How to Manage Metabolite Profiling Requests, the CRO Perspective

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Always good to think about the question

- How to manage metabolite profiling requests, the CRO perspective
  - Working with a CRO on metabolite profiling
  - Managing/enabling metabolite profiling within a CRO

Introduction

- Regulatory and scientific drivers
- Defining what we mean by “metabolite profiling and identification”

- Working with a CRO on metabolite profiling
  - Project planning
  - Scientific approaches

- How we manage metabolite profiling and ID within a CRO
  - Working with different clients
  - Scientific control and governance
  - Dealing with different reporting requirements
  - Managing project finance
  - Capital investment and staffing

Regulatory and scientific drivers

- Guidance and opinions
  - MIST, FDA, EMA, Industry papers

"Further information ….. should be made available to compare human and animal metabolic pathways" (European Medicines Agency)

"We encourage the identification of differences in drug metabolism between the animals used in nonclinical safety assessments and humans as early as possible during the drug development process" (FDA, 2008)

"…studies in humans using radiolabelled forms (RI) are required to determine the metabolite formation…" (Naito *et al, 2007*)

Early information regarding major metabolites and excretion patterns is essential for rational planning of studies, e.g. for special populations." (Sunzel, 2004)

The absolute mass (in excreta) or concentration (in circulation) of metabolites is important … for underwriting the safety of human study subjects with preclinical safety data. (Smith et al, 2007)

Regulatory and scientific drivers

Comparative metabolism

Man vs tox species
Inter species variation
Inter subject variation
In vivo vs *in vitro*

Toxicological concerns

Structural issues
Pharmacologically active metabolites
Dose dependant metabolism
Tissue accumulation

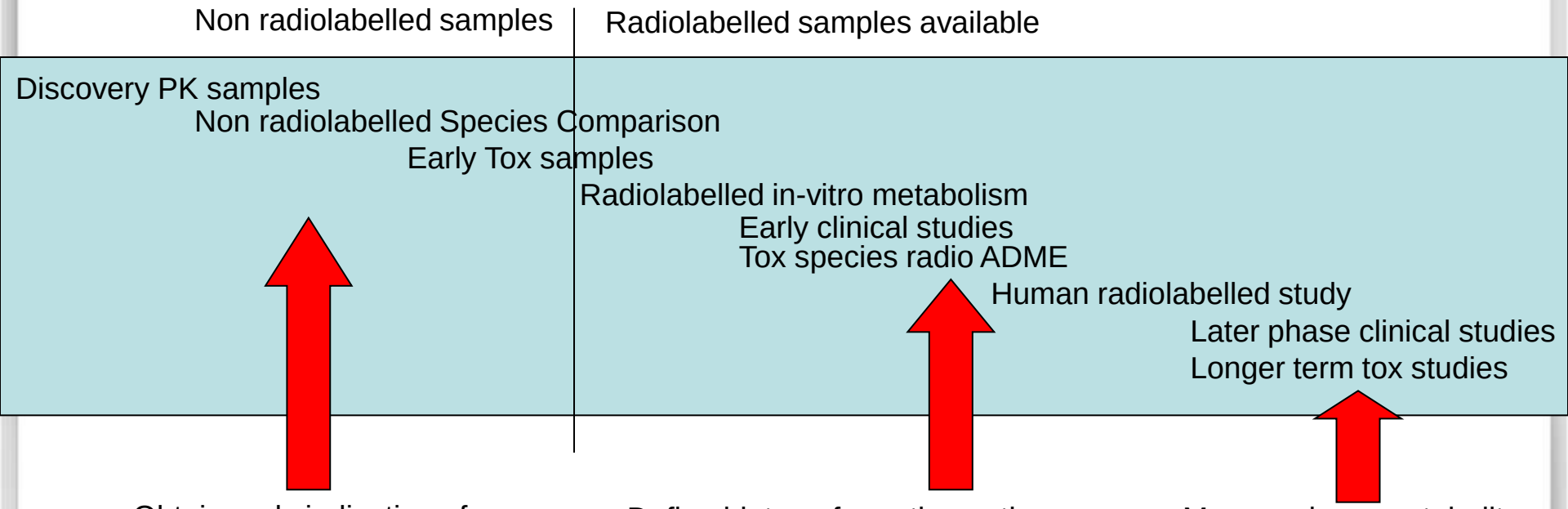
Feedback to Discovery

Structure modification
Improve PK
Reduce clearance

Human Safety

Understanding metabolites of concern
Reference standards can be available
Able to make valid measurements

Regulatory and scientific drivers



Obtain early indication of biotransformation pathways and metabolite quantities

Define biotransformation pathway and relative quantities of metabolites in toxicology species and man

Measure keys metabolites with validated methods and reference standards

Structural alerts

Active metabolites

Unique/disproportionate metabolites

Establish route to production of reference standard for metabolites of concern

Development and validate robust analytical methods for parent and key metabolites

What do we mean by ‘metabolite profiling?

