

**Estimation of Manganese, Zinc and Copper in
Multimineral Preparation Containing Vitamins: A
Complexometric Approach**

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ABSTRACT

A simple, precise, complexometric titration method has been developed for the estimation of essential trace metals- manganese, zinc and copper, in marketed multimineral pharmaceutical dosage form containing vitamins. The selectivity of the method is accomplished by masking the minerals with suitable masking agent (NaCN) and determining the metal ion using Complexon III (EDTA). The method having high recovery range has successfully been applied for the determination of these trace metals in presence of other minerals and vitamins. The values of different statistical parameters (e.g., standard deviation, coefficient of variation, standard error, confidence interval) were also determined, which reflect the accuracy and validity of the proposed method.

Key words: Multimineral, complexometric titration, Na₂EDTA.

INTRODUCTION

Manganese, zinc and copper, three very important members of the trace metals, play significant roles in the enzyme activities and a few very important structural and regulatory functions of the body (Remington. 2001). Owing to the increasing need in nutrition, there is a need for methods for determination of these metals in pharmaceutical preparations and other food products. The marketed multimineral pharmaceutical dosage form with vitamins is a preparation containing 32 active ingredients (20 minerals and 12 vitamins) where the amount of manganese, copper and zinc are 1 mg, 37.04 mg and 2 mg respectively.

A number of methods are reported by several authors (Batsura. 1969; Cousins. 1985; Gacher et al. 1990; Hall et al. 1979; Klein et al. 1991; Knobloch et al. 1994; Lee et al. 1989; Nriagu et al. 1988; Sharrett et al. 1982; Solomon. 1985; Stein et al. 1976) for the determination of manganese, zinc and copper from different type of preparations. The British Pharmacopoeia (1988) and United States Pharmacopoeia (1985) describe methods for the determination of manganese, zinc and copper from single component preparation. The present study reports a simple and precise method having high recovery rate for the estimation of these trace metals in marketed multi-mineral pharmaceutical dosage forms containing vitamins.

EXPERIMENTAL

Samples were collected from the retail pharmacy shops in a random manner and coded S1, S2,....S7.

Preparation of sample solution

Ten tablets from each sample were taken, weighed on an electronic balance (AA-200, Denver instrument, UK) and the average weight of each tablet was determined. The coating on the tablet was removed simply by washing under tap water, then by soaking with filter paper. Then Tablets were triturated into fine powder using mortar and pestle. Then powder equivalent to 1 tablet was taken in a 100 ml beaker, 10 ml of 1N Hydrochloric acid was added and heated on a water bath for 5 minutes with constant stirring with a glass rod. The solution was then cooled and filtered in a vacuum filter; the residue and the beaker were washed several times, filtered and collected.

The filtrate was taken in a 100ml beaker and to it 1 ml concentrated nitric acid (Merck, specific gravity –1.52) and 1 ml concentrated hydrochloric acid (Merck, specific gravity – 1.19) were added and heated to dry on a water bath . Then 2 ml of 33% hydrogen per oxide was added and extracted thrice with 15 ml of ether each time. The ether layer was discarded which contains iron and the water layer was heated on a water bath till the volume was 25ml. The solution was neutralized by adding 3N sodium hydroxide. Then a few drops of acetic acid were added to make the solution acidic. To this solution 3% ammonium oxalate was added and centrifuged at 2000 rpm for 5 minutes. Supernatant fluid was decanted in an Erlenmeyer flask. Few ml of ammonium oxalate was added again and centrifuged at 2000 rpm for 10 minutes and again the supernatant was decanted as before.

Estimation of Manganese

Few crystals of ascorbic acid were added to the decanted liquid and the volume was adjusted to 100 ml with demineralised water. 10 ml of the solution was taken in a conical flask and 3-4 ml of buffer solution of pH 10 (ammonia ammonium chloride buffer), 1 ml of 1M sodium cyanide and excess quantity of 0.001 M complexon III (Na₂EDTA) was added. A blank solution was prepared by adding 3-4 ml buffer of pH 10, 1 ml of 1M NaCN and the same amount of complexon III as in the sample solution. Both the sample & blank were heated to 60⁰C on a water bath. Then the excess EDTA was titrated with 0.001M magnesium sulfate using Eriochrome black T as an indicator. Results obtained are entered in Table-1.

