SAMPL8 Preview

GCC 2020 and SAMPL Satellite Workshop

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Automated High Throughput pK_a and Distribution Coefficient Measurements of Pharmaceutical Compounds for SAMPL8 Blind Prediction Challenge

Overview - Goals

SAMPL8 Project

- **Aim #1: Develop automated approach for determining ionization constants (pK_a) on multiple small molecules**
- **Aim #2: Develop automated high throughput method for measuring distribution coefficients (logD)**
- **Aim #3: Measure logD for 20-30 compounds for a variety of solvent pairs**

Why?

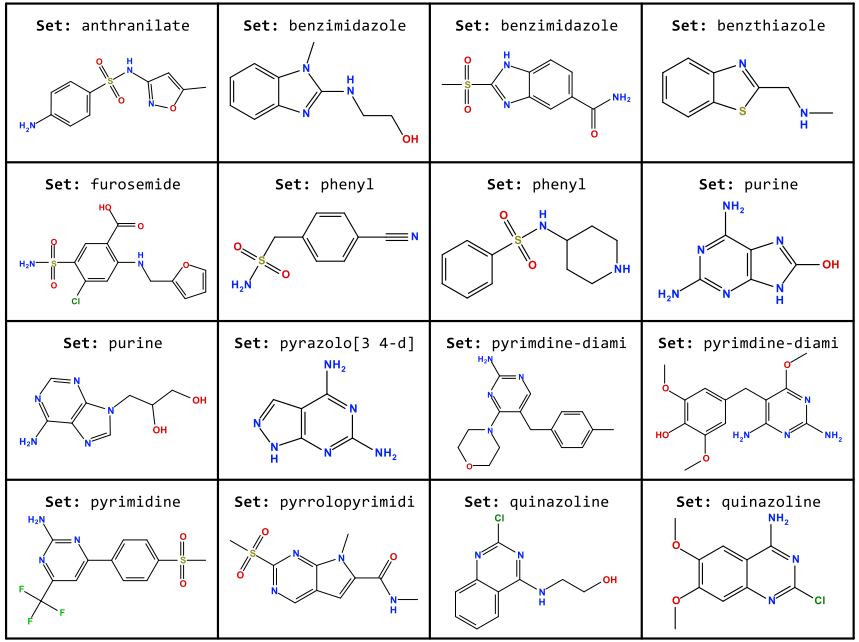
- Understanding the protonation state of a small molecule helps to build computational models for predicting how macromolecules will behave (https ://doi.org/10.1007/s1082 2-018-0168-0)
- Distribution coefficient values provide for computational models that predict protein-ligand binding affinities or hydration/solvation free energies (http://dx.doi.org/10.1101/757393)

88 Compounds Selected for Evaluation

Molecular weight ranges from 135 to 476 Dalton

Several Key Characteristics

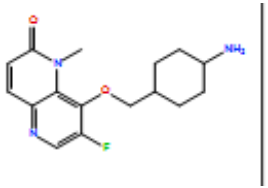
- **Two long range polar groups (separated by > 3 bonds)**
- **Molecules chosen for having a wide variety of scaffolds**
- **100 mg of available powder needed for full testing**



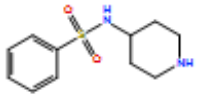
Example representative structures

Experimental pK_a Challenges

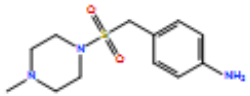
- **Not all of the selected compounds were successful in pKa determination…**
- **The team started with 88 small molecules, but succeeded in measuring 24… Why?**
  - **Some of the compounds had degraded over time, while in storage**
  - **Some of the compounds provided had insufficient amounts available for testing**
  - **Several of the compounds were semi-solids and could no be dispensed for testing**



Only able to determine intrinsic solubility which cannot be used to determine pK_a of compound



Low solubility. Did not dissolve in solution, hence a standard curve could not be generated



Standard HPLC method did not retain on the column, despite its ability to dissolve

Why Automate?

