

The use of Laser Nephelometry in early stage Drug Development

Solubility determination

Drug Discovery

- HTS has revolutionised Drug Discovery
 - Successfully automated and able to test very large number of compounds
- Only after leads have been identified are the physical properties (e.g. solubility, pKa and lipophilicity) determined

Aqueous solubility

- Is invaluable in the selection of drug candidates
- Can help resolve problems in interpretation of suspect or anomalous screen results

Insoluble drugs

- Developing low solubility drugs is more time consuming and expensive than for a compound with more suitable properties
- More difficult to get to market and repay their development costs

Measuring solubility

- 'Traditional' Shake the compound with the solvent for 24 hours, filter and determine concentration. Repeat until constant value
- 'Forced' do not wait for equilibrium

Measuring solubility

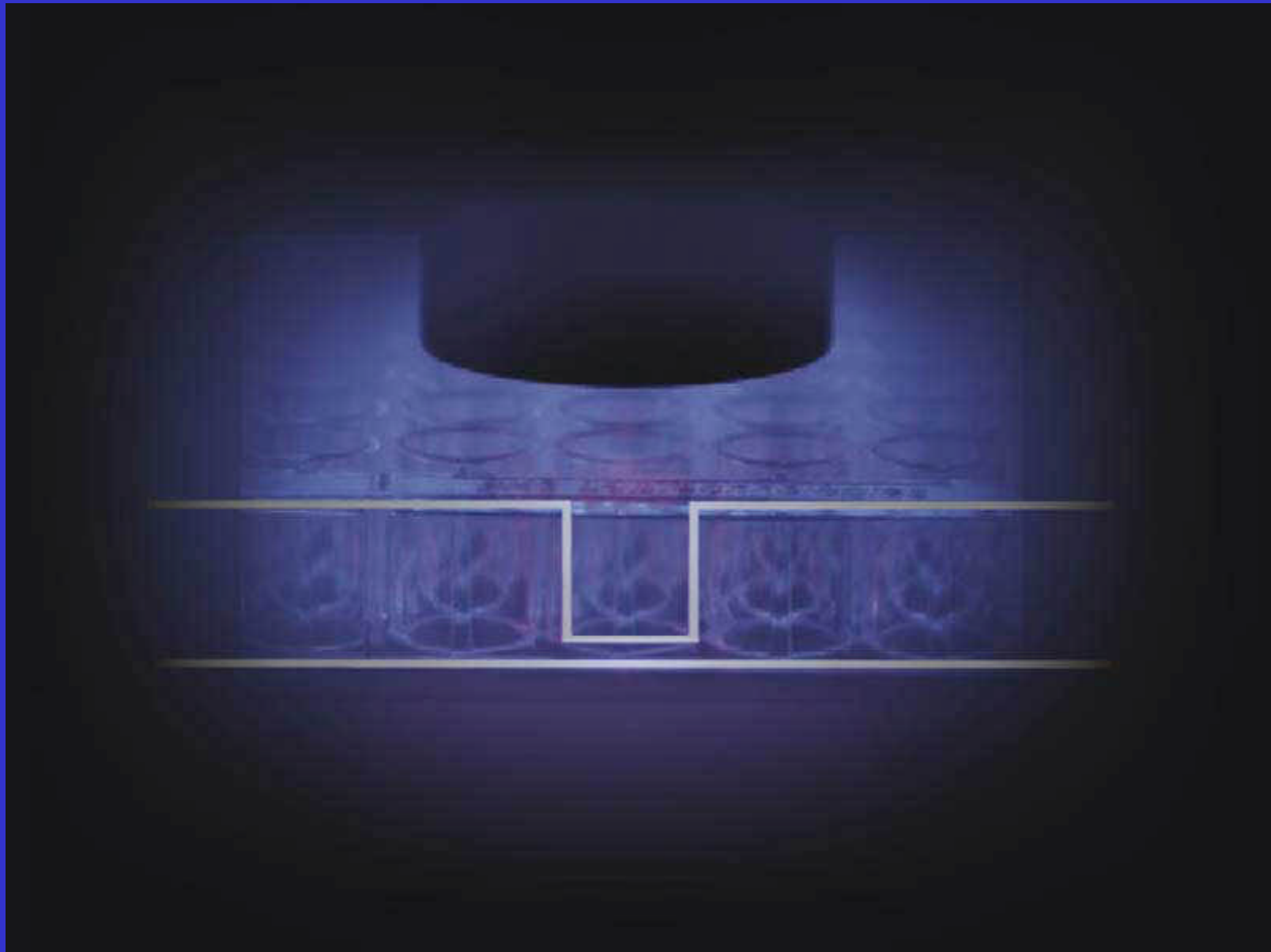
- Inappropriate in a modern Drug Discovery setting because
 - Weighing hundreds of solid samples is too manpower and time consuming
 - Samples are routinely supplied as DMSO solutions and not solids
 - Typically, the analysis is not integrated with the activity screening