- Clear definitions facilitate good communication and understanding

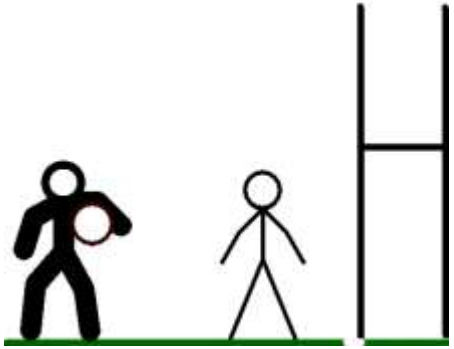
- What do we mean by “metabolite profiling”?
  - Qualitative non radiolabelled metabolite scouting
  - Semi quantitative non radiolabelled metabolite scouting
  - Profiling of radioactive components in samples from ADME studies
  - Can even be used to describe metabolomics

- Metabolite identification means full structural elucidation
  - Below the level of full structural elucidation we would talk about characterisation or assignment

Working with a CRO on metabolite profiling

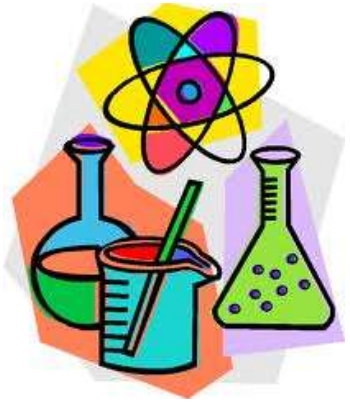
- Early planning
  - Plan metabolite profiling and ID as part of the project
  - Careful planning at the ‘live phase’ can facilitate a successful analytical phase

- Goals
  - Set clear goals
  - Set milestones
  - Avoid mission creep or be clear that the scope of the project is changing
  - Consider what is needed over what is interesting



Working with a CRO on metabolite profiling

- Make the most of your CRO partner's input
  - Variety of approaches
  - Skills obtained from working with different industry sectors
  - Be clear on your preferences or accept your CRO partner's preferred approach
- Methods
  - Be open to method development, CROs tend to be very good at this
  - Do the MS analysis up front