Current Manual Process

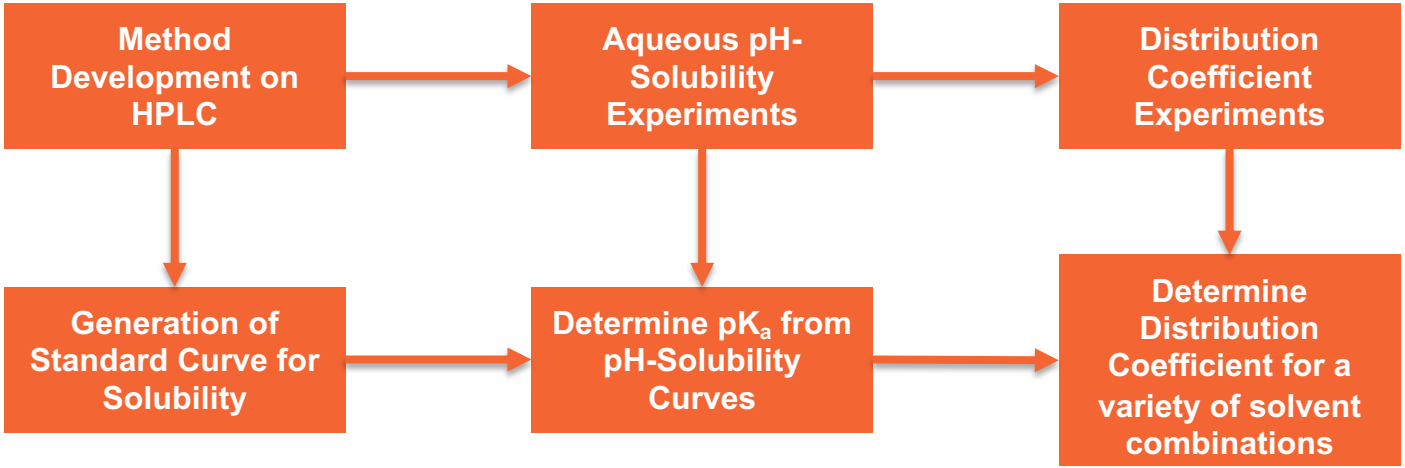
- Less data generated
- Only one or two reactions at one time
- Time consuming
- Data inaccuracy / human error
- Resource and material heavy

Automated Process

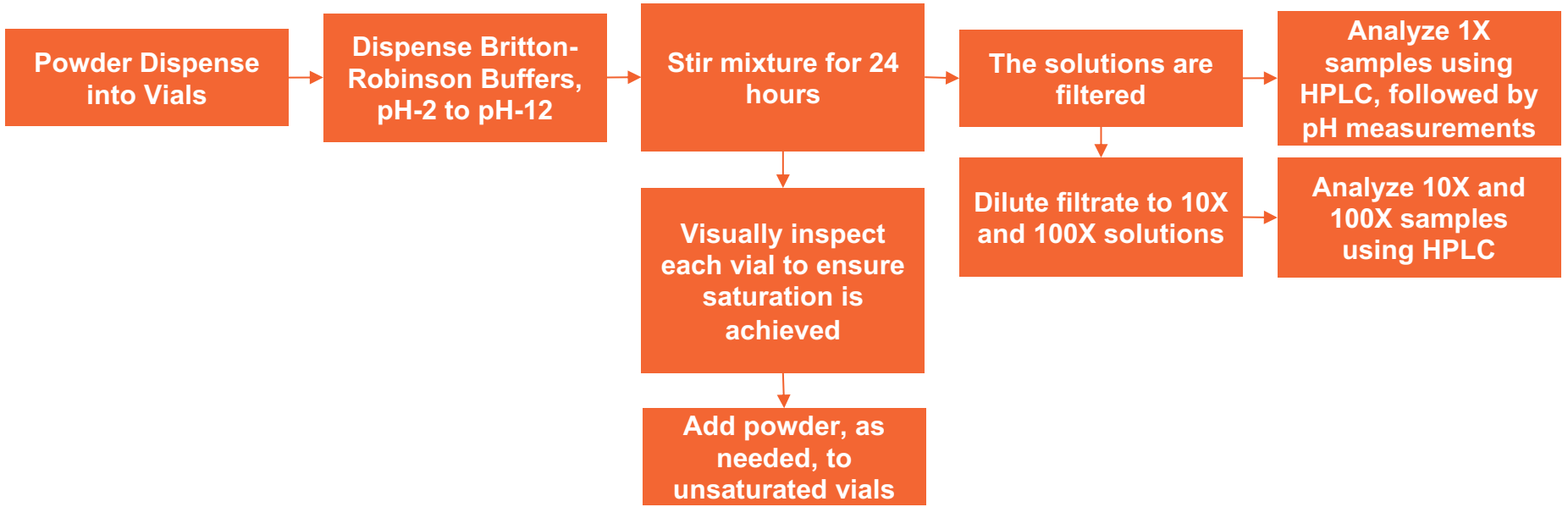
- Greater data generation
- Higher throughput
- Greater accuracy / precision
- Reduce resource required
- Combine multiple analytical techniques