Estimation of Zinc

To the sample solution used earlier for manganese estimation, 1 ml of 1M solution of chloral hydrate was added. Then the solution was titrated with complexon III and the amount of zinc was calculated. Results obtained are entered in Table-1.

Estimation of Copper

Then acetic acid was added until pH of the solution was 6. Excess amount of EDTA was added and titrated against MgSO₄. Again, for blank titration, in a conical flask, 3-4 ml of buffer pH 10, 1 ml of NaCN (M) and excess amount of disodium EDTA (0.001M). Then the solution was titrated against MgSO₄ (0.001M) using Eriochrome black T as indicator. After reaching end point, 1 ml of 1M chloral hydrate was added in the solution and acetic acid was added until the pH of the solution was 6. Then again excess amount of EDTA was added and titrated against MgSO₄. Results obtained are entered in Table-1.

Recovery Experiment

Four 100 ml beakers were taken and labeled 1, 2, 3 and 4. In each of them, an amount of powder equivalent to one tablet was taken and 0.0 mg, 5 mg, 10 mg and 15 mg manganese sulfate, zinc sulfate and copper sulfate were added respectively. Then the proposed method was followed to determine the amount of manganese sulfate, zinc sulfate and copper sulfate in each of the sample (Sane et al. 1982).

RESULTS AND DISCUSSION

The proposed method was successfully applied for the analysis of manganese, zinc and copper in multivitamin multi-mineral tablets (Coded S₁ - S₇). All the samples assayed by the proposed method were found to meet the BP and USP requirements. Results obtained are entered in Table-1. Multi-vitamin tablets containing multi minerals are sugar coated. The sugar coat was removed by washing the tablets with water. By filtering the acid solution of the tablet powder all the insoluble substances including vitamin A, D, E and K were eliminated. Water soluble vitamins which remain in the sample solution do not interfere with the selective complexation of manganese, zinc and copper with Na₂EDTA solution. Among the minerals present in the preparation ferrous sulfate, manganese sulfate, potassium sulfate, zinc sulfate/oxide, calcium and copper all are soluble in acid water system. The solution was made free from iron by extracting the solution several times with ether; calcium was excluded by precipitation (as calcium oxalate) and centrifugation. The final solution contains manganese, zinc, copper and potassium from which manganese and zinc were determined by selective masking of other minerals. This selectivity was obtained by the use of specific masking agent which requires no complicated reaction procedure. The concentration of EDTA solution (0.001M) used in this method was 50 times diluted than that stated in BP and 100 times diluted than that in USP method.

Table 1: Estimation of manganese, zinc and copper in multivitamin multimineral preparation

SL	Manganese (mg)			Zinc (mg)			Copper (mg)		
	Amount declared	Amount determined	% Potency	Amount declared	Amount determined	% Potency	Amount declared	Amount determined	% potency
S ₁	1.00	1.10	110	37.04	37.04	100.00	2.00	1.94	97.0
S ₂	1.00	1.08	108	37.04	36.78	99.30	2.00	1.89	94.5
S ₃	1.00	1.01	101	37.04	37.12	102.00	2.00	1.99	99.5
S ₄	1.00	1.04	104	37.04	36.78	99.30	2.00	1.84	92.0
S ₅	1.00	1.01	101	37.04	36.72	99.14	2.00	2.24	112
S ₆	1.00	0.98	098	37.04	36.82	99.40	2.00	2.14	107
S ₇	1.00	1.04	104	37.04	36.65	98.95	2.00	1.89	94.5

Selectively in EDTA titration of mixture of metals can be achieved by adding masking agent, a component that complex strongly with one of the metals, thus decreasing its conditional constant with EDTA so that this metal is not titrable. In this situation, the second metal is available for titration without influence. The masking agent- cyanide used in this method masked copper and zinc, from which manganese was determined. After the determination of Mn, chloral hydrate was added to demask zinc, and then zinc was determined. Then solution was made acidic for the determination of copper.

