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# Supplementary Information Design and Optimization for One Pot Synthesis of Calcium Orotate Using a Quality by Design (QBD) Approach

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**KEYWORDS**
Calcium orotate, One-pot synthesis, Quality by Design (QbD), Process optimization, Experimental design.

**ABSTRACT:**
The research focuses on the design and optimization of a one-pot synthesis method for calcium orotate, utilizing a Quality by Design (QbD) approach. Calcium orotate, a compound with significant pharmaceutical and nutraceutical applications, is synthesized through a streamlined process aimed at enhancing efficiency and product quality. The study employs QbD principles to systematically investigate and control various process parameters, ensuring a robust and reproducible method. Through a combination of experimental design, statistical analysis, and risk assessment, key factors influencing the synthesis are identified and optimized. The resulting process not only improves yield and purity but also demonstrates scalability and industrial applicability. This work contributes to the field by providing a comprehensive framework for the rational development of chemical processes, highlighting the benefits of integrating QbD in synthesis strategies.

***Elemental analysis***

*Characterization of calcium orotate*

The optimized process was repeated and the compound obtained was characterized as described below.

**Structure elucidation using UV-visible spectrophotometery.** A 10 ppm aqueous solution of calcium orotate showed a UV visible spectrum as shown in Fig S1. It indicates maximum absorbance at 278 nm. This is a characteristic feature of the chromophore from the orotic acid moiety in the molecule of calcium orotate.

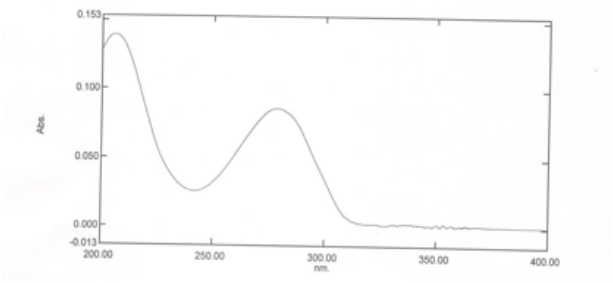


**Figure S1.** UV scan of calcium orotate

**Structure elucidation using IR spectroscopy.** The IR spectrum of calcium orotate is shown in fig S2. The spectrum shows peaks at 1673 $nm^{-1}$ indicative of C=O stretching of the carbonyl functional group. IR spectrum also shows sharp C-H bending with 600-700 $nm^{-1}$ peaks.

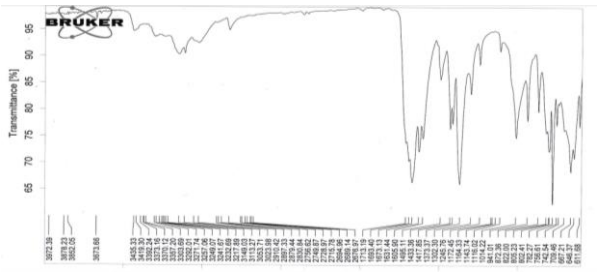


**Figure S2.** IR spectrum of calcium orotate

**Structure elucidation using $^1$H NMR spectroscopy** · The $^1$H NMR spectrum was recorded in DMSO-$d_6$ solvent and at 600 MHz. The spectrum is shown in fig S3. The spectrum shows a chemical shift for every proton in the molecule. The results obtained are of chemical shift values (δ) 11.08 (s, 1H), 9.71 (s, 1H), 5.87 (d, $J$ = 2.0 Hz, 1H).

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The molecule of calcium orotate reflects three types of attachments of protons. The chemical shift values obtained in the spectrum reflect the same. The multiplicity obtained in the spectrum assures the arrangement of protons in the structure. The values and interpreted protons from the structure are detailed in Table S1 and explained in fig S4.

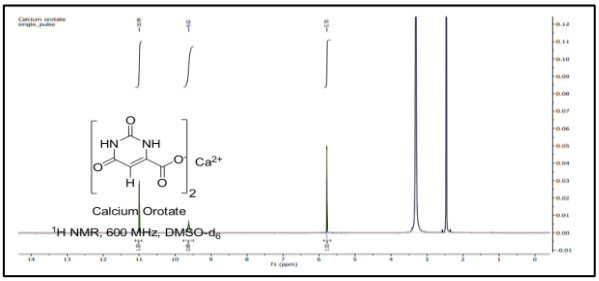


**Figure S3.** $^1$H NMR spectrum

**Table S1.** Interpretation of $^1$H NMR spectrum

| Sr. No. | Chemical shift | Multiplicity | Interpretation proton (H*) |
|---|---|---|---|
| 1. | 11.08 | Singlet | H*-N(C=O)$_2$ |
| 2. | 9.71 | Singlet | H*-N(C=O) -C=C |
| 3. | 5.87 | doublet | H*-C(=C)-C |

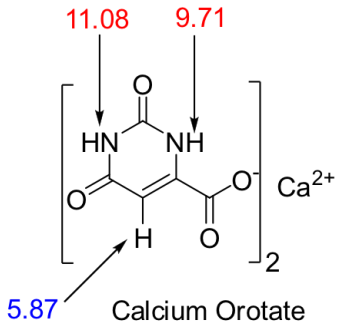


**Figure S4.** Interpretation of $^1$H NMR spectrum

**Structure elucidation using $^{13}$C NMR Spectroscopy.** The $^{13}$C NMR (151 MHz) spectrum was recorded in DMSO-d6 solvent. The spectrum is shown in Fig S5. The spectrum shows chemical shifts at (δ) 165.75, 161.24, 151.52, 149.63, and 100.02. These values represent every C atom present in the molecule.

