Novatia

# CoSMoS Method Development Olympics

Averica Discovery Services and Novatia LLC

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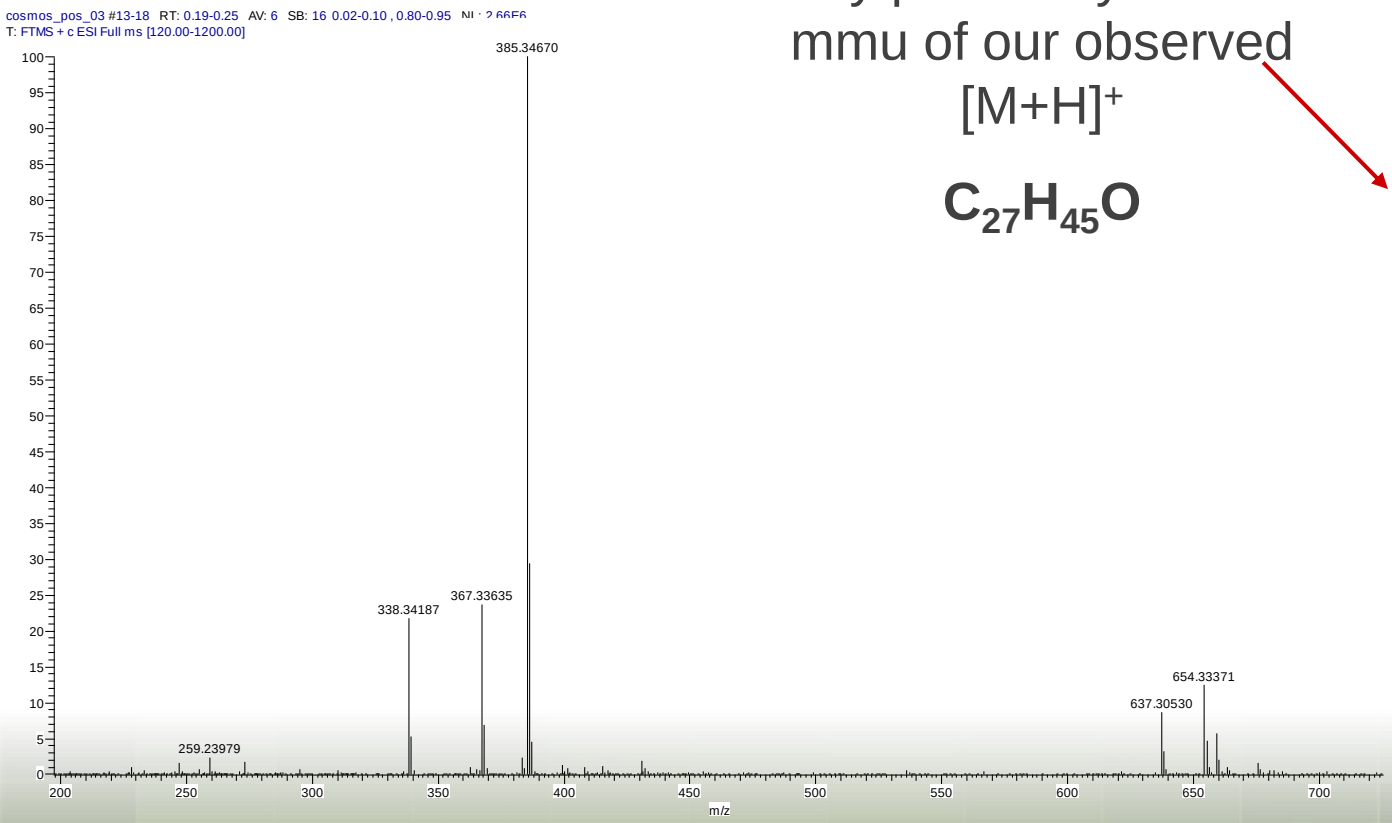
# Plan of Attack

