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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR DETERMINATION OF CALCIUM OROTATE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

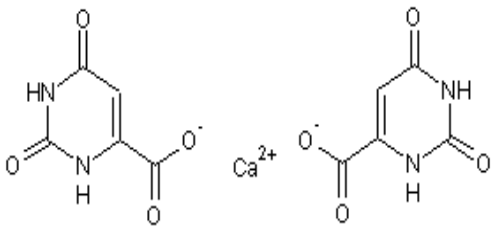
Calcium implicates normal functioning of nerves, cells, muscle, and bone. Deficiencies of calcium results in uptake of calcium from bones, thereby weakening bones. A simple, sensitive and highly accurate UV spectroscopic method has been developed for the determination of Calcium Orotate in bulk and its tablet dosage form. Calcium Orotate in its water solutions was determined at the wavelength range of 240-400 nm by the spectroscopic method. Solution of Calcium Orotate in water shows a maximum absorbance at 278 nm. Beer's law was obeyed in the concentration of 4-12 $\mu g.mL^{-1}$. Correlation coefficient, detection and quantification limit were also calculated. The proposed method has been applied successfully to quantify of Calcium Orotate in pure and tablet dosage form. Results of percentage recovery and placebo (excipients) interference show that the method was not affected by the presence of common excipients. The percentage of recovery of Calcium Orotate in tablet was 99.25-101.25% of the label claimed 400 mg per tablet. The method was then validated successfully as per ICH guidelines (2005) which yielded good results concerning range, precision, accuracy, reproducibility, specificity and robustness.

KEYWORDS: Calcium Orotatetablets, Potency, UV-Spectroscopic method, Estimation, Method validation.

INTRODUCTION

Calcium Orotate **(Fig.1)** is chemically described as calcium 1,2,3,6-tetrahydro-2,6-dioxopyrimidine-4-carboxylate which is used as calcium supplements for human body and effective in relieving discomfort resulting from osteoporosis of the spine.[1] Malignant bone tumors (thereby preventing further metastases) recalcification is successfully possible with calcium orotate as reported by Dr. Nieper.[2] A further paper reported on the benefits of calcium orotate in addressing joint diseases.[3] Calcium Orotate (orotic acid) is a biochemical substance made by all cells. It is a necessary raw material for making the genetic substances RNA and DNA.[4]

The literature survey reveals that there is no method either UV or HPLC or any other for the estimation of Calcium Orotate (COT) in bulk and tablet dosage forms. Since, the drug is not included in any official pharmacopeia, an attempt was taken to develop a simple, cost effective, accurate, precise, reproducible and robust method for estimation of COT in bulk and its tablet dosage form.



Molecular formula $C_{10}H_6CaN_4O_8$. Molecular weight 350.25g

FIG.1: CHEMICAL STRUCTURE OF CALCIUM OROTATE (COT)

MATERIALS AND METHODS

Apparatus

A UV/VIS Spectrometer, Perkin Elmer, Lambda 25, USA, double beam spectrophotometer with 1 cm matched quartz cell was used for spectral measurement. Sartorius CPA224S analytical balance was used for weighing purpose.

Reagents and solutions

Pharmaceutical grade of Calcium Orotate INN was gifted by Alcon Biosciences Private Ltd., India and certified to contain 99.85% w/w of Calcium Orotate. It was used without further purification. Excipients used in tablet formulation were Microcrystalline Cellulose (PH 101),

Lactose Monohydrate, Maize Starch, Povidon (K-30) Croscarmellose Sodium, Magnesium Stearate and Talcum Purified and they were of BP and/USP grade. Water was obtained from double distillation in glass and passage through a Milli-Q® System, Millipore, Milford, MA, USA.

Wavelength selection

Appropriate dilutions (4, 6, 8, 10 and 12 µg.mL^{-1}) were prepared for drug from the standard (200 µg.mL^{-1}) stock solution and the solutions were scanned in the wavelength range of 240 – 400nm. The λ_{max} was found at the wavelength 278 nm.