Laser Nephelometry

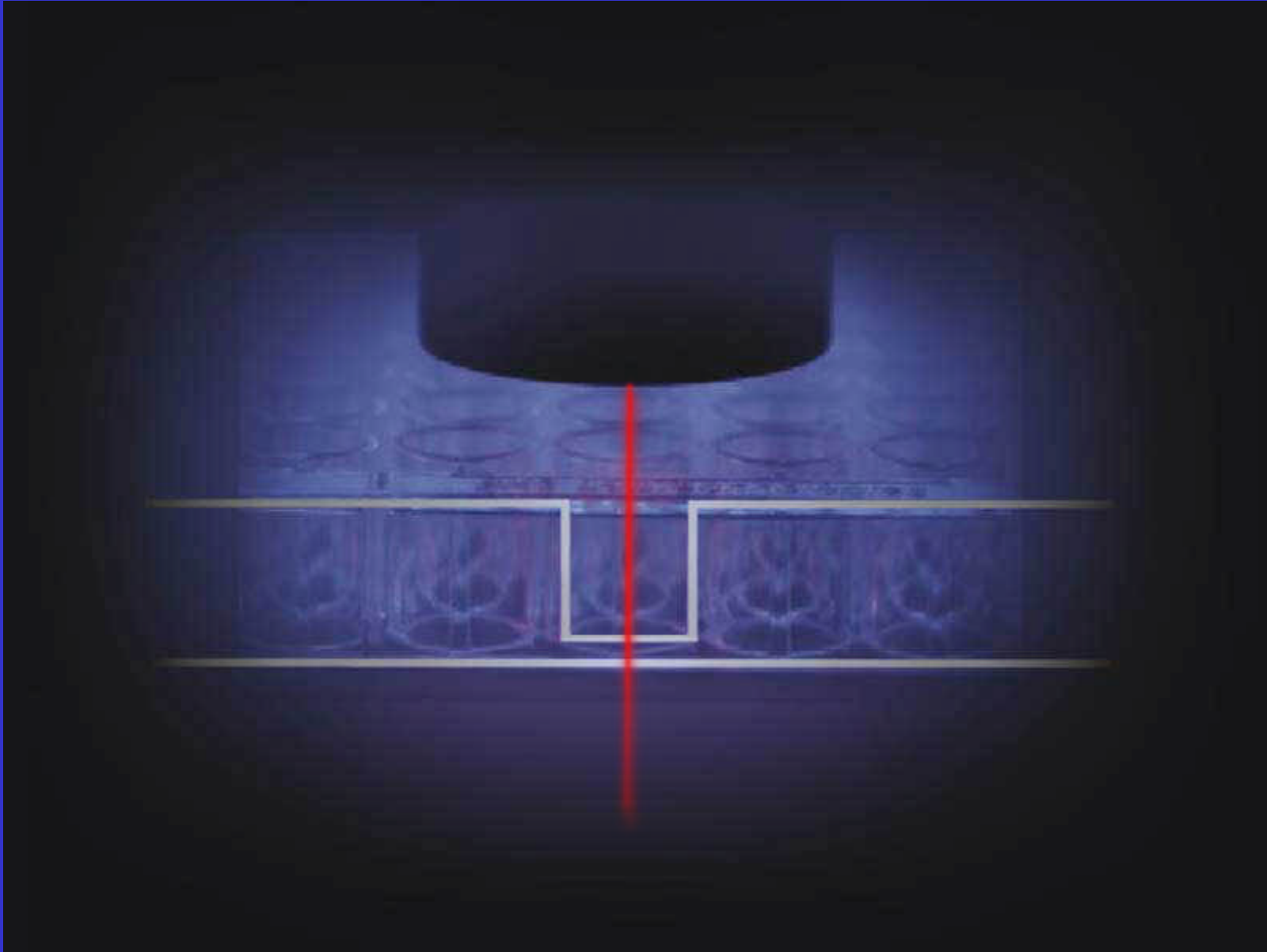
- *Nephele (Greek for cloud)*
- Light travelling through a suspension is scattered. The measurement of the intensity of the scattered light at right angles to the incident beam is the basis of nephelometry