Overview of Experimental Approach

General Overview of the High Throughput Process

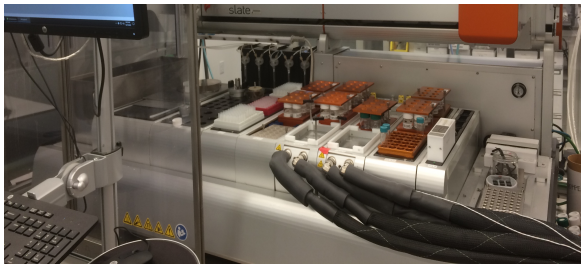


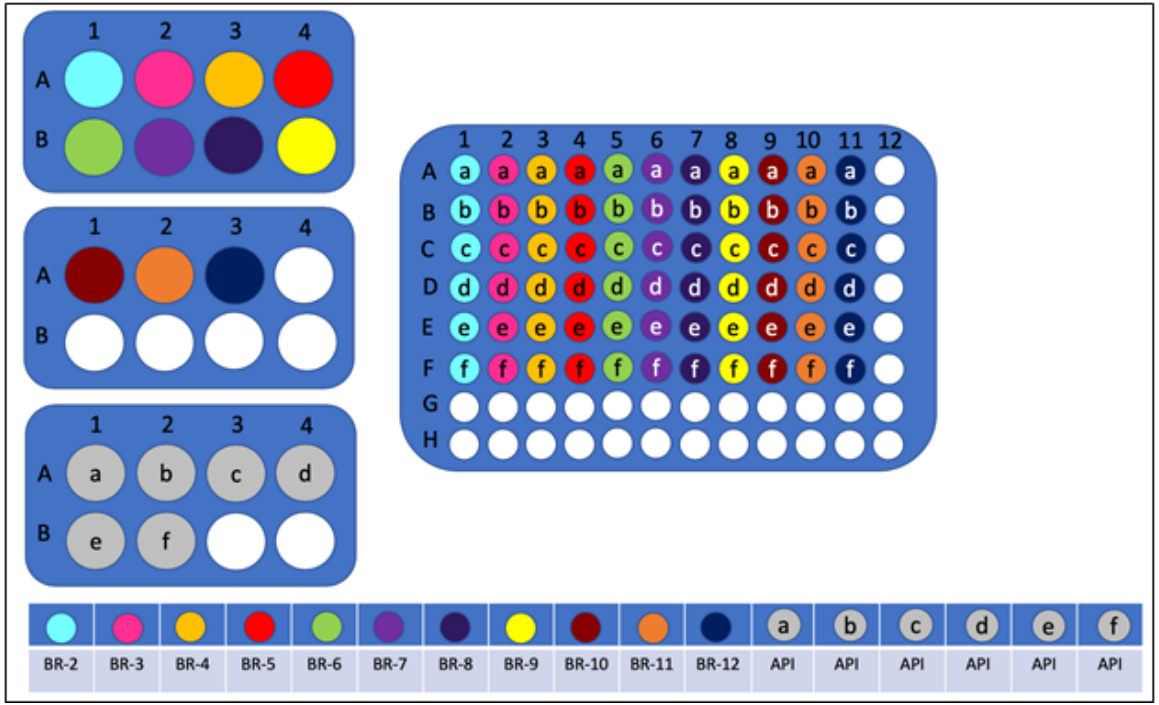
pKa Determination: Generation of pH Solubility Curve