Calcium Orotate stock solution preparation

Calcium Orotate 20 mg API standard was weighed and transferred to volumetric flask of 100 mL capacity containing 70 mL of water and sonicated for 5 minutes. The flask was then heated in a water bath at 80ºC for about 45 min with intermittent shaking. The solution was allowed to cool at room temperature. The flask was shaken and volume was made up to the mark with water to give a solution of 200 µg.mL^{-1}. From this solution, 2 mL was taken and diluted to 50 mL with water to give a solution of 8 µg.mL^{-1} and was used for the estimation of Calcium Orotate.

Analytical concentration range selection

From the standard stock solution (200 µg.mL^{-1}) of Calcium Orotate, appropriate aliquots were pipetted out into 50 mL volumetric flasks and dilutions were made with water to obtain working standard solutions of concentration from 4–12 µg.mL^{-1}.

Calibration Curve for the Calcium Orotate

Appropriate value of aliquots from standard Calcium Orotate stock solutions were transferred to different volumetric flask of 50 mL capacity. The volume was adjusted to the mark with water to obtain concentration of 4, 6, 8, 10 and 12 µg.mL^{-1}. Absorbance spectrum of each solution against water as blank was measured at 278 nm and the graphs of zero order overlain spectra was obtained (**Fig. 2).** The Regression equation and correlation coefficient (R^2) were determined and presented in the**Table 1**.

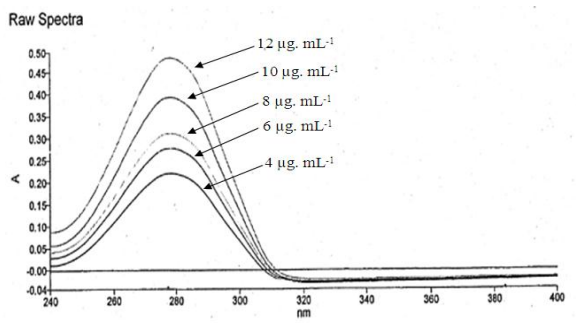


Fig. 2: Spectra of COT at five concentrations (4 µg.mL^{-1}, 6µg.mL^{-1}, 8 µg.mL^{-1}, 10 µg.mL^{-1} and 12µg.mL^{-1}) at 278nm.

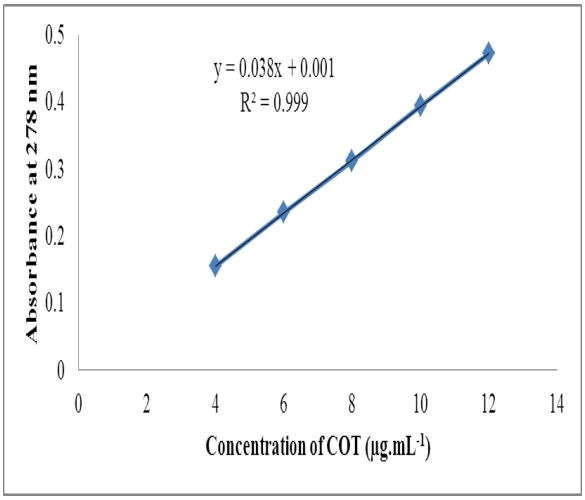


Fig. 3: Calibration curve of COT only (Working standard).

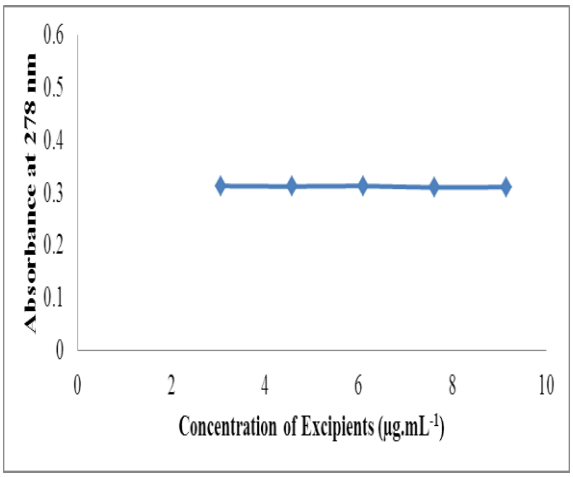


Fig. 4: Absorbance of COT at various concentrations of excipients.