Laser Nephelometry

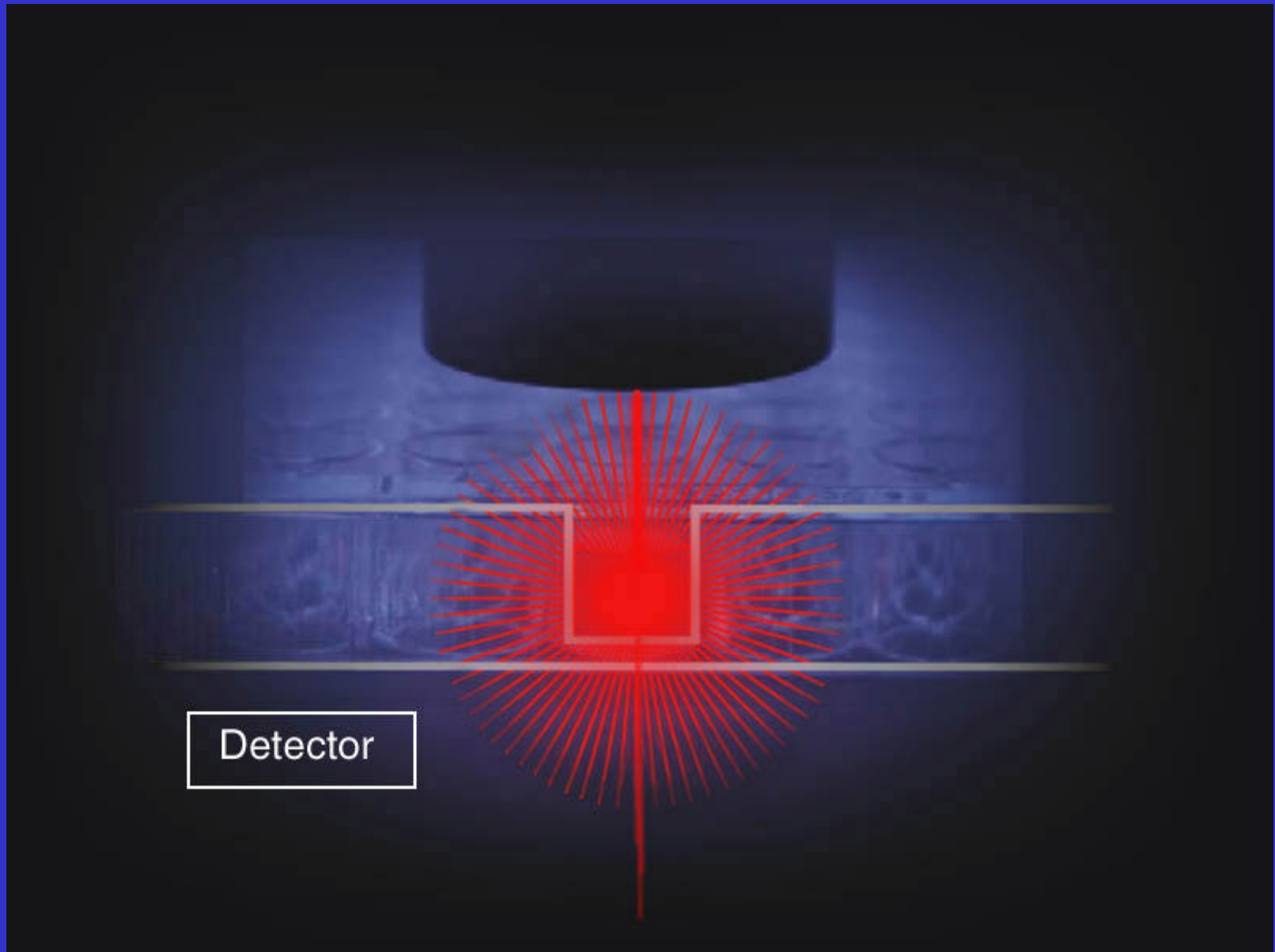
- If a DMSO solution of a water soluble drug is introduced into a aqueous buffer, the mixture will remain clear unless the aqueous solubility limit is reached, at which point precipitation occurs. Progressive incremental dilution of the suspension is performed until the the solute redissolves