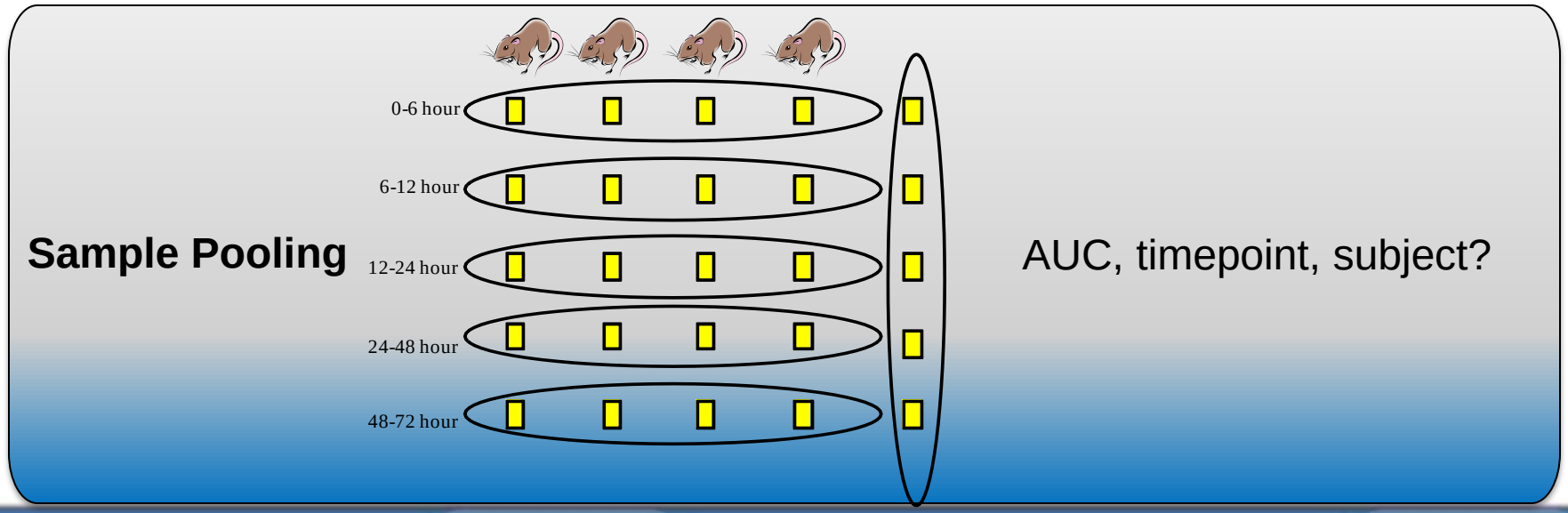


Planning in Advance for Metabolite Profiling and ID

- Metabolite profiling is often added in when samples become available
- If possible discuss profiling and identification before live phase begins and think about
  - Available sample volumes
  - Dose levels and vehicles
  - Sample pooling strategies – dictates the data you get
  - Sample preparation methods
- On radiolabelled studies
  - Position of radiolabel
  - Specific activity
  - LOD and LOQ

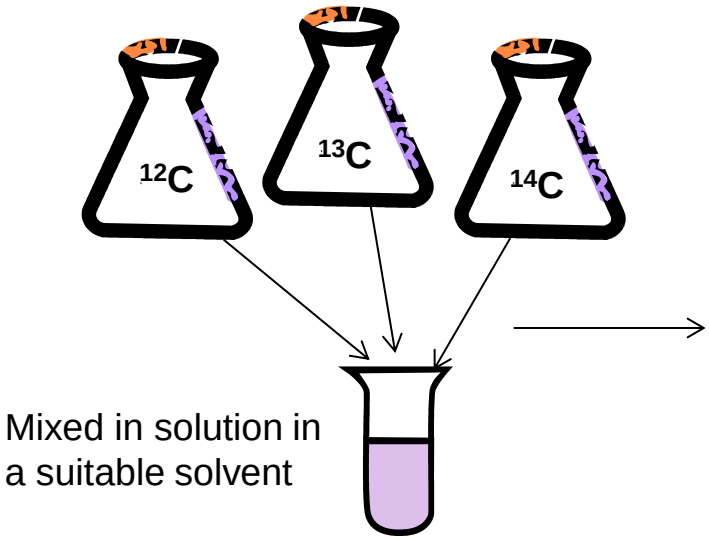
Pooling strategies

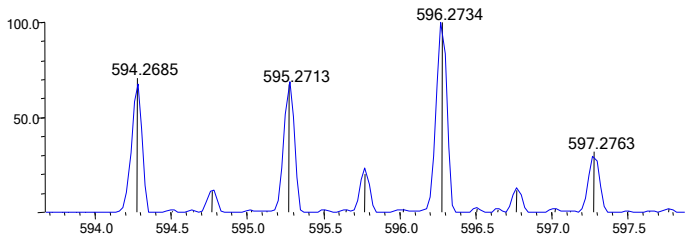
- Advantages of using pooled samples for metabolite profiling
  - Better sensitivity, samples can be concentrated
  - Reduces the number of samples



Specific activity

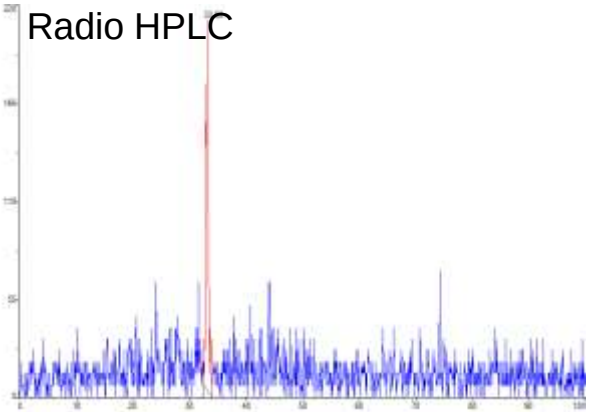
- Use specific activity to help with metabolite profiling
  - Higher spec ac = easier detection, better sensitivity
- Use specific activity to help with metabolite ID
  - Incorporate a diagnostic isotope pattern