Analysis of Tablet

Twenty tablets (20) were weighed and finely powdered. The powder equivalent to 20 mg of Calcium Orotate was accurately weighed and transferred to volumetric flask of 100 mL capacity containing 70 mL of water and sonicated for 5 minutes. The flask was then heated in a water bath at 80ºC for about 45 min with intermittent shaking. The solution was allowed to cool at room temperature. The flask was shaken and volume was made up to the mark with water to give a solution of 200 µg.mL^{-1}. The above solution was filtered through Whatman filter paper (No. 41). From this solution, 2 mL was taken and diluted to 50 mL with water to give a solution of 8 µg.mL^{-1} and it was used for the estimation of Calcium Orotate present in the tablet.

Validation of the proposed method

The developed analytical method was validated subsequently as per ICH guidelines for the following parameters: System suitability, Linearity, Limit of quantification (LOQ), Limit of detection (LOD), Range, Specificity, Placebo (excipients) effects, Accuracy, Solution stability, Precision and Robustness.

a. System suitability
System suitability of the method was evaluated by 10 replicates of the standard solution having concentration of 8 $\mu g.mL^{-1}$. Percentage (%) of RSD of the absorbance was calculated.

b. Linearity checking
Linearity of analytical method was tested by performing three studies[5]: a) regression analysis of COT at different concentration, b) regression analysis of COT in the concentration range of 50% -150% with fixed concentration of excipients and c) regression analysis of COT with different concentration of excipients. Later two experiments were carried out to see whether there was any interaction between the standard and excipients.

c. Limit of Quantification (LOQ)
LOQ was calculated using the regression line (obtained in linearity check) and from the formula 10σ/s, where σ is the standard deviation of y-intercepts of the regression line and 's' is the slope of the calibration curve.[6]

d. Limit of Detection (LOD)
Limit of detection (LOD) is the lowest concentration of the analyte that can be detected.[6] LOD was calculated from the formula 3.3σ/s, where σ is the standard deviation of y-intercepts of the regression line and 's' is the slope of the calibration curve.

e. Range
Analytical range was derived from linearity studies.It was calculated from the upper and lower concentration of analyte in the sample[6] for which it was demonstrated that the analytical procedure had a suitable level of precision, accuracy and linearity.

f. Specificity
The specificity of the method was evaluated by monitoring a standard (raw material) solution of COT, its tablet, blank sample and placebo (excipients) materials.[7] Sample of standard and tablets showed λ_{max} at 278 nm separately in UV while blank and placebo (excipients) did not show λ_{max} at 278 nm. Percent recovery of COT in the absence and in the presence of excipients was calculated.

g. Placebo (excipients) effects
Placebo (excipients) effect was studied by testing the blank, placeboand active solution in UV spectrophotometer.

h. Accuracy
In case of assay of the drug in the formulated product, accuracy of the method was determined first. To do so a blank matrix (placebo); the excipients (all ingredients except API as per formulation of COT tablet) simulated COT sample (excipients + API) (50%, 100% and 150%) were tested separately in three replicates in the UV and recovery was studied for each replicate.[8]

i. Solution Stability
The sample solution was allowed to stand at ambient temperature (25ºC) for different time intervals (0, 12, 24 hrs) to see the stability of COT.[6] Percentage of RSD for absorbance was calculated to measure the stability of sample solution over a period of 24 hours.

j. Precision
The precision of the assay was studied with respect to repeatability, intermediate precision and reproducibility.[6] Repeatability precision was determined by six independent determinations of fixed test concentration (8$\mu g.mL^{-1}$) of a solution COT of on the same day. Values of RSD (%) were calculated from these determinations and the obtained RSD (%) value was checked to see whether it was within the limit (NMT 2%) of ICH guidelines. The experiment was repeated by assaying freshly prepared solution of the same concentration on two consecutive days by another analyst with different equipment within same laboratory to determine intermediate precision and similarly reproducibility were carried out by 3rdanalyst of 6 (six) determinations immediately one after the other under different conditions (Laboratory) as per ICH guidelines.

k. Robustness of method
Robustness (or Ruggedness) of the method was determined by making small deliberate change in the wavelength (±2 nm) of the operating parameters of the method.[9]

RESULTS AND DISCUSSIONS
Performance of the analytical system was confirmed by system suitability test where % RSD of absorbance was calculated as 0.345 (**Table 2).** It complied with the recommended range (NMT 1%) CDER.