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Detector

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Plate Quality

- Any imperfections on the base of the plate will scatter light and produce a false positive signal
- This may be overcome by statistically rejecting wells, by prescanning plates or using manufacturers who can supply plates without imperfections

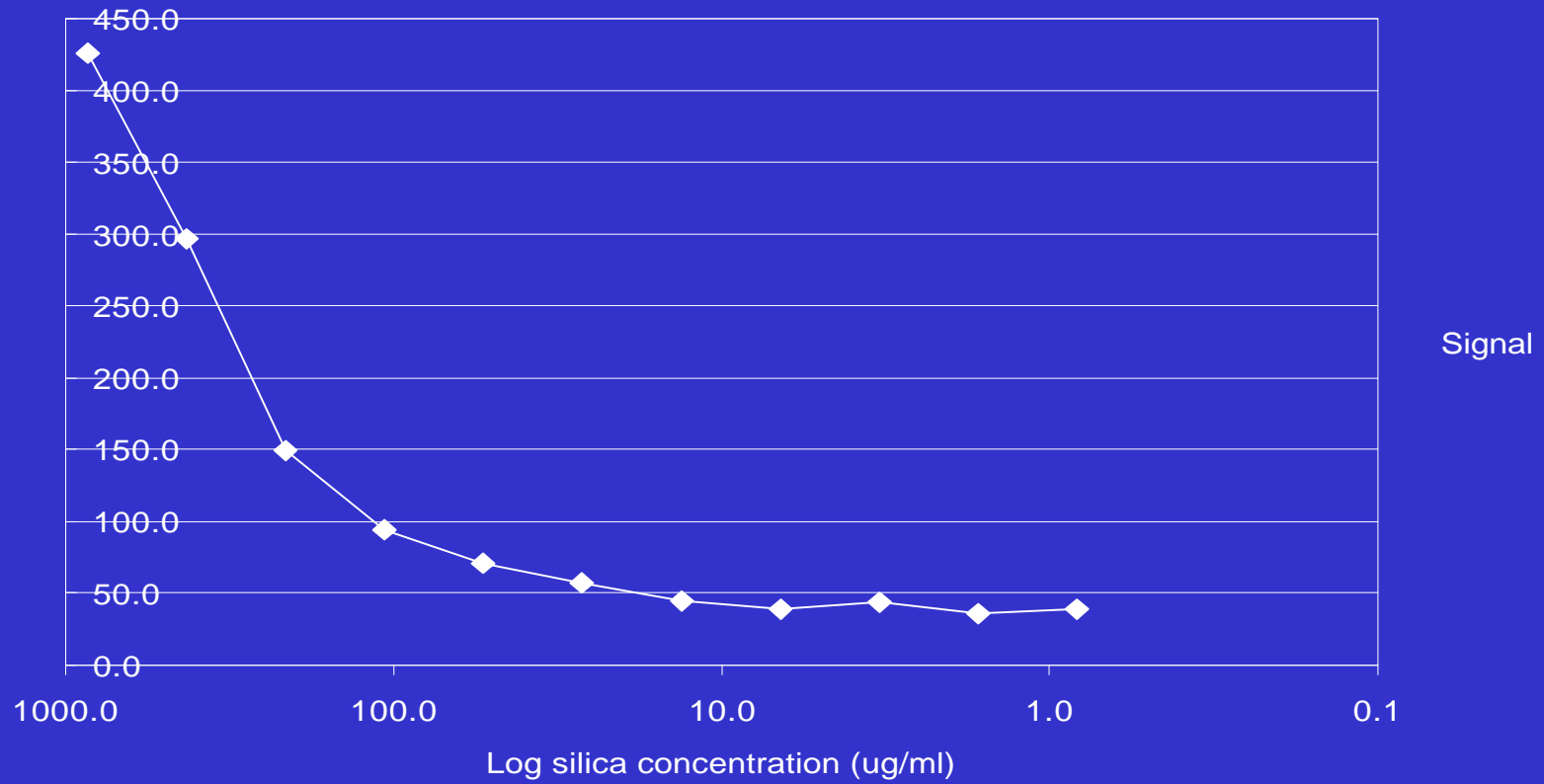
Effect of colour on signal

- Colour of solution may absorb the incident red laser light
- Red and yellow solutions do not affect the signal
- A blue solution can absorb the incident red laser light, fortunately, few potential drugs produce intense blue solutions

