for eye or ear surgery. All patients were otherwise healthy, had normal liver and kidney function, and were taking no other medication. Each received 10 mg of diazepam and 0.3 mg of scopolamine intramuscularly 30 min prior to etomidate injection.

An indwelling cannula was placed in an antecubital fossa vein, and a 10-ml control blood sample was withdrawn. Etomidate (0.3 mg/kg iv) was then administered over 30 sec into an antecubital fossa vein of the other arm. After induction with etomidate, anesthesia was maintained with nitrous oxide–oxygen and enflurane.

Blood samples of 10 ml were withdrawn through the cannula into heparinized glass syringes. Samples were taken at 1, 2, 4, 8, 15, and 30 min after injection and then hourly to 6 hr. The blood samples were immediately transferred to 15-ml glass-stoppered centrifuge tubes containing heparin and 10 μl of saturated potassium fluoride solution (to inhibit esterase activity) (6). The plasma was separated by centrifugation at 2000 rpm for 15 min and then frozen and stored at −15° until extracted.

Because of the wide range of etomidate concentrations, the 0–30-min samples were processed separately from later samples. Standard plasma samples containing etomidate concentrations ranging from 1.25 to 0.10 μg/ml were extracted with the 0–30-min patient plasma samples. Aliquots, 3 ml, of 0.05 *M* phosphate buffer, pH 7.4, and 1.0 ml of 1.0-μg/ml propoxate hydrochloride internal standard solution were first added to a 1.0-ml aliquot of each plasma sample. These samples were then subjected to the extraction procedure described below.

Patient plasma samples from later sampling times (1–6 hr) were analyzed with a separate set of standard plasma samples containing 0.25–0.001 μg of etomidate/ml. Aliquots, 1 ml, of a 0.2-μg/ml propoxate hydrochloride internal standard solution were added to 4.0-ml aliquots of these samples. These samples were then subjected to the following extraction procedure, which is a modification of that suggested by Wynants *et al.* (7):

[1] Courtesy of Janssen Pharmaceutica, Beerse, Belgium.
[2] Finnegan model 3200E.
[3] Supelco, Inc., Supelco Park, Bellefonte, Pa.
[4] Finnigan model 1015-80 PROMIM.