In vitro Interaction of Nandrolone with Calcium Nitrate, Magnesium Sulfate and Potassium Permanganate in Aqueous Medium

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ABSTRACT: Nandrolone is an anabolic steroid drug that increases production and urinary excretion of erythropoietin. The drug is indicated with calcium nitrate, magnesium sulfate and potassium permanganate as concomitant uses. Therefore, there is a chance of interactions during concomitant use. Hence, we evaluated the interactions monitored by UV, conductometric titration and Ardon's spectrophotometric methods of nandrolone with calcium nitrate, magnesium sulfate and potassium permanganate at the ratio of 1: 1 at different pHs. It was found that nandrolone did not form any stable complexes at the ratio of 1:1 with the above mentioned salts.

Key words: Nandrolone, drug-drug interaction, drug-metal interaction.

INTRODUCTION

Now-a-days, various mineral supplements along with drug therapy are a common and useful practice for the treatment of diseases where minerals and drugs are given concurrently. The drugs may exhibit effects independently or may interfere or interact with each other. The interaction may be agonist or antagonist of one drug by another. Sometimes, the combination therapies are beneficial to the patients and sometimes it causes serious negative effects. Patients with diseases like kidney/heart transplantation or failure, diabetes mellitus and hypertension, anemia, bone and lipid disorders and so on are frequently prescribed numerous medications. Concomitant use of a large number of medications may have increased risks for drug interactions. Drug interactions are classified into two classes-pharmacodynamic and pharmaco-kinetic interactions. Pharmacodynamic interactions include those that result in additive or antagonistic pharmacological effects. Pharmacokinetic interac-tions involve induction or inhibition of metabolizing enzymes in the liver or elsewhere, displacement of drug from plasma protein binding sites, alterations in gastrointestinal absorption, or competition for active renal secretion.[1,2] Therefore, drug interactions definitely alter the pharmacological effects. The effects of a moderate interaction may cause deterioration in the patient's clinical status, resulting in additional treatment, hospitalization, and/or an extended hospital stay. The effects of a major interaction are potentially life-threatening or can lead to permanent damage.[3,4] Therefore, in our research we studied the *in vitro* interaction of nandrolone with calcium nitrate, magnesium sulfate and potassium permanganate in aqueous medium.

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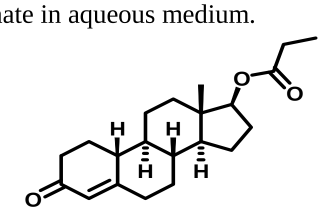


Nandrolone

Nandrolone is an anabolic steroid drug which increases production and urinary excretion of erythropoietin. It may also have a direct action on bone marrow. Nandrolone binds to the androgen receptor to a greater degree than testosterone. Nandrolone is used in the treatment of refractory deficient red cell production anemias, breast carcinoma, hereditary angioedema, antithrombin-III deficiency, fibronogen excess, growth failure and Turner's syndrome. It is also indicated in the prophylaxis of hereditary angioedema. It was also used to treat osteoporosis in post menopausal women.[5,6] Calcium nitrate is a source of calcium which is needed for building our bones.[7] Magnesium and manganese both exert synergistic effect in the pharmacological activity of calcium by increasing the deposition rate of the calcium in bones.[5,8]

The drug is indicated with calcium nitrate, magnesium sulfate and potassium permanganate to the patient sufferings from refractory deficient red cell production anemias, breast carcinoma, hereditary angioedema, antithrombin-III deficiency, fibronogen excess, growth failure and Turner's syndrome with osteoporosis.[7] Therefore, there is a chance of interactions among them during concomitant use. Hence, we carried out the research work to have an idea about the concomitant use of these drugs. We evaluated the interactions by monitoring UV, conductometric titration[9-11] and Ardon's spectrophoto-metric methods[12] of nandrolone with calcium nitrate, magnesium sulfate and potassium permanganate at the ratio of 1:1 at different pHs.

MATERIALS AND METHODS

Apparatus. UV-visible spectrophotometer (Model- UV 1601, Shimadzu, Japan), Conductometer (Jenway, Switzerland), Cyclic voltameter (Shimadzu, Japan), centrifuge machine, pH meter (Mettler Toledo, Switzerland) were used for the analysis.