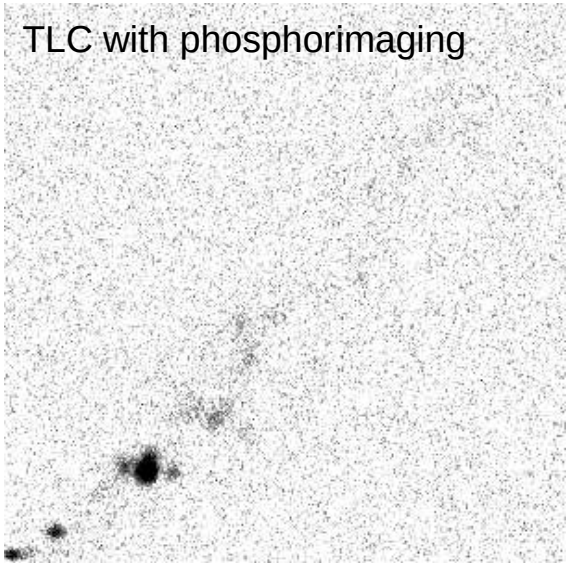




LOQ

- You know what you can see but what are you not seeing?
- Influence on measured concentrations





Separation	Detection	Limit of Sensitivity
HPLC/UPLC	On-line detection	200 dpm
HPLC/UPLC	Fraction collection and LSC	20 dpm
HPLC/UPLC	TopCount	10 dpm
TLC/2DTLC	Phosphorimaging	<10 dpm

Metabolite Quantification

- Radiolabelled metabolite quantification
- Clients expectations can differ from the standard procedures used at the CROs
  - Normalisation or not for extraction and procedural recovery?
  - % Region of interest or % total radioactivity for profiling data
- Should we validate radio-profiling methods?
  - Stability during extraction and profiling
  - Robustness of chromatography methods
  - Usually based on parent
  - Some clients may consider it an unnecessary additional cost
- Non-radiolabelled metabolites?

Metabolite Scouting

How do we get approximate quantities of metabolites without radiolabelling or a reference standard?

MS signal?

Variable ionisation potential prevents comparison between metabolites

What about comparing a single metabolite across time or between sexes/dose levels/species?

Yes – but what about matrix effects?

Variable matrix effects prevents comparison across matrices or between species

How do we control / eliminate matrix effects?