- Do as little as possible
- Assess drug substance chromatographically
- Identify drug substance
- Develop sample specific chromatographic method
- Develop extraction protocol
- Assay the pills

# Initial Screen

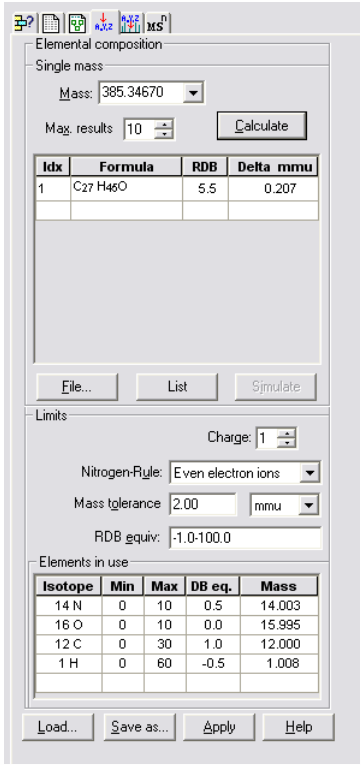
- Screened by SFC and LC by standard screening protocols, multiple columns, solvents, and pH's
- LC/MS screen: 385 amu = $[M+H]^+$, 367 amu = $[-H_2O]$
- What was learned?
  - **Neutral molecule, lipophilic, contains labile hydroxyl group, chromatographs well by both SFC and HPLC, and shows only one peak**

