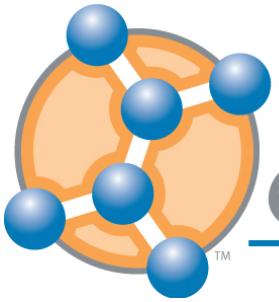

chemtos

CoA of Reference Standards

Khalid Thakur, Ph.D.

CHEMTOS

February 2020

Key assumptions of Ref Std analyzed

- Compound is uniform and homogeneous (*CoA does not analyze and report sample homogeneity*)
- Has been vacuum dried to constant weight
- 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, or solvent trituration, or vacuum drying, or lyphillization, or compound could be amorphous

Key Analysis Requirements for a CoA

- **Confirmation of Identity of compound**
  - 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 need reference data for validation.
- **Determine Potency**
  - Accuracy needed depends on application of Ref Std compound
  - 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**
  - These are needed prior to human or animal consumption
  - Could be trace metal ions or bacterial contaminations
  - Not required for Analytical reference standards (Chemtos products)

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 as a separate and proportional response
- Traditionally, (i) Organics, (ii) Inorganics, (iii) Salts, (iv) Residual solvents, each have to be separately assayed using distinct analytical tools and accounted for
- It is like determining the size of the elephant by separately analyzing sizes of trunk, tusks, tail, ears, legs, etc.

chemtos

Potency 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 solvents in crystalline structure
  - KF titration for water of hydration and absorbed/adsorbed water
  - Organic residual solvents by Headspace GC or proton NMR
  - Remember, organic solvents can also be locked in crystal structure
  - TGA works in some instances
- Salts and counter ions
  - Can be a significant fraction of the solid and requires appropriate analysis

Potency Determination …

- Accurate potency value determination using old school / traditional methods require significant effort and remain prone to errors
- Fortunately, there is an 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.
  - 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.
  - We have spent four years optimizing our processes

Quantitative Proton NMR (qNMR)

- **qNMR** is a powerful tool when implemented correctly
- 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

chemtos

Crystalline states

- Typical CoA does not analyze or report crystalline polymorphic state or amorphous state
- DSC or XRD would be an analytical tool 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.
- 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 – USP grade
- Polymorphs have different physical properties, even though chemically identical

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

chemtos

Re-certification interval?

- For well-defined pure API crystals, shelf life often expected to be >3 years
- Reference standards via small-scale synthesis methods often 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 one year retest date for initial CoA
- If analytical values match prior CoA values, or compounds are known to be crystalline or stable, we typically list three year retest dates

Chemtos solution ampoule Ref Stds

- We are slowly 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 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 DEA scheduled compound reference standards, most of them as DEA Exempt products

chemtos

Key Points on CoA for Ref Stds

- Typical CoA focusses on molecular structure purity of a compound, and does not address crystalline state
- Compound used as qualitative Ref Std require less stringent analysis – e.g. UV HPLC, LC-MS, and proton NMR often adequate for SIL compounds
- Accurate potency determination is challenging for compounds with lower % purity and for those not in a unique stable crystalline 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, most of which are US DEA exempt products

chemtos