pKa Determination: Generation of pH Solubility Curve

Using Freeslate (Unchained Labs) CM3, samples are prepared using several source and destination vials in a mapping scheme illustrated here





Curve Fitting to Determine pK_a

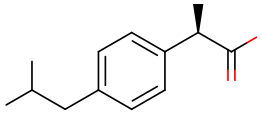
Henderson-Hasselbalch Equations

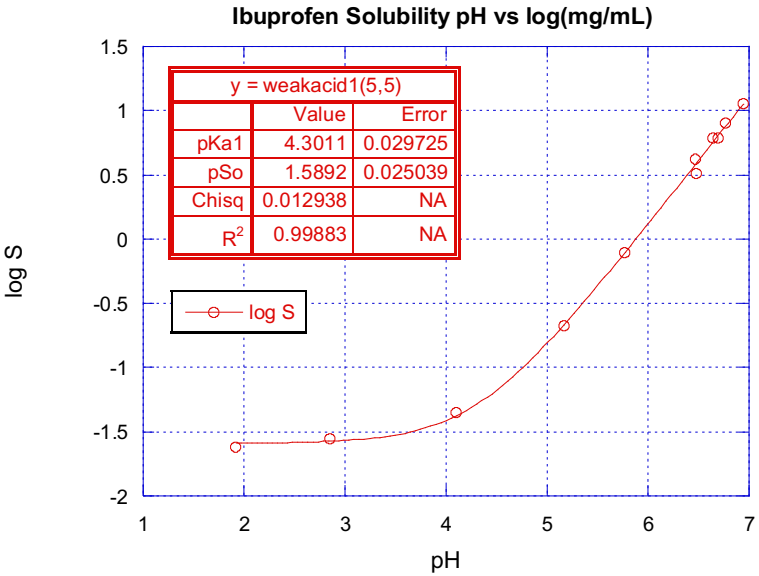
$$pH = pK_a + log_{10}\frac{[A^-]}{[HA]}$$