Drugs and chemicals. The working standards of nandrolone propionate, calcium nitrate (Merck, Germany), magnesium sulfate (Merck, Germany) and potassium permanganate (Merck, Germany) with potency of 98.80%, 98.50%, 99% and 99%, respectively, were used. The nandrolone propionate was a gift of Techno Drugs Ltd., Dhaka, Bangladesh. Hydrochloric acid (37%), potassium dihydrogen orthophosphate and orthophosphoric acid were purchased from Active Fine Chemicals Ltd., Bangladesh. Potassium chloride, potassium hydroxide, sodium hydroxide, potassium bromide and heparin were of reagent grade and purchased from Merck, Germany.

Preparation of buffer solutions[9, 10]

pH 1.4. To prepare 1 litre of pH 1.4 buffer, 6.57 g of potassium chloride was taken in a 1000 ml volumetric flask and dissolved in 600 ml of demineralized (DM) water. 119 ml of 0.1M HCl was added into the solution slowly. Finally the volume was made up to the mark with DM water. The pH was adjusted to 1.4 by using HCl.

pH 3.4. This buffer was prepared by mixing of 302.80 ml of 0.1M formic acid with 95 ml of 0.1M KOH solution and diluted to 1000 ml with DM water.

pH 6.4. This buffer was prepared by mixing of 302.1 ml of 0.02M KH_2PO_4 with 131.90 ml of 0.01 M Na_2HPO_4 and diluted to 1000 ml by adding DM water.

pH 7.4. This buffer was prepared by mixing of 65.40 ml of 0.02M KH_2PO_4 with 289.70 ml of 0.01M Na_2HPO_4 and diluted to 1000 ml with DM water.

Preparation of stock solutions

Nandrolone. 100 ml stock solution of 5×10^{-3}M was prepared by dissolving 0.165 g of nandrolone propionate in DM water and added 2-3 drops of methanol and chloroform to make it soluble. Finally, the volume was made up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solutions.

Calcium nitrate. 100 ml stock solution of 5×10^{-3} M was prepared by dissolving 1.18 g of calcium nitrate in DM water and made the volume up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solutions.

Magnesium sulfate. 100 ml stock solution of 5×10^{-2}M was prepared by dissolving 1.2825 g of magnesium sulfate in DM water and made the volume up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solutions.

Potassium permanganate. 100 ml stock solution of 5 x 10^{-2}M was prepared by dissolving 0.79 g of potassium permanganate in DM water and made the volume up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solutions.

Analyses

UV analysis. The ultraviolet absorption characteristics of nandrolone, calcium nitrate, magnesium sulfate and potassium permanganate and their 1:1 mixtures in different pHs (pH 1.4, 3.4, 6.4 and 7.4) were compared with those of each interacting drug. The concentrations of the samples were kept at very dilute levels in each case and the measurements were recorded between 200-400 nm using an UV-Vis spectrophotometer. The experiment was repeated thrice.

Conductometric analysis. Conductometric titrations were done to detect the complex formation of nandrolone, calcium nitrate, magnesium sulfate and potassium permanganate as well as to find the molar ratios of the interacting agent to the drug molecule in the complex. 40 ml of 0.005M nandrolone solution was taken in a 100 ml beaker and was titrated individually with gradual addition of 0.05M solution of calcium nitrate, magnesium sulfate and potassium permanganate from a burette. Reversely 40 ml each of 0.05M calcium nitrate, magnesium sulfate and potassium permanganate were titrated with gradual addition of 0.005M nandrolone under similar conditions. The conductance values (mS) were plotted against molar ratios between the two agents in the system. The titrations curves showed break at the points of possible interaction. All the titrations were performed with solutions adjusted to pH 1.4, 3.4, 6.4 and 7.4.