# Accurate Mass and Molecular Formulae

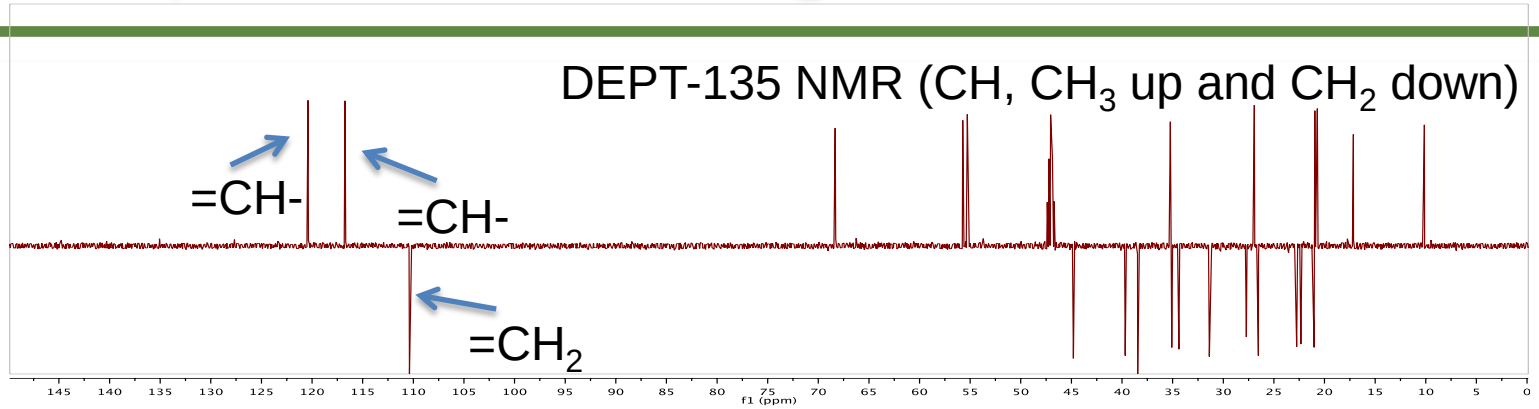


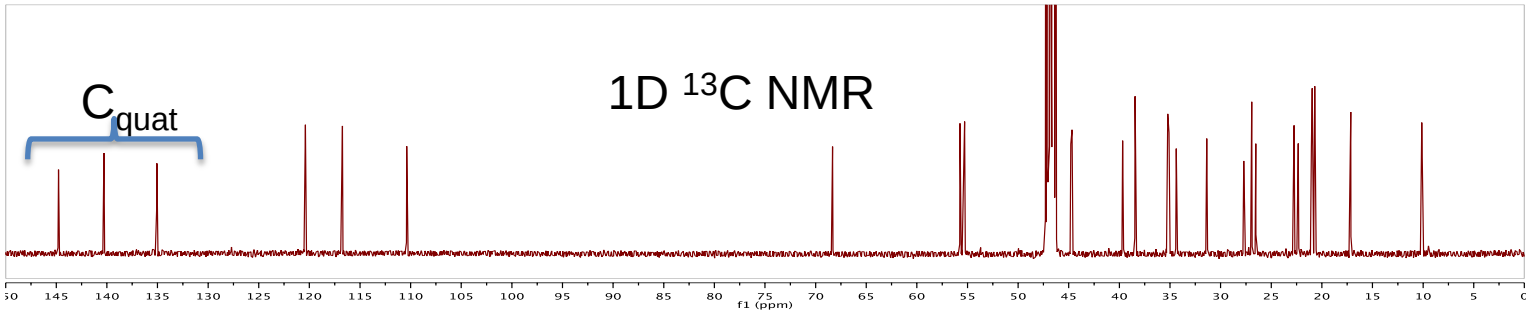

Only possibility within 2 mmu of our observed $[M+H]^+$

$C_{27}H_{45}O$



# 1D $^{13}C$ and DEPT-135 NMR Spectra of Drug Substance



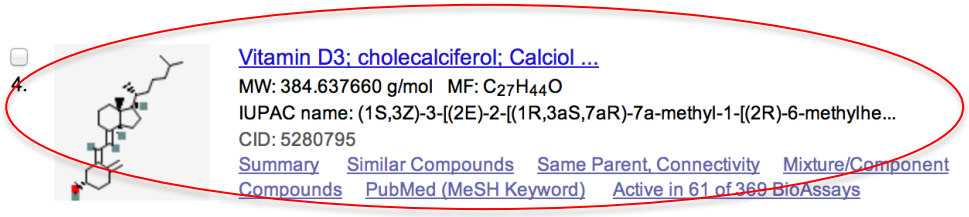

## 1D $^{13}C$ and DEPT-135 NMR Data revealed

1. *27 Carbon (44 Protons from H1 data)*
2. *4 Methyls*
3. *3 Double Bonds*
4. *A terminal olefinic CH2*