The characteristic chemical shift of the C atom assists in confirming the structure of calcium orotate. The molecule of calcium orotate contains C atoms bonded in five different ways.

The chemical shift values obtained in the spectrum indicate each of them. The chemical shift values and their corresponding C atom is shown in Table S2 and explained in fig S6.

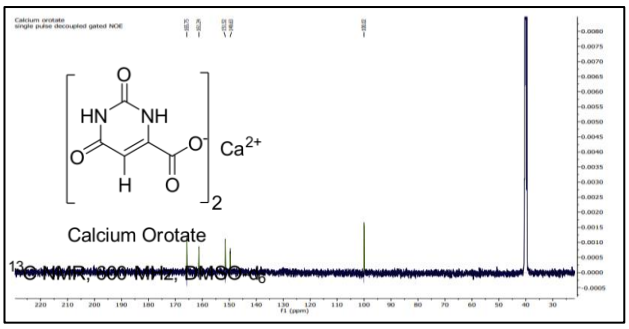


**Figure S5.** $^{13}$C NMR spectrum

**Table S2.** Interpretation of $^{13}$C NMR spectrum

| Sr. No. | Chemical shifts | Representative C (C*) atom |
|---|---|---|
| 1. | 165.75 | O-C*(=O)-C |
| 2. | 161.24 | N-C*(=O) - N |
| 3. | 151.52 | N-C*(=O)-C |
| 4. | 149.63 | N-C*(=O)-C |
| 5. | 100.02 | C-C*=C |

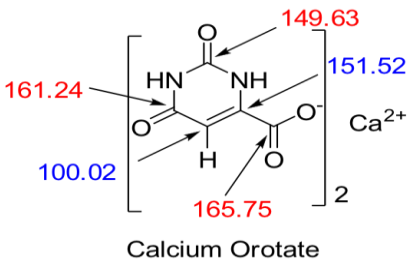


**Figure S6.** Interpretation of $^{13}$C NMR spectrum

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***Repeatability and scale-up studies***

The optimized process was repeated for synthesizing three batches of 400 gms each. These were analysed for description, identification test by IR spectrum, loss on drying, assay, calcium content and orotic acid content. The results of repeatability of optmized batch is shown in table S3.

**Table S3.** Results of repeatability of optimized batch

| Sr. No. | Test | Observation | | | Limits |
|---|---|---|---|---|---|
| | | SC/PHD/CO/R1 | SC/PHD/CO/R2 | SC/PHD/CO/R3 | |
| 1 | Batch size | 400 gms | 400 gms | 400 gms | |
| 2 | Description | Complies | Complies | Complies | White to almost white crystalline powder |
| 3 | Identification | Complies | Complies | Complies | a) IR spectra of the sample should be in compliance with that of standard |
| 4 | Loss on drying | 0.38% | 0.33% | 0.35% | Not more than 1.0% |
| 5 | Assay | 99.62% | 100.01% | 99.98% | Not less than 97.0 % and not more than 103.0 % |
| 6 | Calcium content | 10.34% | 9.89% | 10.78% | Not less than 09.5 % and not more than 11.5 % on dried basis |
| 7 | Quantification of orotic acid | 90.31% | 90.12% | 89.87% | Not less than 88.5 % and not more than 90.5% on dried basis |

This process was also scaled up to 10x (4kg) which was validated for its repeatability parameter and tested completely as per specifications mentioned above. The results showed that all three batches of scale up size were consistent with test results. These results concluded that the calcium orotate which is synthesized is of the desired specifications. The results are shown in table S4. Since the process synthesis is one pot method, only a larger vessel size was used for scale up studies and no other parameters were changed.

**Table S4.** Test results for scale up studies for calcium orotate

| Sr. No. | Test | | | | Limits |
|---|---|---|---|---|---|
| | | SC/PHD/CO/S1 | SC/PHD/CO/S2 | SC/PHD/CO/S3 | |
| 1 | Batch size | 4 kgs | 4 kgs | 4 kgs | |
| 2 | Description | Complies | Complies | Complies | White to almost white crystalline powder |
| 4 | Identification | Complies | Complies | Complies | a)IR spectra of the sample should be in compliance with that of standard |
| 5 | Loss on drying | 0.34% | 0.33% | 0.32% | Not more than 1.0% |

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| | | | | | |
|---|---|---|---|---|---|
| 10 | Assay | 98.99% | 99.21% | 99.38% | Not less than 97.0 % and not more than 103.0 % |
| 11 | Calcium content | 10.98% | 10.96% | 10.84% | Not less than 09.5 % and not more than 11.5 % on dried basis |
| 12 | Quantification of orotic acid | 89.25% | 89.81% | 89.77% | Not less than 88.5 % and not more than 90.5% on dried basis |

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