$$pH = pK_a + log_{10}\frac{S - S_0}{S_0} \quad \text{Weak mono-acids}$$

$$pH = pK_a + log_{10}\frac{S_0}{S - S_0} \quad \text{Weak mono-bases}$$

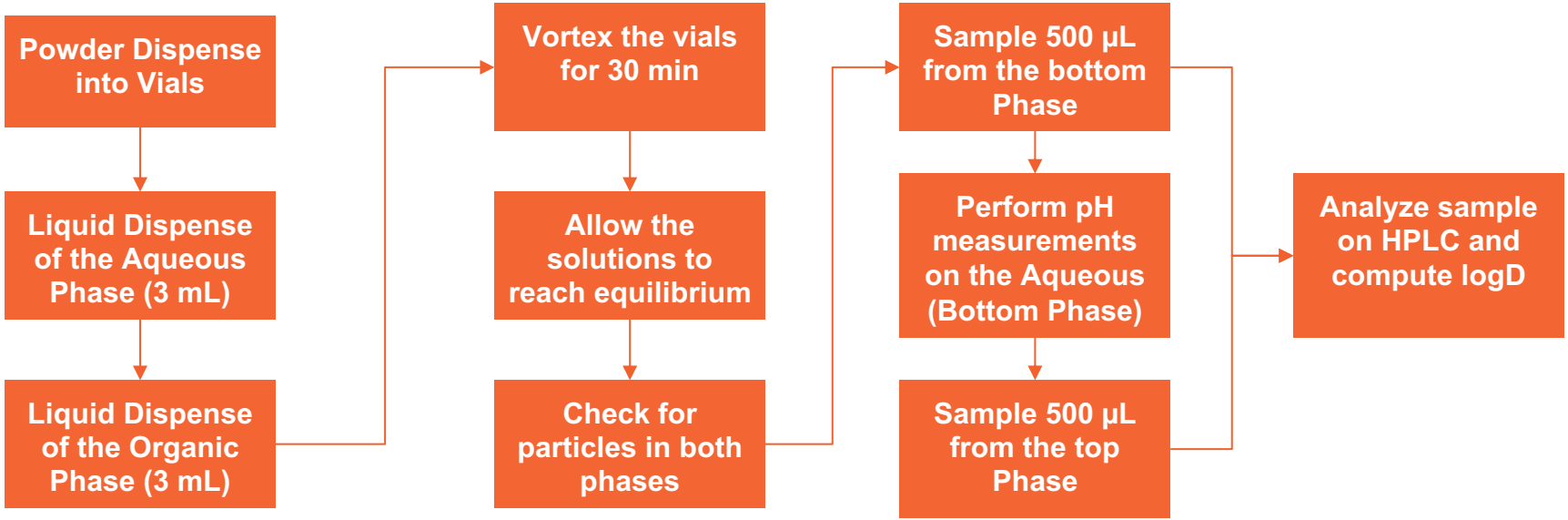
Where S_0 is the intrinsic solubility (equilibrium solubility) and the S is the total solubility





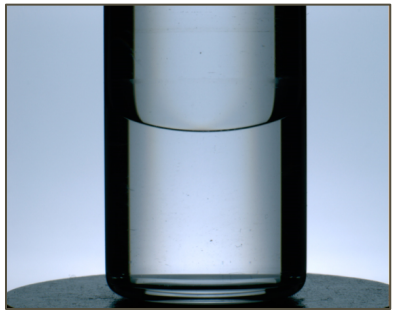
Overview of Experimental Approach

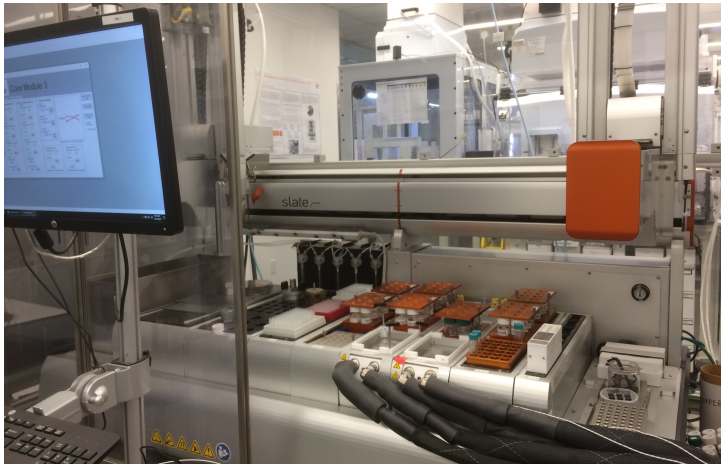
High Throughout Approach for Distribution Coefficient Measurements



