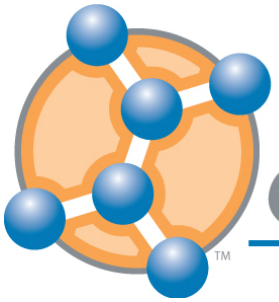

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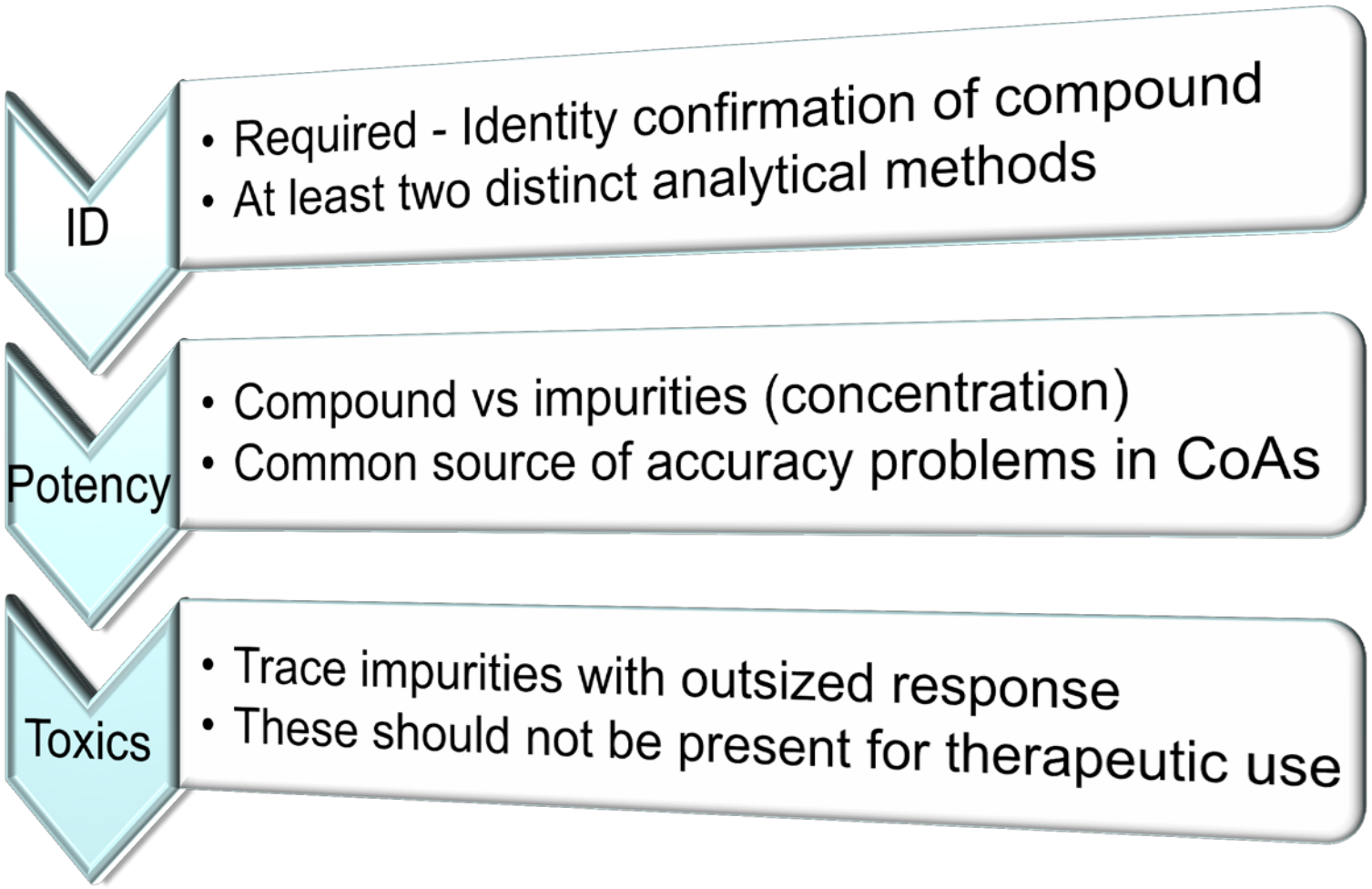
CoA of Reference Standards

Khalid Thakur, Ph.D.