The Ardon's method for analysis of drug-drug interaction.[12] In this method, concentrations of drugs were varied while keeping the concentrations of the ligand was fixed. The absorbance of solutions having pH 1.4, 3.4, 6.4 and 7.4 were measured at 257 nm for nandrolone using UV-Vis spectrophotometer. For calculations, the following Ardon's equation[10] was used -

$$1/(D-E_A C) = 1/(KC(E_{com} - E_A)[B]^n + 1/C(E_{com} - E_A)$$

Where,

D = absorbance of mixture

C = molar concentration of drug,

B = molar concentration of ligand (targeted drug)

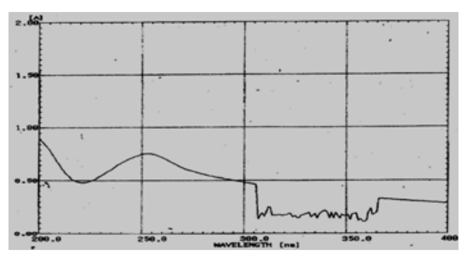
E_{com} = molar extinction co-efficient of the complex

E_A = molar extinction co-efficient of the drug

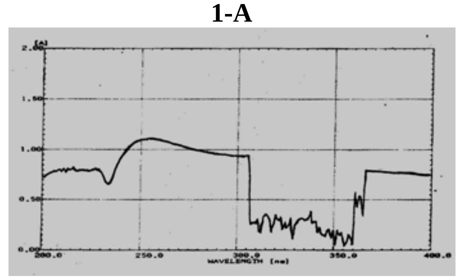
The value of n was chosen as 1, which is an essential condition for validation of the method. The value for $1/(D-E_A C)$ was plotted versus 1/[B] to get the straight lines. The concentration of nandrolone was kept constant at 5×10^{-5}M (denoted by C in the equation). The 1:1 complex gave a straight line in the plots with an intercept and slope. The stability constant of the complex was given by K=intercept/slope. It is noted that the method is only valid for the systems where 1:1 complexes are found.

RESULTS AND DISCUSSION

When separated mixtures of nandrolone with calcium nitrate, magnesium sulfate and potassium permanganate at the ratio of 1:1 at the pH of 1.4, 3.4, 6.4 and 7.4 were studied at the range of 200 to 400 nm in UV-Vis spectrophotometer, the absorption maxima of nandrolone were found to be shifted (Figures 1 and 2). This alteration in spectral patterns might be regarded as an indicator for the primary interaction of drugs.

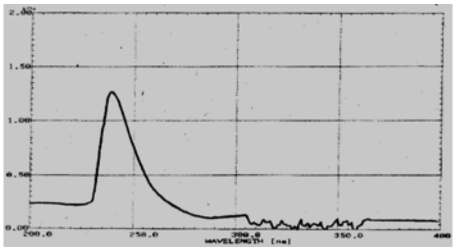


1-A

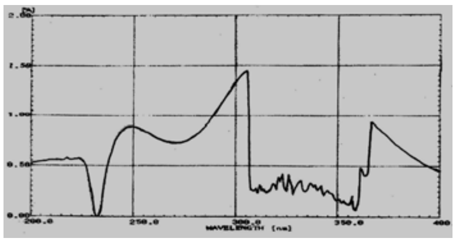


1-B

Figure 1. UV spectra of nandrolone pure drug (1-A) and nandrolone with calcium nitrate at pH 1.4 (1-B) at 257 nm.



2-A



2-B

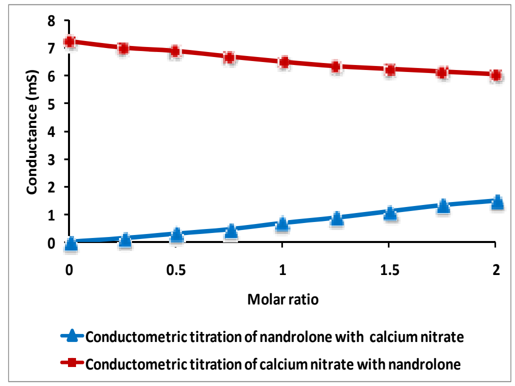
Figure 2. UV spectra of nandrolone with magnesium sulfate (2-A) and potassium permanganate (2-B) at pH 6.4 at 257 nm.

The conductometric titrations in DM water at pH 6.4 were carried out to find the molar ratios at which complexation occurred. For each combination two titrations were carried out- one was titrated against the other and vice-versa. For each reacting species, the same process was followed. The conductance at each addition was recorded. Then the conductance was plotted versus the molar ratios of the titrants for obtaining conductivity curves (Tables 1- 3, Figure 3).