Table 1: Optimized conditions, optical characteristics and statistical data of the regression equation in zero order spectroscopic method

Parameters	UV Method
λ_{max}(nm)	278
Range ($\mu g.mL^{-1}$)	4 – 12
Molar extinction coefficient ($M^{-1}.cm^{-1}$)	13.49×10^{3}
Regression equation (Y)	y = 0.038x + 0.001
Slope (b)	0.038
Intercept (a)	0.001

Correlation Coefficient (R^2)	0.999
LOD ($\mu g.mL^{-1}$)	0.232
LOQ ($\mu g.mL^{-1}$)	0.703

Table 2: Results of System Suitability test.

No. Sample (Replicates)	Absorbance (278 nm)	RSD (%) of Absorbance		Remarks
		Result	*CDER Limit	
01	0.311	0.345	NMT 1	Complied and the system is suitable to carry out the analysis
02	0.309			
03	0.311			
04	0.312			
05	0.309			
06	0.312			
07	0.311			
08	0.310			
09	0.311			
10	0.310			

* CDER: Center for Drug Evaluation and Research, USA.

Table 3: Results of Specificity test

Sample Information	Content	Absorbance (278 nm)	Content of COT (%)			Remark
			Theoretical	Observed	ICH Limit	
						Complied and method is specific
Blank	Water only	-	-	-	-	
Control	Water + excipients	-	-	-	-	
Standard	Water + COT INN (8 $\mu g.mL^{-1}$)	0.312	99.85	99.68	98-102	
Tablet	Water + COT INN (8 $\mu g.mL^{-1}$) + excipients (6.10 $\mu g.mL^{-1}$)	0.314	100	100.88	-	

Table 4: Percent recovery of COT from simulated tablet contents.

COT ($\mu g.mL^{-1}$)	% of test concentration	Absorbance (278 nm)	Recovery from sample ($\mu g.mL^{-1}$)	Recovery (%)	Average Recovery (%)	ICH Limit (%)	Remark
							Complied and the method is accurate
4	50	0.155	3.97	99.25	100.19	98 -102	
4		0.157	4.02	100.05			
4		0.156	3.99	99.75			
8	100	0.313	8.08	101.00			
8		0.312	8.10	101.25			
8		0.314	7.98	99.75			
12	150	0.468	11.99	99.92			
12		0.469	12.11	100.92			
12		0.467	11.98	99.83			

Table 5: Relative standard deviation of six determinations of COT contents in simulated tablet amount

Sample	Concentration ($\mu g.mL^{-1}$)	Absorbance at 278 nm	Result (%)	RSD (%)	ICH Limit of RSD (%)	Remarks
						Repeatability of COT measurement complied
01	8	0.318	101.08	0.438	NMT 2	
02	8	0.315	100.53			
03	8	0.317	101.04			
04	8	0.318	101.08			
05	8	0.312	99.98			
06	8	0.315	100.53			

Plot of absorbance versus concentration of COT (**Fig.3**) of regression analysis resulted in the linear regression equation y = 0.038x + 0.001(R^2=0.999). It is clear from the F**ig. 3** that the response was linearly dependant on the concentration of COT. The linearity of the regression line is also evident from correlation coefficient (R^2 = 0.999). Similar dose-response relationship of COT was observed even in presence of excipients (data are not shown). And with a fixed concentration of API, the response for COT (absorbance) was not changing (**Fig. 4**) with the increase of excipient concentration. It means that there is no interference on COT response from the excipients.

It is important to mention here that the proposed UV method for COT estimation was found linear in the range of 4-12$\mu g.mL^{-1}$ (**Fig. 3**) but beyond that range linearity was not found (data are not shown). Lower limit of quantitation (LLOQ) is therefore 4 $\mu g.mL^{-1}$or 50% while the upper limit of quantitation (ULOQ) is 12 $\mu g.mL^{-1}$ or 150%. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0.232 and 0.703 $\mu g.mL^{-1}$ respectively.

