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In vitro **ADME & PK**

Turbidimetric Solubility

Background Information

'While thermodynamic solubility is useful when preparing a data package for a proposed development candidate, kinetic solubility seems most appropriate for high throughput *in vitro* assays in discovery.'

[1]Kerns EH and Di L. (2005) *Journal of the Association for Laboratory Automation* **10 (2)**; 114-123.

- Poor solubility can limit the quality of the data generated in other *in vitro* assays. Therefore, it is essential to evaluate solubility at an early stage in drug discovery.
- The solubility of a compound is an important factor in determining its absorption from the gastrointestinal tract and ultimately its oral bioavailability.
- Compounds with poor solubility can pose a development challenge and result in prolonged development time frames and increased cost.
- Turbidimetric solubility is now an accepted early stage screen in drug discovery. Cyprotex's Turbidimetric Solubility assay investigates the kinetic solubility of compounds by diluting a test compound solution prepared in DMSO into aqueous buffer. Turbidimetry is used as the end-point by measuring absorbance at 620 nm.
- Turbidimetric solubility allows a rapid determination of solubility using small amounts of compound.

Prediction of Human Intestinal Absorption

Cyprotex's Turbidimetric Solubility data can be used in conjunction with Cyprotex's Caco-2 Permeability data to predict dose dependent human intestinal absorption. Please refer to our Human Intestinal Absorption Model section for further details.

Protocol

Final Test Article Concentration
1 µM, 3 µM, 10 µM, 30 µM and 100 µM

Buffer
0.01 M phosphate buffered saline pH7.4 (alternatives available on request)

Final DMSO Concentration
1%

Number of Replicates
n = 7 per concentration

Incubation Time
2 hr

Incubation Temperature
37°C

Test Article Requirements
150 µL of a 10 mM DMSO solution

Analysis Method
Absorbance at 620 nm

Data Delivery
Estimated solubility range (lower and upper bound and calculated mid-range in µM).

To find out more contact **enquiries@cyprotex.com**

Cyprotex's Turbidimetric Solubility assay is performed at 37°C which is more relevant to the physiological situation and is comparable with conditions set in other *in vitro* biological assays.

Turbidimetric Solubility

12 Compounds were screened through Cyprotex's Turbidimetric Solubility assay in quadruplicate on 4 separate occasions. These data are highly reproducible for both poorly and highly soluble compounds.

Data generated in Cyprotex's Turbidimetric Solubility assay compare well with third party data.

Table 1

Comparison of Cyprotex's Turbidimetric Solubility with third party solubility data.

Compound	Cyprotex's Turbidimetric Solubility (µg/ml)	Blind Trial Solubility by LC-UV/MS (µg/ml)
1	0.5	<1
2	0.7	<1
3	0.8	<1
4	1.6	<1
5	5.6	2.2
6	26.5	89
7	30.8	43.9
8	31.8	9.3
9	>19.2	81
10	>26.2	48.8
11	>30.8	158.9
12	>33.3	113.6
13	>33.8	176.7
14	>41.2	200.3
15	>41.4	>41.4
16	>42.7	119.6
17	>44.2	222
18	>44.3	177.8
19	>45	237.7
20	>45.1	203.1
21	>46.4	>46.4
22	>47.1	166.4
23	>50	260
24	>52.1	279

In a blind trial, Cyprotex's solubility results for discovery compounds compared well to a customer's own solubility data. All compounds ranked in close agreement with regards to their solubility profile.

Figure 1

Mean solubility data generated by Cyprotex's Turbidimetric Solubility assay at pH7.4 (error bars represent the standard deviation of 4 replicates).

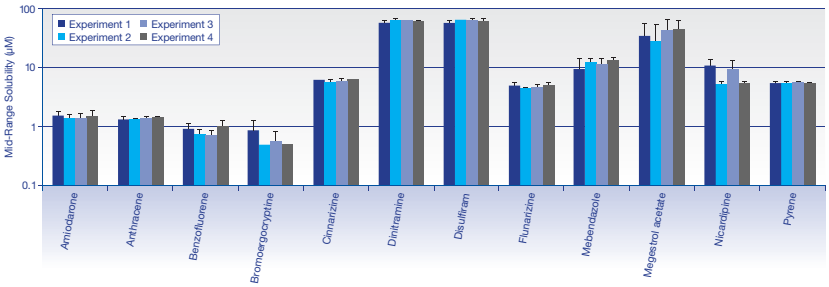
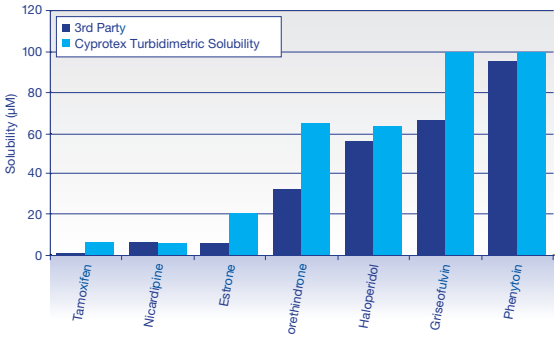


Figure 2

Comparison of Cyprotex's Turbidimetric Solubility results with third party data generated by HPLC-UV/VIS



References

[1] Kerns EH and Di L. (2005) *Journal of the Association for Laboratory Automation* **10 (2)**; 114-123.