

Cap and CASE – Chasing Structures in the Drug Delivery Process

Ruth Boetzel^a, Anna Codina^a & Katherine Mackay^{a,b}

^a Analytical Research & Development, Pfizer PGRD, Sandwich, Kent, UK

^b School of Engineering & Physical Sciences, Heriot-Watt University, Edinburgh, UK

Introduction

Identifying impurities down to a low level and characterising them as fully as possible is a critical part of the drug development process. This often involves working with amounts in the µg range, a challenge for complete structure elucidation by NMR.

A typical characterisation of an impurity consists of the following steps:

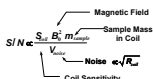
- LC-MS (accurate mass)
- LC-MS-MS (molecular formulae)
- NMR (1D & 2D experiments)
- Manual or computer-assisted (CASE) structure elucidation [1]

Our goals are two-fold:

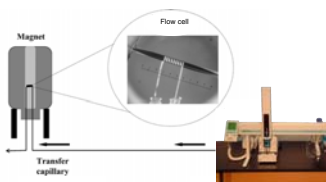
- To speed up routine structure verification processes
- To improve structure elucidation for 'tricky' molecules and small amounts of material

Here we report combining capillary (cap) NMR [2] and CASE using ACD-Labs™ Structure Elucidator to determine the structures of 'tricky' molecules with a limited number of protons.

Capillary (cap) NMR

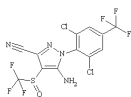


- Increase in sensitivity by using a microcoil [3] which has a smaller diameter and is closer to the sample
- Higher mass sensitivity for mass-limited, highly soluble samples
- Less solvent necessary (5-10 µl)



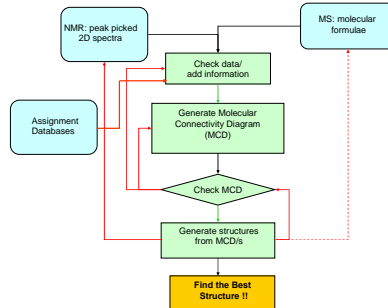
[Adapted from A. Webb, *Anal. Bioanal. Chem.*, **388**, 525-528 (2007)]

Fipronil™ & Arylpyrazoles



- Insecticide developed by Rhône-Poulenc
- On the market since 1993
- Member of the arylpyrazole family
- Arylpyrazoles: similar antiparasitic properties
- Extremely challenging for NMR due to lack of protons and connectivities

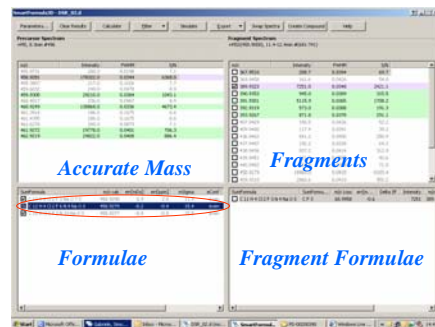
Structure Elucidation Process



MS – SmartFormula3D™

- Accurate mass = '1D' = many formulae
- Isotope patterns = '2D' = reduces number of formulae
- MSMS = '3D' = further reduction in number of formulae (often down to one)

Example: Fipronil™ (C₁₂H₅Cl₂F₆N₄OS) (Bruker MicroTOF + Bruker SmartFormula3D™)



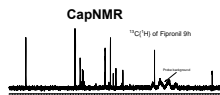
Result:

- Correct formula is No. 2
- No. 1 formula can be excluded since two CF₃ groups have been identified in the ¹³C and ¹⁹F NMR spectra

NMR

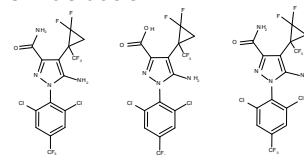
Fipronil:

- Not an 'ideal NMR molecule'
- Only 2 C-H and 2 N-H protons
- Lack of connectivities
- C-F correlations have to be used
- Related arylpyrazoles have similar characteristics



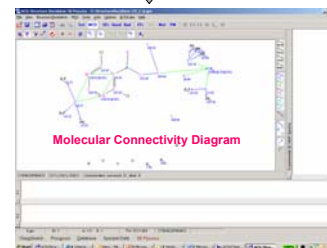
Can it be solved by CASE?

Structure Elucidation

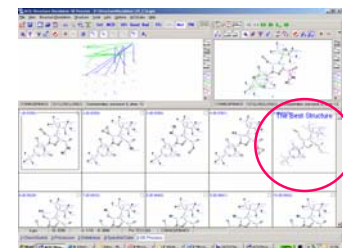


	C ₁₂ H ₅ O ₂ N ₄ F ₆ Cl ₂	C ₁₂ H ₅ O ₂ N ₄ F ₆ Cl ₂	C ₁₂ H ₅ O ₂ N ₄ F ₆ Cl ₂
Total Amount	12mg	530µg	<500µg
Ex. Data	¹³ C HSQC ¹³ C/ ¹⁵ N HMBC	¹³ C HSQC/HMBC	¹³ C HSQC/HMBC
User Data	¹⁵ N HSQC	¹⁵ N HSQC/HMBC ¹⁹ F HSQC	¹⁹ F HSQC
SE Protocol	•ACD fragments •N properties •'good list' & 5-membered ring	•ACD & arylpyrazole fragments •Ring information	•2 connectivities drawn •'good list': 3 fragments

ACD Structure Elucidator Connectivities



ACD Structure Elucidator Calculation



Conclusions

- Structures of 'tricky' molecules can be solved in Structure Elucidator.
- More user input is required if there are incomplete experimental data.
- A good database of related/similar compounds is essential.

References

1. M. E. Elyashberg, A. J. Williams & G.E. Martin. *Progr. NMR Spectr.*, **53**, 1-104 (2008).
2. F. C. Schroeder & M. Gronquist, *Angew. Chem. Int. Ed.*, **45**, 7122-7131 (2006).
3. A. Webb. *Anal. Bioanal. Chem.*, **388**, 525-528 (2007).