Experiments must be appropriately designed with suitable controls

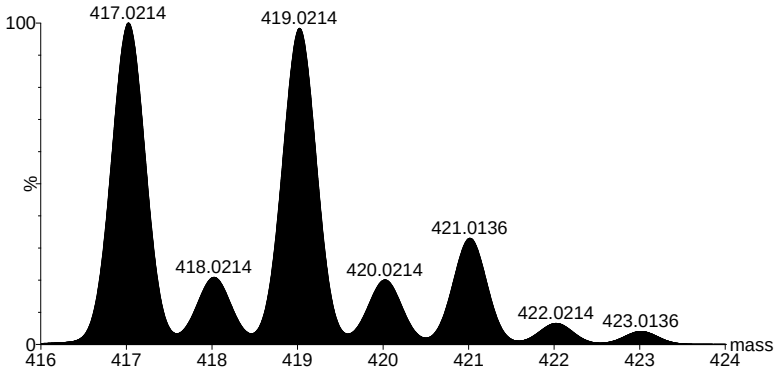
Metabolite Identification

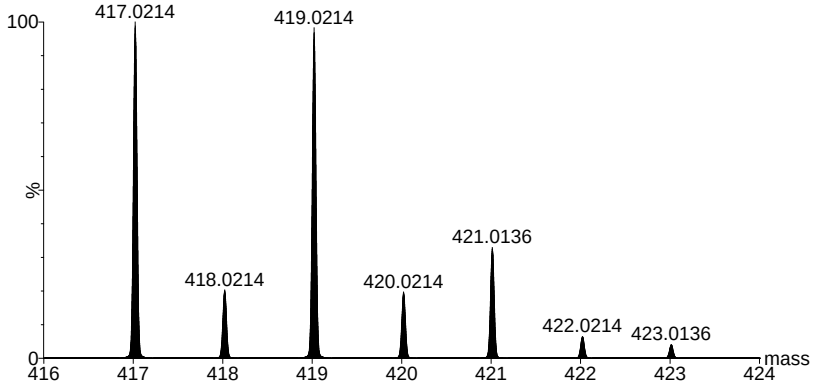
- A tool box of techniques are available to help us with structure elucidation
  - Extraction characteristics (particularly with pH changes)
  - Chromatographic characteristics
  - Co-chromatography with authentic reference standards
  - **Mass spectrometry** (can be applied in many different ways)
  - Enzyme hydrolysis for conjugated metabolites
  - DAD (sulphate conjugate)
  - Spray reagents on TLC
  - Forced chemical change
    - Derivatisation
    - Hydrolysis
  - NMR

Key is to find the path of least resistance

Many MS Configurations are Available

QqQ	Ion trap	IT-TOF	Synapt G2S
MS/MS	MS^n	MS^n	MS^e Ion Mobility
100 ppm	100 ppm	5 ppm	1-2 ppm
Res.: 2000	Res.: 2000	Res.: 7500	Res.: 40,000





MS Systems can be used in a variety of ways

Accurate mass

Isotope Matching

Neutral Loss

SIM

Fragmentation

Ion Mobility

Mass Defect

MRM

MS^n

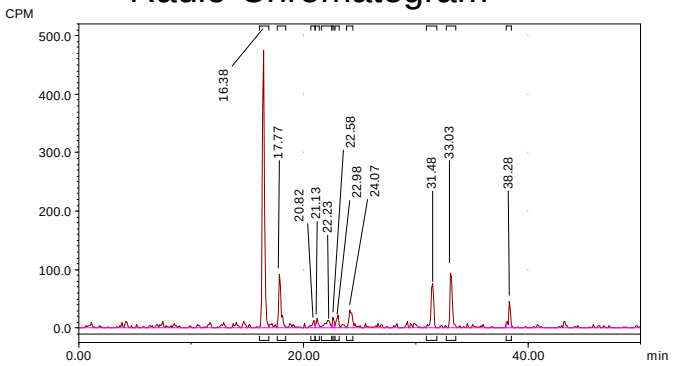
Project goals and chemistry should dictate the approaches taken

Reporting Requirements

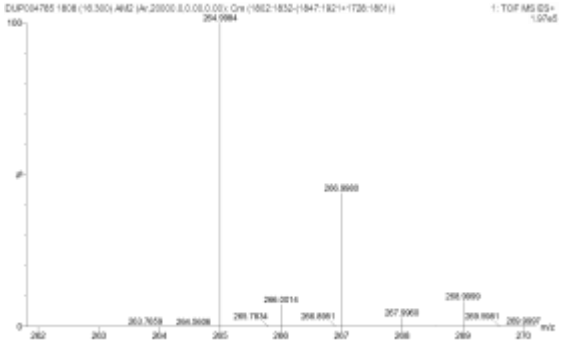
- Content
  - Metabolite quantification data and discussion
  - Include all data that supports an assignment
- Format
  - Regulatory agencies have specific reporting requirements
  - Clients may have their own format preferences
  - Report specialists rather than scientific staff
- Active discussion by phone, web meeting etc