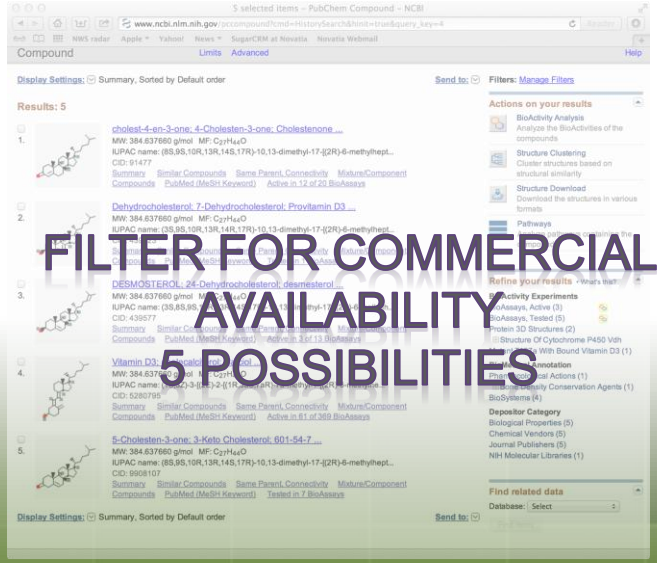
# PubChem Database Search

MS Formula = $C_{27}H_{44}O$

INITIAL PUBCHEM SEARCH
593 POSSIBILITIES




FILTER BY NMR FUNCTIONALITIES
1 POSSIBILITY




FILTER FOR COMMERCIAL AVAILABILITY
5 POSSIBILITIES

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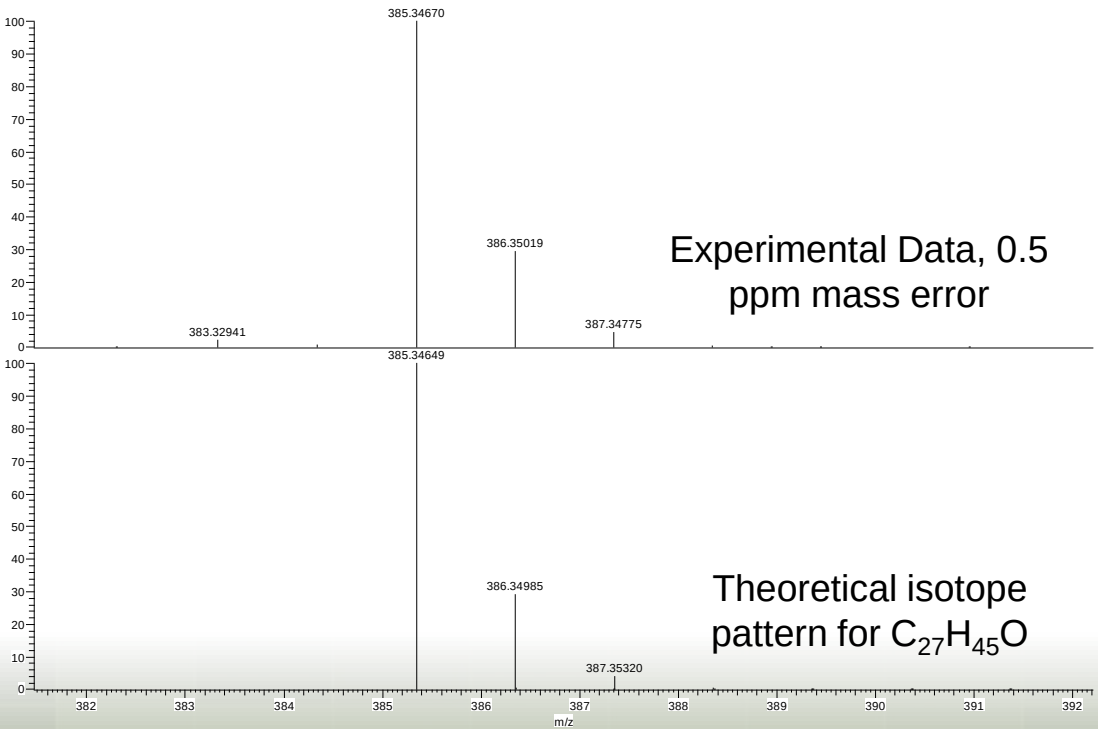
# Rigorous MS and NMR Characterization