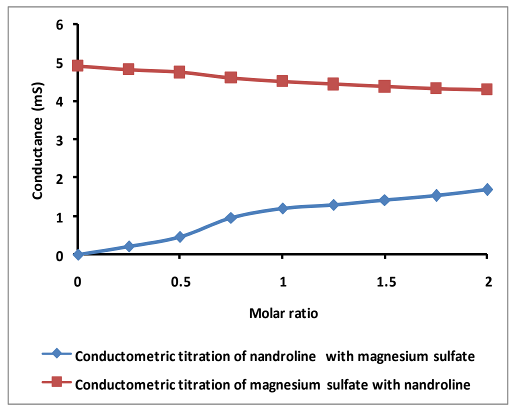
When nandrolone was titrated with calcium nitrate at pH 6.4, one distinct break corresponding to nandrolone-calcium nitrate molar ratios of 1:1 was found in the curve. The reverse titration also showed break at 1:1 molar ratio which indicated that nandrolone formed unstable complex with calcium nitrate at the molar ratio of 1: 1.

Table 1. Data for conductometric titration of nandrolone-calcium nitrate at pH 6.4. Initial concentration of nandrolone [B] = 0.005 M. Initial volume of nandrolone solution = 40 ml. Concentration of added species (calcium nitrate) = 0.05M

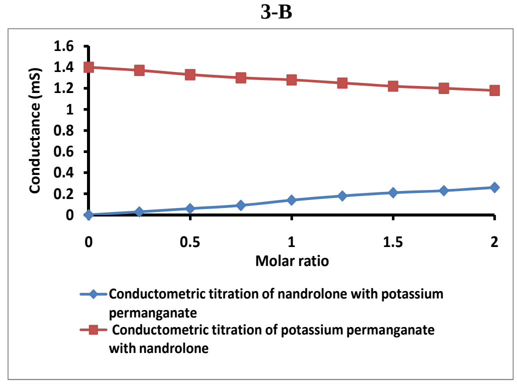
Volume of added species (ml)	Conductance (mS) for normal titration	Conductance (mS) for reversed titration	Molar ratio (calciun nitrate/nandrolone) [A]/[B]
0	0.0	7.25	0.0
1	0.12	7.01	0.25
2	0.30	6.90	0.5
3	0.45	6.69	0.75
4	0.69	6.51	1.0
5	0.88	6.35	1.25
6	1.10	6.24	1.5
7	1.33	6.15	1.75
8	1.5	6.05	2.0



3-A



3-B



3-C

Figure 3. Curve for conductometric titration of nandrolone with calcium nitrate (3-A), magnesium sulfate (3-B) and potassium permanganate (3-C) at pH 6.4.

The same titration of nandrolone with mixture of magnesium sulfate was carried out at pH 6.4. It showed a distinct break at 1:1 molar ratio, where as the reverse titration also showed a break at 1:1 molar ratio which also indicated the complex formation of nandrolone with magnesium sulfate. The nandrolone solution was also titrated with potassium permanganate at pH 6.4 showed distinct breaks at 1:1 molar ratio, which the reverse titration also showed.

This indicated that the nandrolone also formed an unstable complex with potassium permanganate at 1:1 molar ratio. From the above discussions revealed that nandrolone does not form any stable complex with calcium nitrate, magnesium sulfate and potassium permanganate at the molar ratio of 1:1 at pH 6.4.

Table 2. Data for conductometric titration of nandrolone-magnesium sulfate at pH 6.4. Initial concentration of nandrolone [B] = 0.005 M. Initial volume of nandrolone solution = 40 ml. Concentration of added species (magnesium sulfate) = 0.05M

Volume of added species (ml)	Conductance (mS) for normal titration	Conductance (mS) for reversed titration	Molar ratio (magnesium sulfate /nandrolone) [A]/[B]
0	0.0	4.90	0.0
1	0.20	4.80	0.25
2	0.45	4.73	0.5
3	0.95	4.60	0.75
4	1.20	4.50	1.0
5	1.30	4.42	1.25
6	1.42	4.36	1.5
7	1.52	4.31	1.75
8	1.68	4.28	2.0

Table 3. Data for conductometric titration of nandrolone-potassium permanganate at pH 6.4. Initial concentration of nandrolone [B] = 0.005 M. Initial volume of nandrolone solution = 40 ml. Concentration of added species (potassium permanganate) = 0.05M.