The Full “Narrative” in MS Data Reports

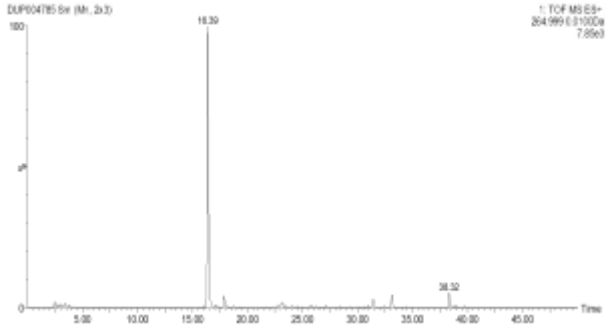
Radio-Chromatogram



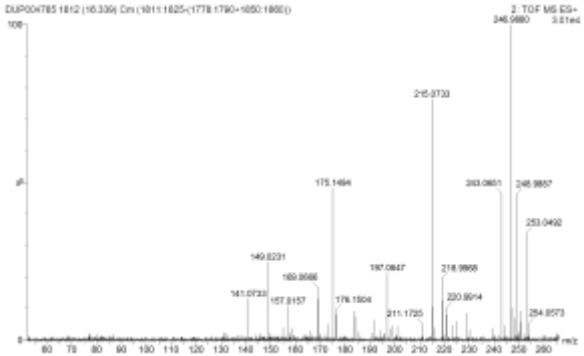
Full Scan



Extracted Ion Chromatogram



Daughter Ions



Delivering metabolite profiling and identification within the CRO

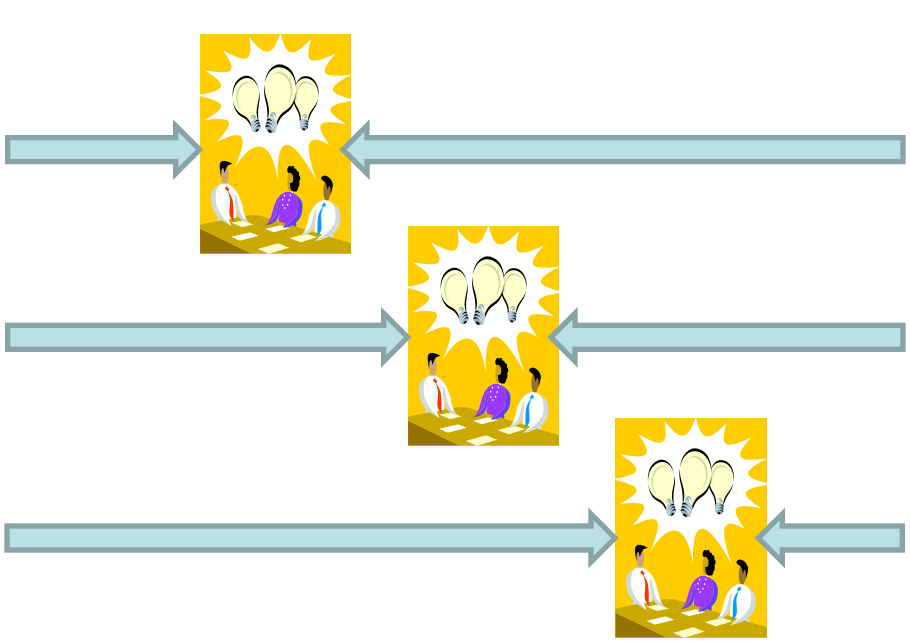
- Different clients
- Selection and training of staff
- Communication
- Project finance
- Capital Investment

Working with Different Clients

- Experienced biotransformation chemists
- Bioanalytical chemist or pharmacokineticist who has biotransformation as an additional responsibility
- Project manager or clinician who “just needs the Data”
- CRO staff need to be able to be flexible and work with different clients

Finding the Meeting Point

CRO



Experienced biotransformation chemist

BA chemist or pharmacokineticist

'Just needs data'

Staff Selection and Training

- Carefully selected and developed in house

- Biotransformation Chemist (Study Director)
  - Skilled in a range extraction and purification techniques
  - Skilled in a range of analytical techniques including method development
  - Able to interpret MS data
  - Understanding of biotransformation chemistry (able to predict metabolites)

- Mass Spectrometrist
  - Tend to recruit synthetic organic chemists and train them in mass spectrometry, biotransformation and analytical chemistry

- Both positions would require excellent interpersonal skills and good soft skills. These are looked at during interview and then built on during in house training

Non Technical Scientific Training Programme

QUALITY

COMMUNICATION

Communication

- CRO staff will be trained to communicate
- Establish a communication plan
  - When
  - How
- Establish project milestones
  - Preliminary profiles
  - Quantification using definitive profiles
  - Identification

Project Finance

- Biotransformation studies are very difficult to cost
- Clear expectations
- Different approaches can be used
  - Fixed pricing
  - Weekly charge
  - FTE rates
- All approaches demand a close relationship with the client company
  - Regular communication
  - Trust

Capital Investment

- Metabolite profiling and identification needs investments in equipment
- To keep pace with mass spectrometer technology requires planned capital expenditure every 4 to 6 years.
- Significant spend
- Validation, training time and costs also need to be considered

Summary

- The key to successful metabolite profiling and identification with a CRO partner lies in….
  - Early planning
  - Communication of required outcomes, results and any challenges
  - Experienced biotransformation scientists
  - Suitable methodology and instrumentation