## Experimental vs Theoretical MS Comparison

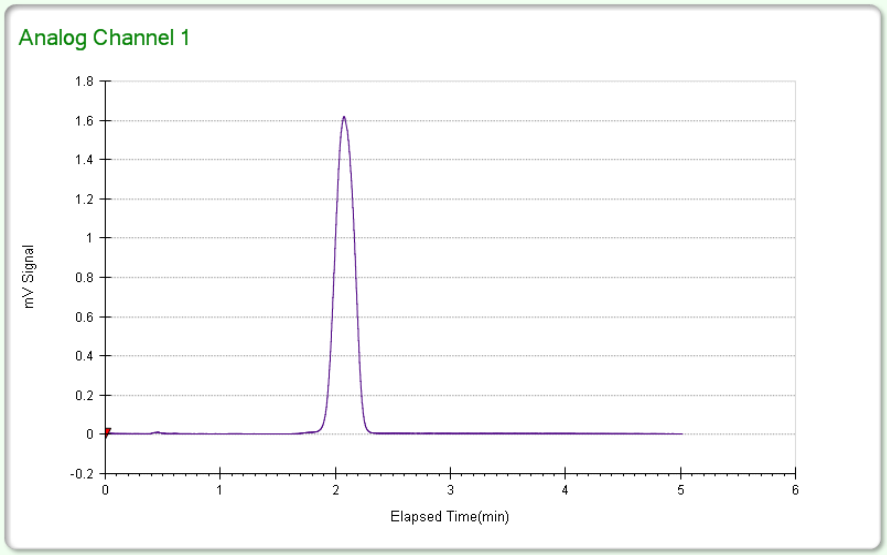



## Full NMR Chemical Shift Assignments

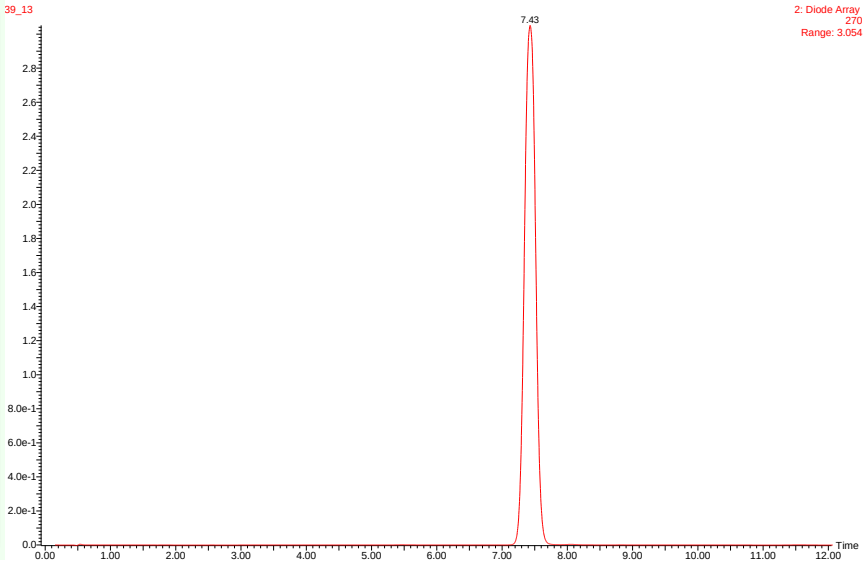
| Position | 1H | 13C |
|---|---|---|
| 1 | 2.41,2.11 | 33.8 |
| 2 | 1.97,1.54 | 36.8 |
| 3 | 3.76 | 70.7 |
| 4 | 2.54,2.19 | 47.2 |
| 5 | - | 137.5 |
| 6 | 6.22 | 122.8 |
| 7 | 6.03 | 119.1 |
| 8 | - | 142.7 |
| 9 | 2.86,1.69 | 30.1 |
| 10 | - | 147.2 |
| 11 | 1.67,1.55 | 24.7 |
| 12 | 2.03,1.33 | 42.1 |
| 13 | - | 47.1 |
| 14 | 2.01 | 57.7 |
| 15 | 1.48 | 23.4 |
| 16 | 1.91,1.30 | 28.9 |
| 17 | 1.30 | 58.1 |
| 18 | 0.56 | 12.5 |
| 19 | 5.04,4.75 | 112.8 |
| 20 | 1.39 | 37.6 |
| 21 | 0.95 | 19.5 |
| 22 | 1.40,1.04 | 37.5 |
| 23 | 1.39,1.19 | 25.1 |
| 24 | 1.15 | 40.8 |
| 25 | 1.54 | 29.3 |
| 26 | 0.88 | 23.3 |
| 27 | 0.88 | 23.1 |