Key Analysis Requirements for a CoA

- **Confirmation of Identity of compound (the WHO)**
  - This is an absolute must for all CoA
  - Need to confirm molecular weight, molecular structure, presence of one major component
  - Chiral compounds require additional analysis and reference data.
- **Determine Potency (the HOW MUCH)**
  - Accuracy of value depends on application of Ref Std compound
    - *Qualitative (± 10%, SIL) versus Quantitative (must be accurate)*
  - This is a common source of problems in a CoA
  - There is no analytical tool/technique or detector that has a uniform (mole% or weight%) response for everything that could be present!
- **Detection of trace toxic or biologics (the NOT TO BE PRESENT)**
  - These can have outsized biological response
  - Cannot be present in compounds for human or animal consumption
  - Could be trace metal ions/ bacteria/ virus/ parasite/ peptide/ organics
  - Not a major concern for “Analytical Ref Stds” (Chemtos Products)

Simplify CoA into three categories



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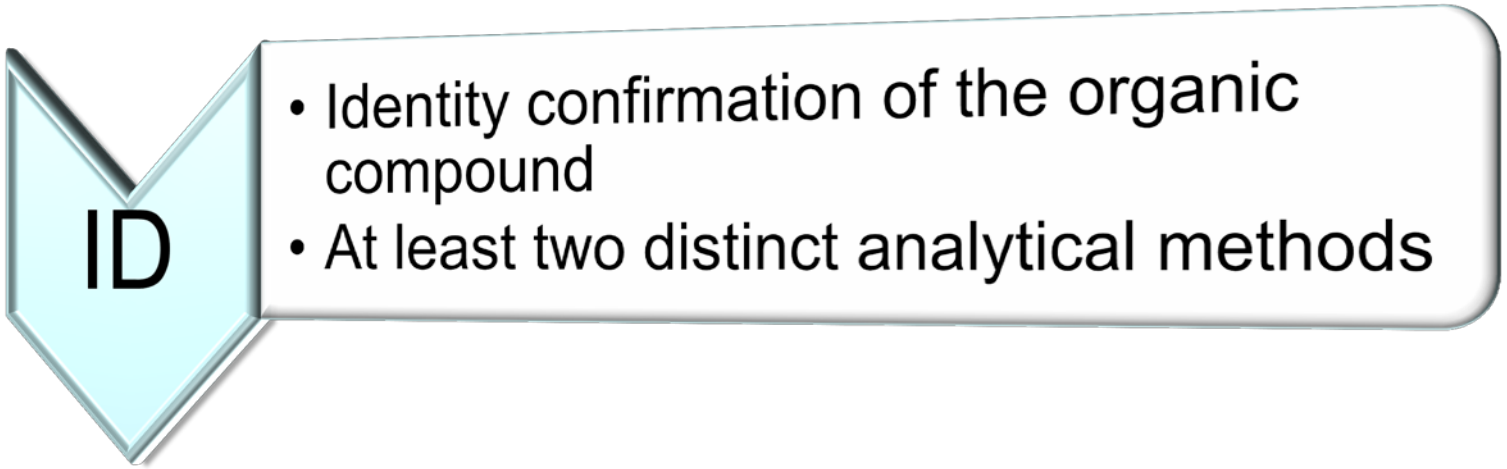
Minimum requirement for a CoA



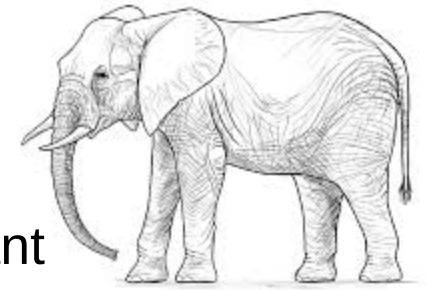
- LC-MS to confirm m/z of the compound
- Proton NMR to confirm molecular structure of compound
- FT-IR confirm if reference data is available for matching
- Chromatography (UV HPLC, GC, TLC) used to confirm presence of one major compound (this is relative % purity of those detected – this is not potency of cmpd)

Potency determination is prone to errors

Potency

- Compound vs impurities (concentration)
- Common source of accuracy problems in CoAs

- Different analytical tools for compound vs impurities
- Like detection of elephant size by separately detecting trunk, tail, legs, ears, body, etc
- qNMR provides accurate wt% potency
  - qNMR gaining acceptance and use!
  - This is like direct detection of size of elephant
  - Chemtos offers this analysis using ~5 mg of reference standard