Overview of Experimental Approach

High Throughout Method for Distribution Coefficient -Automated Platforms



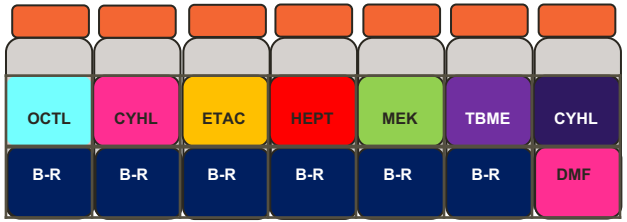


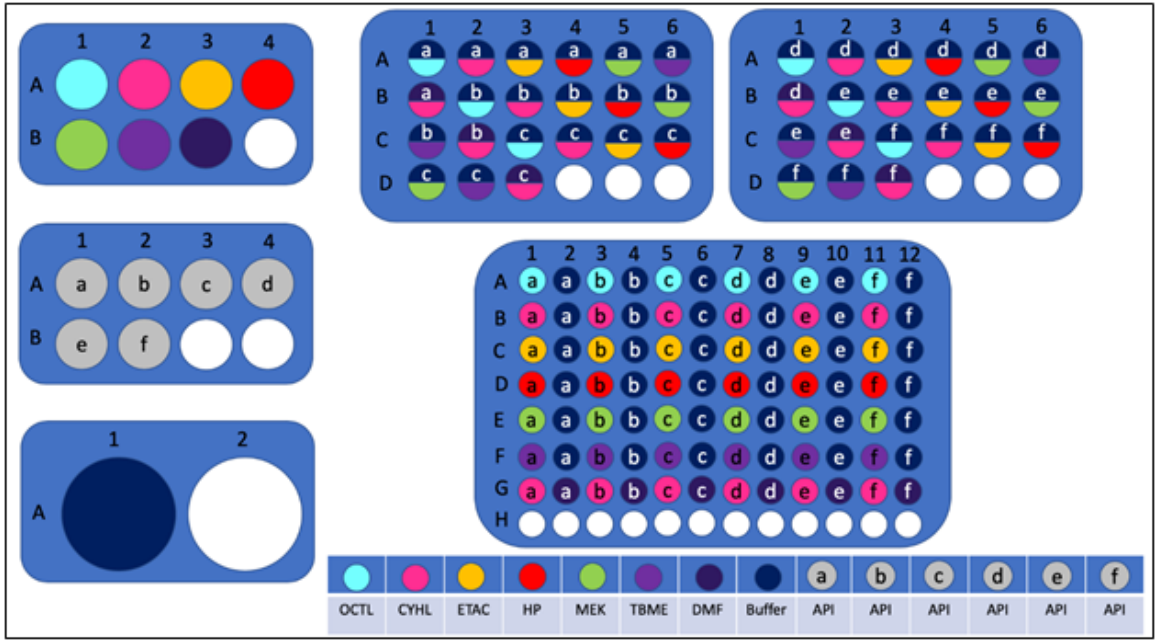
Experimental Sample Preparation

Overview of Experimental Approach

High Throughout Method for Distribution Coefficient Measurement

– Using Freeslate (Unchained Labs) CM3, samples are prepared using several source and destination vials in a mapping scheme illustrated here



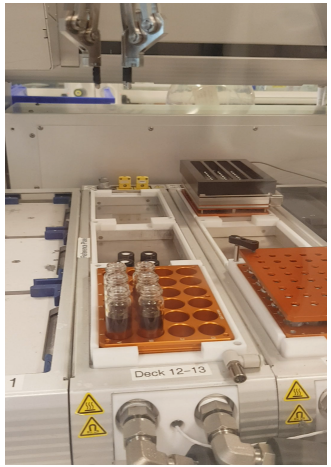


Workflow in Action

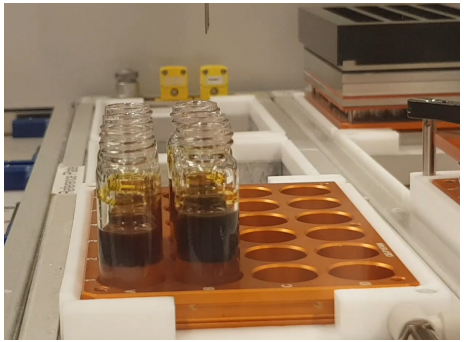
Density vials weighed

Samples transferred to pre-tared vials

Images taken of reaction vials



Density sample taken from reaction vials



Take-Home Messages

- Created a high-throughput approach to HPLC method development
- Developed an automated pH-solubility workflow for pKa determination
- Developed a fully automated distribution coefficient workflow
- Successfully sampled from organic and aqueous phases
- Increased amount of data generated in a short time

Acknowledgements

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