The specificity of the method was checked by monitoring a standard API solution of COT, its tablet, blank sample and excipients (placebo) materials. Sample of standard and tablets showed λ_{max} at 278 nm when tested separately in UV while blank and excipients did not show any λ_{max} at 278 nm. These results indicate that COT could be detected by the present method and it is able to estimate COT even in the presence of excipients quantitatively (**Table 3**). Percent recovery of COT as API was found within the limit of ICH guidelines (**Table 3**) and in the presence of excipients, % recovery of COT was also close to the recovery of API only of COT. These results thus mean that the developed method is specific for quantification of COT.

Accuracy was assessed using nine determinations over three different concentration levels covering the predetermined range (4-12$\mu g.mL^{-1}$) of analysis. And there were three replicates of each concentration (**Table 4**). From these determinations, it was found that the values of recovery for each estimation were within the range (98%-102%) of ICH percentage recovery guideline. Thus, it indicates that the proposed method is accurate for the analysis of the drug COT.

Repeatability precision was carried out by six independent determinations of a fixed test concentration (8 $\mu g.mL^{-1}$) of a solution (**Table 5**) of COT. Values of RSD were calculated from these determinations and the obtained RSD value was checked to see whether it was within the limit (NMT 2%) of ICH guidelines. In the present case, % RSD was found as 0.438% (**Table 5**) which was within the limit (NMT 2%) of ICH guidelines and hence the repeatability was complied for the present method of analysis of COT. Similarly, it was found that the intermediate precision and Reproducibility criteria were also as per ICH guidelines (data are not shown).

The sample solution was allowed to stand at ambient temperature (25ºC) for different time intervals (0, 12, 24 hrs) to see the stability of COT. The obtained relative standard deviation was a measure of the stability of sample solution over a period of 24 hours. In the present study, the % RSD for sample solution was found 0.408% (ICH limit NMT 2%) which indicates that the working sample solution was stable for at least 24 hours.

Robustness of the method was judged by changing the wavelength (±2 nm) of the operating parameters and found no remarkable change in the test results. Percentage (%) of RSD of the test results at different wavelengths was calculated and found as 0.615% which is within the ICH limit (NMT 2%) indicating that the method is sufficiently robust to analyze COT.

In the light of validation parameters results, it can be said that the developed method is valid for the estimation of COT from the bulk and its tablet formulation.

CONCLUSION

The UV-Vis method ((λ_{max}=278 nm) was developed for the analysis of COT in bulk and formulated tablet. The developed method was compiled with the ICH guidelines. It was successfully applied for the estimation of API, bulk and tablet dosage form without interference of excipients in relevant system.

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REFERENCES

1. Nieper HA. The anti-inflammatory and immune-inhibiting effects of calcium orotate on bradytrophic tissues. Agressologie, 1969; 10(4): 349-57.
2. Nieper HA. Recalcification of bone metastases by calcium diorotate. Agressologie, 1970; 11(6): 495-502.
3. Nieper HA. The clinical effect of calcium-diorotate on cartilage tissue, the specific function dependent upon the pentose metabolism of bradytrophic tissue [in German], 1973; 3(4): 82-9.
4. Lifelinknet.com: Calcium Orotate.
5. U.S. Pharmacopoeia: The United State Pharmacopoeial Convention, USP 35 NF 30, Vol. 1: 2012, p. 880-880.
6. ICH Harmonized Tripartite guideline, Validation of Analytical Procedures: Text and Methodology Q2: 2005, pp. 1-13.
7. British Pharmacopoeia Commission: British Pharmacopoeia, SWIS 95Z, Vol. 5: 2012, pp. A626-A628.
8. Nash RA, Watchler AH. Pharmaceutical Process Validation. 3rded. (revised and extended): 2011, pp. 507-524.
9. Quality Assurance of Pharmaceuticals: A compendium of guideline and related materials. 2ndEdition, Vol. 2: 2007, pp. 131-131.