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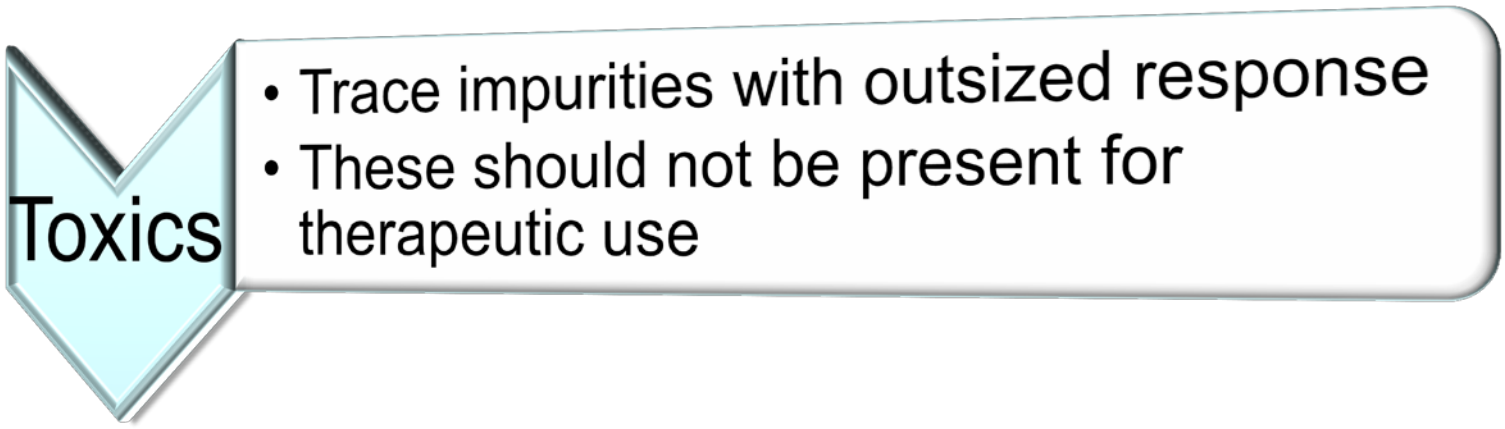
Toxics not be present in GMP products



- Trace toxics and biologics can have an outsized response masking or altering the response of the API
- These should not be present if administered or GMP
- Include trace metals, trace organics, bacteria, virus, etc
- Not as crucial for “Analytical Reference Standards” that are not for human consumption

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Key assumptions of Ref Std analyzed

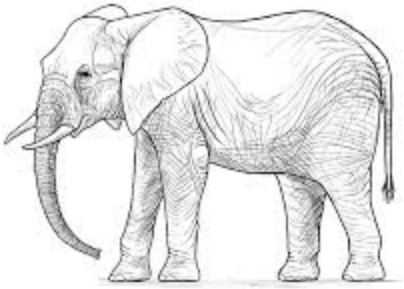
- Organic Compound is uniform and homogeneous
  - ***CoA usually does not analyze and report sample homogeneity***
- Has been vacuum dried to constant weight
  - ***residual solvents present are likely trapped within solid matrix***
- Could be an API, impurity, or metabolite
  - ***that may or may not prefer to be in crystal form***
- Could either be free base/free acid or be in some salt form
- Could be product of a small scale R&D synthesis or large scale controlled synthesis as for an API
- Final isolation could have been via:
  - ***Crystallization (true crystals, but could be hydrate or solvate)***
  - ***Solvent trituration (could be mixed crystals and be mixed hydrate or solvate)***
  - ***Chromatographic isolation and vacuum drying (??)***
  - ***Lyophillization (often amorphous)***
  - ***Distillation (often liquid)***

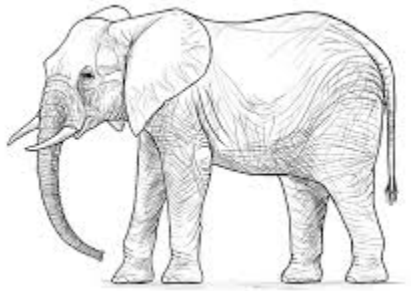
Identity Confirmation

- **Molecular weight is typically confirmed via m/z analysis**
  - in a Mass Spec detector, often via LC-MS
- **Molecular Structure is confirmed via NMR**
  - NMR can independently confirm molecular structure, without need for reference
  - Often used to determine molecular structure of unknowns, but that requires significant effort
- **Confirm presence of one major component**
  - via UV HPLC for polar and higher MW compounds
  - via GC for volatile and lower MW compounds

Potency Determination

- Unfortunately, there is no analytical tool that can detect everything that might be present, with each component having a separate/distinct and proportional weight% response
- Traditionally, (i) Organics, (ii) Inorganics, (iii) Salts, (iv) Residual solvents, each have to be separately assayed using distinct analytical tools and accounted for – some have mole% response but often used as wt% value
- It is like determining the size of the elephant by separately analyzing sizes of trunk, tusks, tail, ears, legs, etc.





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Purity Determination …

- ***Percent purity*** often determined using any combination of
  - UV HPLC (multi-wavelength DAD at Chemtos)
  - GC with a detector such as TCD, FID, MS, etc
  - Proton NMR
- **This is not the potency!** It is ***relative purity*** among those detected
  - Low errors as long as %purity level is high (>98%)
  - Detector response of impurities often different from detector response for the major component
- ***Residual solvents and hydrates/solvates***
  - KF titration for water of hydration and absorbed/adsorbed water
  - Headspace GC or proton NMR for organic residual solvents
  - Remember, organic solvents can also be locked as solvates in crystal structure
- ***Inorganics and counter ions***
  - Can be a detectable fraction of the solid and requires appropriate analysis such as residue on ignition, elemental analysis, ICP-MS, etc.