# Identity Verification

SFC, 5% MeOH, 5 min



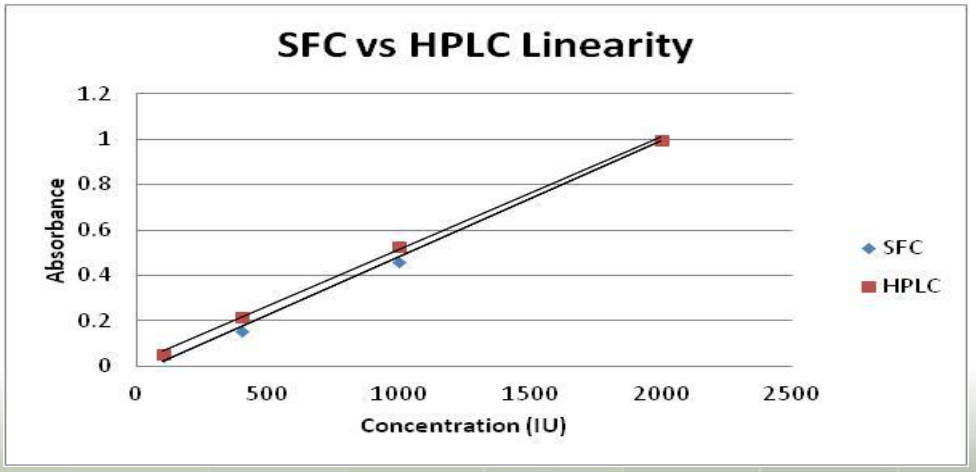

RP-HPLC, gradient 80-100% ACN, 12 min




- Co-injection of standard with commercially available Vitamin $D_3$ by SFC and LC methods

# Chromatographic Methods Development

- SFC was chosen
  - SFC chosen due to rapid elution and solvent compatibility
  - SFC method was developed to resolve compounds with similar structure such as 7-dehydrocholesterol (biological precursor) and vitamin $D_2$
  - SFC and RP-LC results were comparable with respect to linearity
    - SFC $R^2$= 0.998
    - HPLC $R^2$ = 0.999



# Extraction Protocol

- USP method available
  - Compedial method too complex
- Literature search provided more practical protocol*
- Our simplified method
  - Pills dissolved in dilute phosphoric acid/ethanol followed by hexane extraction, drying of extract, re-dissolution in methanol

*Klaczkow et al., Simultaneous Determination of Vitamins A, D3 and E in Multiple Pharmaceutical Preparations by HPLC Method. Acta Pharmaceutica-Drug Research, Vol. 57, No. 3, pp-167-170.

# Quantitation

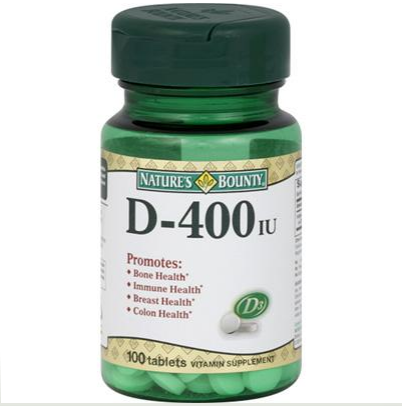
- Assay of supplied drug substance 94.3% potent
- Quantitated against commercially available Vitamin $D_3$ due to subpotent drug substance
- Put standard through extraction protocol used to develop standard curve.
  - Curve: 100-2000 IU (knowing Vitamin to be either 400 or 1000 IU)

# Results

- Assay (average from 5 tablets) result 370 IU 10
- Conclusion: 400 IU tablets

# Extraction Protocol Test

- Purchased 400 IU Vitamin $D_3$ to test extraction protocol
- Our results pointed to 1000 IU pills
- Conclusion: Mislabeled bottle, free crate of Vitamin $D_3$!



# Acknowledgements

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