Volume of added species (ml)	Conductance (mS) for normal titration	Conductance (mS) for reversed titration	Molar ratio (potassium permanganate /nandrolone) [A]/[B
0	0.0	1.40	0.0
1	0.03	1.37	0.25
2	0.06	1.33	0.5
3	0.09	1.30	0.75
4	0.14	1.28	1.0
5	0.18	1.25	1.25
6	0.21	1.22	1.5
7	0.23	1.20	1.75
8	0.26	1.18	2.0

Ardon's plot confirmed the formation of 1:1 complexes of nandrolone with calcium nitrate, magnesium sulfate and potassium permanganate. Stability constants (K) for nandrolone-calcium nitrate, nandrolone-magnesium sulfate and nandrolone-potassium permanganate systems were obtained from the ratio between the intercepts (c) and slopes (m) of the Ardon's plots, i. e. K=c/m. The calculated stability constants (K) for different systems are given in the table (Tables 4-6, Figures 4 and 5). It was found that the values of stability constant for the systems remain quite close to each other at pHs 1.4 and 6.4 except for nandrolone-calcium nitrate system which was found to be different than other (3.49 and 1.31, respectively).

Table 4. Stability constants (K) for different systems.

Systems	Stability constants K x 10^{-5}/mole	
	pH 1.4	pH 6.4
Nandrolone-calcium nitrate	3.49	1.31
Nandrolone-magnesium sulfate	2.23	2.87
Nandrolone-potassium permanganate	6.75	4.06

Table 5. Values for Ardon's plot at pH 1.4. Concentration of nandrolone [C] = 5x10^{-5}M. Absorbance of 5x10^{-5}M nandrolone = 0.4028. Molar extinction co-efficient of nandrolone, E_A = 8056

Table 5A. Nandrolone-calcium nitrate system

Conc. of calcium nitrate [B], Mx10^{-5}	Abs. of mixture [D]	1/(D-E_AC)	1/([B] x 10^{-5})
1.00	0.4682	15.29	10.00
1.50	0.4857	12.06	6.67
2.00	0.4957	10.76	5.00
2.50	0.5089	9.43	4.00
3.00	0.5247	8.20	3.33
3.50	0.5328	7.69	2.86
4.00	0.5469	6.94	2.50
4.50	0.5528	6.67	2.22
5.00	0.5617	6.29	2.00

Table 5B. Nandrolone-magnesium sulfate system

Conc. of magnesium sulfate [B], Mx10^{-5}	Abs. of mixture [D]	1/(D-E_AC)	1/([B] x 10^{-5})
1.00	0.4829	12.48	10.00
1.50	0.5158	8.85	6.67
2.00	0.5349	7.57	5.00
2.50	0.5526	6.68	4.00
3.00	0.5782	5.70	3.33
3.50	0.5928	5.26	2.86
4.00	0.6147	4.72	2.50
4.50	0.6358	4.29	2.22
5.00	0.6528	4.00	2.00

Table 5C. Nandrolone-potassium permanganate system.

Conc. of potassium permanganate [B], Mx10^{-5}	Abs. of mixture [D]	1/(D-E_AC)	1/([B] x 10^{-5})
1.00	0.4662	15.77	10.00
1.50	0.4726	14.33	6.67
2.00	0.4829	12.48	5.00
2.50	0.4898	11.49	4.00
3.00	0.4925	11.15	3.33
3.50	0.4991	10.38	2.86
4.00	0.5017	10.11	2.50
4.50	0.5147	8.94	2.22
5.00	0.5182	8.67	2.00

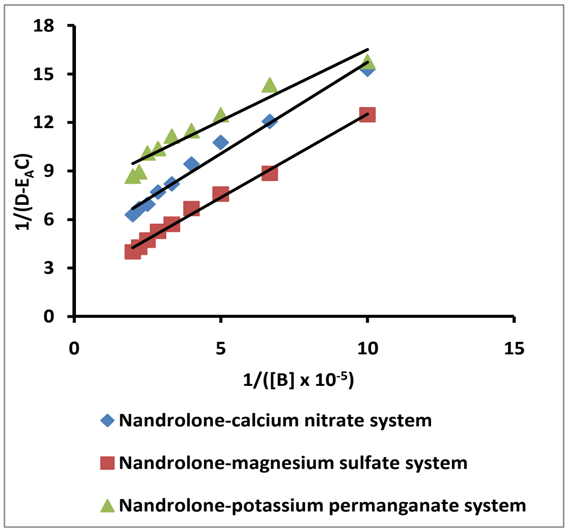


Figure 4. Ardon's plot at pH 1.4.