From the result of recovery experiment it was found that the recovery is more than 98% for manganese (98.4%), zinc (98.96%) and copper (99.16%) that is there was almost no loss even after several steps of the proposed method. Moreover more than 98% recovery proves the accuracy and reproducibility of the proposed method in addition to its simplicity (Table– 2).

Table–2: Recovery experiment for manganese, zinc and copper by the proposed method

Metals	SI No	Amount present	Amount added (X)	Amount calculated(Y)	XY	X ²	* (%) recovery
Mn	1	1.00	0.00	1.30	0.00	0.00	98.40
	2	1.00	5.00	5.91	29.55	25.00	
	3	1.00	10.00	11.28	112.8	100.00	
	4	1.00	15.00	15.90	238.50	225.00	
Zn	1	37.04	0.00	37.12	0.00	0.00	98.96
	2	37.04	5.00	41.73	208.65	25.00	
	3	37.04	10.00	47.74	477.74	100.0	
	4	37.04	15.00	51.64	774.15	225.00	
Cu	1	2.00	0.00	1.848	0.00	0.00	99.16
	2	2.00	5.00	6.942	34.71	25.00	
	3	2.00	10.00	12.03	120.3	100.00	
	4	2.00	15.00	16.68	250.2	225.00	

$$*(\%) \text{ Recovery} = \frac{N \sum XY - \sum X \cdot \sum Y}{N \sum X^2 - \sum X \cdot \sum X} \times 100$$

The results of recovery experiment had been analyzed statistically and expressed in terms of standard deviation, standard error, coefficient of variation and confirmed by the test of significance at different levels of confidence interval. The values of different statistical parameter (Table–3)

reflect that the proposed method is accurate enough to give valid and acceptable results. For two degrees of freedom the tabulated value for 5% and 1% level of significance are 2.776 and 4.602. All the calculated values of "t" were below this level which means the difference between the labeled potency and the determined potency by the proposed method was in closer agreement (Schwarzenbach, 1954).

Table-3: Statistical parameters of the recovery experiment of manganese, zinc and copper

Sample No	Sl No	Standard deviation	Coeff of variation	Standard error	Calculated "t"	Confidence interval	
						95%	99%
A ₁ (Mn)	1	0.190	2.85	0.218	1.970	0.86 - 1.77	0.567 - 2.07
	2	0.075	2.149	0.084	1.866	5.71 - 6.05	5.59 - 6.17
	3	0.080	1.20	0.089	3.807	11.07- 11.442	10.94-11.57
	4	0.077	0.822	0.085	2.691	15.54 – 16.00	15.53 16.12
A ₃ (Zn)	1	0.273	2.080	0.305	0.220	36.04 – 38.17	35.30 -38.87
	2	0.049	0.321	0.055	1.762	43.00 – 43.39	42.86- 43.52
	3	0.085	0.505	0.095	5.910	47.39 – 48.04	47.22- 48.35
	4	0.155	0.853	0.853	1.680	51.00 – 52.22	50.61- 52.70
A ₅ (Cu)	1	0.370	25.06	0.415	0.86	1.21-2.48	0.78-2.90
	2	0.233	4.19	0.260	0.51	6.54-7.34	6.27-7.60
	3	0.149	1.54	0.167	0.40	11.77-12.30	11.61-12.46
	4	0.149	1.11	0.167	4.28	16.42-16.94	16.25-17.10

CONCLUSION

The proposed method is simple and selective involving no complex technique or any critical reaction condition and has been successfully applied for the analysis of manganese, zinc and copper in pharmaceutical preparations containing various minerals and vitamins.

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