Potency Determination …

- Accurate potency value determination using such old-school / traditional methods require significant effort and remains prone to errors!
- The percent HPLC purity is used with weight fractions of all other impurities detected in order to estimate potency.
  - Orthogonality of each analysis is implicitly assumed
  - Everything present needs assay value as weight%
  - Value incorrect if even one impurity has been missed!
- Fortunately, there is an accurate alternative available
  - This is like directly detecting the size of elephant
- ***"Quantitative Proton NMR" or "qNMR" can directly detect the potency of the organic compound***
  - but it does not detect all the impurities that might be present.

Quantitative Proton NMR (qNMR)

- **qNMR** is a powerful tool when implemented correctly
  - Another reference standard of known purity, potency, and weight is added to a known weight of organic compound. This is thoroughly dissolved in an appropriate NMR solvent and analyzed by proton NMR. When done correctly, it yields very accurate potency values.
- Provides weight% values for each compound detected
- NMR Data acquisition requires adequate recycle times (magnetization recovery between pulses), narrow resonances (magnetic field and RF field homogeneity), good S/N ratio (longer data acquisition, good probes), clean baseline (good console and correct timings)
- NMR Data processing needs to done correctly – phased correctly, baseline corrections done correctly, integrations done correctly to account for ^{13}C satellites, etc.
- Very few facilities can do this as accurately as we can at Chemtos

Crystalline states

- Typical CoA does not analyze or report crystalline polymorphic state or amorphous state
- DSC or XRD would be an analytical tool often used
- Each polymorphic state is considered a distinct entity since the physical properties are often different for each polymorphic state of a compound
- An HCl salt is different from HBr salt of a compound and can have different crystal packing structure and different properties
- Similarly, various hydrates will have distinct crystal structures
- Presence of a mix of crystalline states creates sampling inhomogeneity, including during analysis for CoA
- True API will be isomorphic i.e. be of one crystalline state – and hence relatively easy to analyze for its CoA.
- But metabolites, impurities and SILs can be a mix of crystalline states, especially when %purity is lower

API production vs. Small scale synthesis

Large Scale API production

- Well defined high purity crystal structure (after significant effort in process optimization)
- High purity tightly packed crystals less prone to degradation – hence long shelf life
- Polymorphs have different physical properties, even though chemically identical
- Not available for metabolites or for compounds that may be unstable.

Small scale ref std synthesis

- Isolation/purification not optimized
- Impurities often present
- Mixed crystal structures possible
- More contact with atmosphere - hence higher probability of degradation/contamination
- Metabolites/impurities more prone to degradation

As you know, Polymorphs are separately patentable

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Re-certification interval?

- For well-defined pure API crystals, shelf life often expected to be >3 years
- Reference standards via small-scale synthesis methods may contain impurities/crystal imperfections, and hence are more prone to degradation/contamination
- Higher probability of contamination when storage container is frequently opened and temperature cycled from freezer to room temp
- At Chemtos, in absence of stability information or prior CoA, we list at least one year retest date for initial CoA
- If analytical values match prior CoA values for that batch, or compounds are known to be crystalline or stable, we typically list three year retest dates

Chemtos solution ampoule Ref Stds

- We are gradually adding reference standard products as dilute solution in flame sealed ampoules
- Potency is confirmed using at least 2x qNMR analysis
- The values are accurate and independently verified at molecular level
  - Not dependent on another ref std of same compound
- Being a solution, homogeneity is assured
- Stability studies conducted at 45 °C, RT, 4 °C, and -20 °C for at least four weeks
- Priced lower than competitors
- Includes numerous scheduled compound reference standards solutions as US DEA Exempt products

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Key Points on CoA for Ref Stds

- Typical CoA focusses on molecular structure purity of a compound, and does not address crystalline polymorphic state
- Compound used as qualitative Ref Std require less stringent/ fewer analysis – e.g. UV HPLC % purity, LC-MS m/z, and proton NMR structure confirmation often adequate for SIL compounds
- Accurate potency determination is challenging for compounds with lower % purity and for those that are in a mixed polymorphic state
- *Quantitative Proton NMR (qNMR)* is an effective analytical tool to accurately determine potency while using minimal quantity (<5 mg) for the analysis
- Traditional / Old school methods require analysis using multiple tools and larger amounts of compound for potency determination, while still remaining prone to errors
- Chemtos now offers solution ampoules ref stds with accurate potency, which are either unscheduled or US DEA exempt products