Table 6. Values for Ardon's plot at pH 6.4. Concentration of nandrolone [C] = 5x10^{-5}M. Absorbance of 5x10^{-5}M nandrolone = 0.3849. Molar extinction co-efficient of nandrolone, E_A = 7698

Table 6A. Nandrolone-calcium nitrate system

Conc. of calcium nitrate [B], Mx10^{-5}	Abs. of mixture [D]	1/(D-E_AC)	1/([B] x 10^{-5})
1.00	0.4628	12.84	10.00
1.50	0.4800	10.52	6.67
2.00	0.4961	8.99	5.00
2.50	0.5429	6.33	4.00
3.00	0.5682	5.46	3.33
3.50	0.5891	4.90	2.86
4.00	0.6048	4.55	2.50
4.50	0.6453	3.84	2.22
5.00	0.6812	3.37	2.00

Table 6B. Nandrolone-magnesium sulfate system

Conc. of magnesium sulfate [B], Mx10^{-5}	Abs. of mixture [D]	1/(D-E_AC)	1/([B] x 10^{-5})
1.00	0.5482	6.12	10.00
1.50	0.5924	4.82	6.67
2.00	0.6348	4.00	5.00
2.50	0.6725	3.48	4.00
3.00	0.7129	3.05	3.33
3.50	0.7426	2.80	2.86
4.00	0.7928	2.45	2.50
4.50	0.8254	2.27	2.22
5.00	0.8567	2.12	2.00

Table 6C. Nandrolone-potassium permanganate system

Conc. of potassium permanganate [B], Mx10^{-5}	Abs. of mixture [D]	1/(D-E_AC)	1/([B] x 10^{-5})
1.00	0.4465	16.23	10.00
1.50	0.4552	14.22	6.67
2.00	0.4649	12.50	5.00
2.50	0.4764	10.93	4.00
3.00	0.4802	10.49	3.33
3.50	0.4889	9.62	2.86
4.00	0.4928	9.27	2.50
4.50	0.5098	8.01	2.22
5.00	0.5148	7.70	2.00

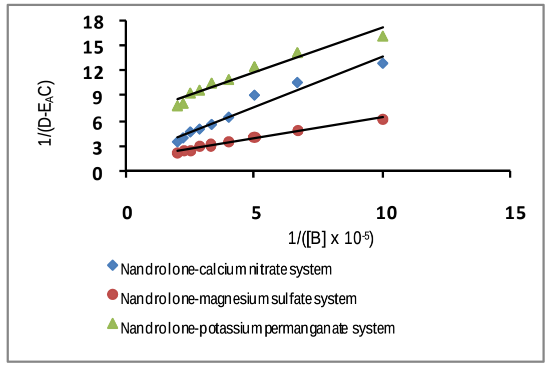


Figure 5. Ardon's plot at pH 6.4.

CONCLUSION

Since nandrolone has been prescribed frequently with the minerals like calcium nitrate, magnesium sulfate and potassium permanganate for concurrent use, the principal purpose of the present study was to investigate *in vitro* complex formation of nandrolone with such minerals and to study the nature and strength of the complexes which could be formed from the interactions. The interactions were studied in the aqueous systems at room temperature at different pHs by a variety of physical methods to detect and confirm the nature of complexation of these drugs. It was found that nandrolone did not form any stable complexes at the ratio of 1:1 with the salts. Initially, absorption spectral analysis showed that unstable complexes were formed but conductometric titration indicated that there was a very rare chance of the formation of complexes between nandrolone and these metal ions. The Ardon's spectrophotometric analysis confirmed that the values of stability constant for the nandrolone-calcium nitrate, nandrolone-magnesium sulfate and nandrolone-potassium permanganate systems remain quite close to each other at the ratio of 1:1 at pHs 1.4 and 6.4 except for nandrolone-calcium nitrate system which was found to be different than other (3.49 and 1.31, respectively). Therefore, it was concluded that at pH 1.4 and 6.4, nandrolone-calcium nitrate system formed stable complexes while other systems formed relatively weak complexes.

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