Laser Nephelometry

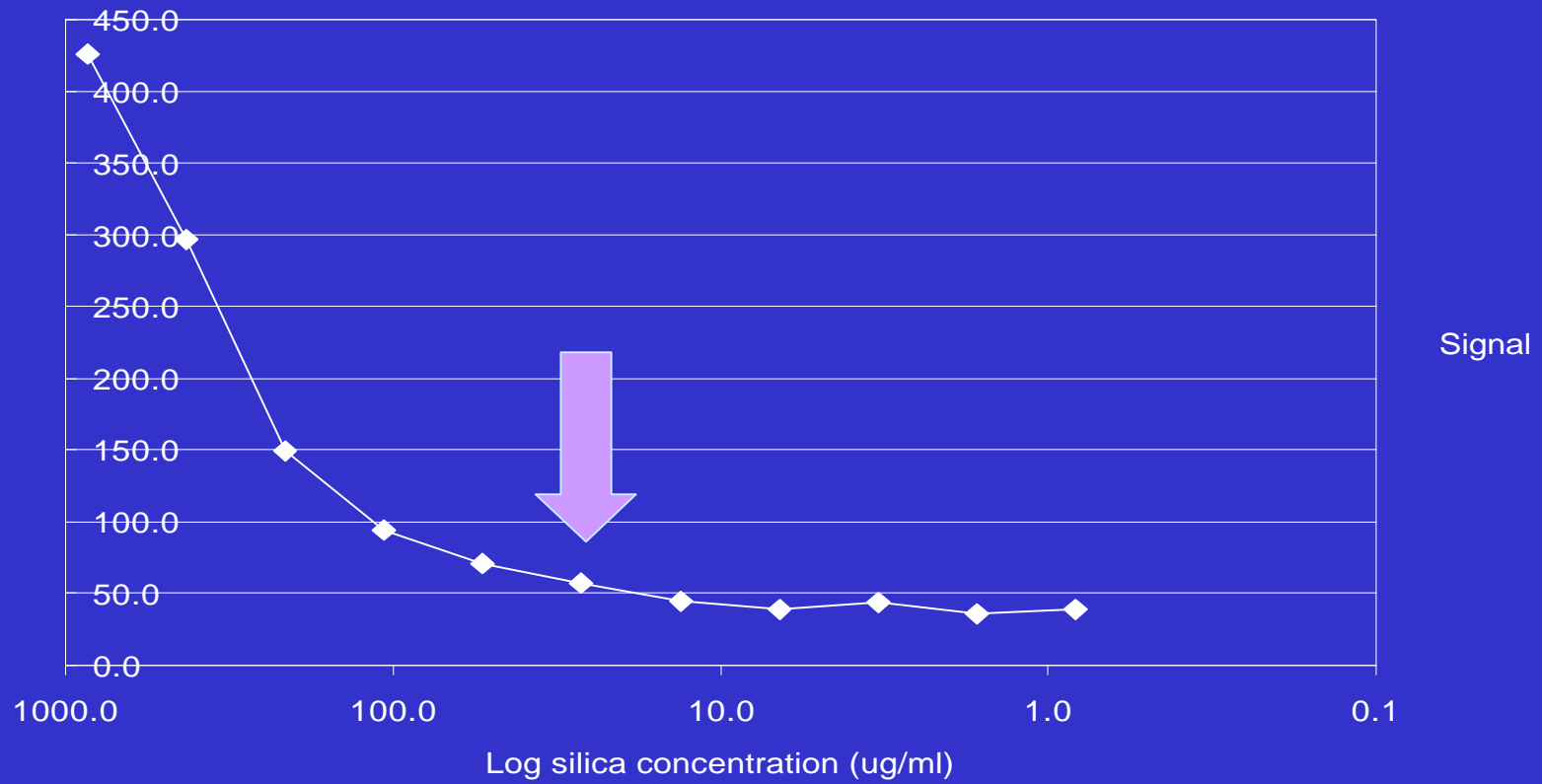
- A model insoluble compound *Ludox silica suspension* was chosen
- The silica colloid will not dissolve in DMSO or PBS buffer used
- A solution at 0.85mg/ml in water was prepared
- A series of 10 double dilutions with PBS buffer (8 replicates)

Silica LOD



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Silica LOD



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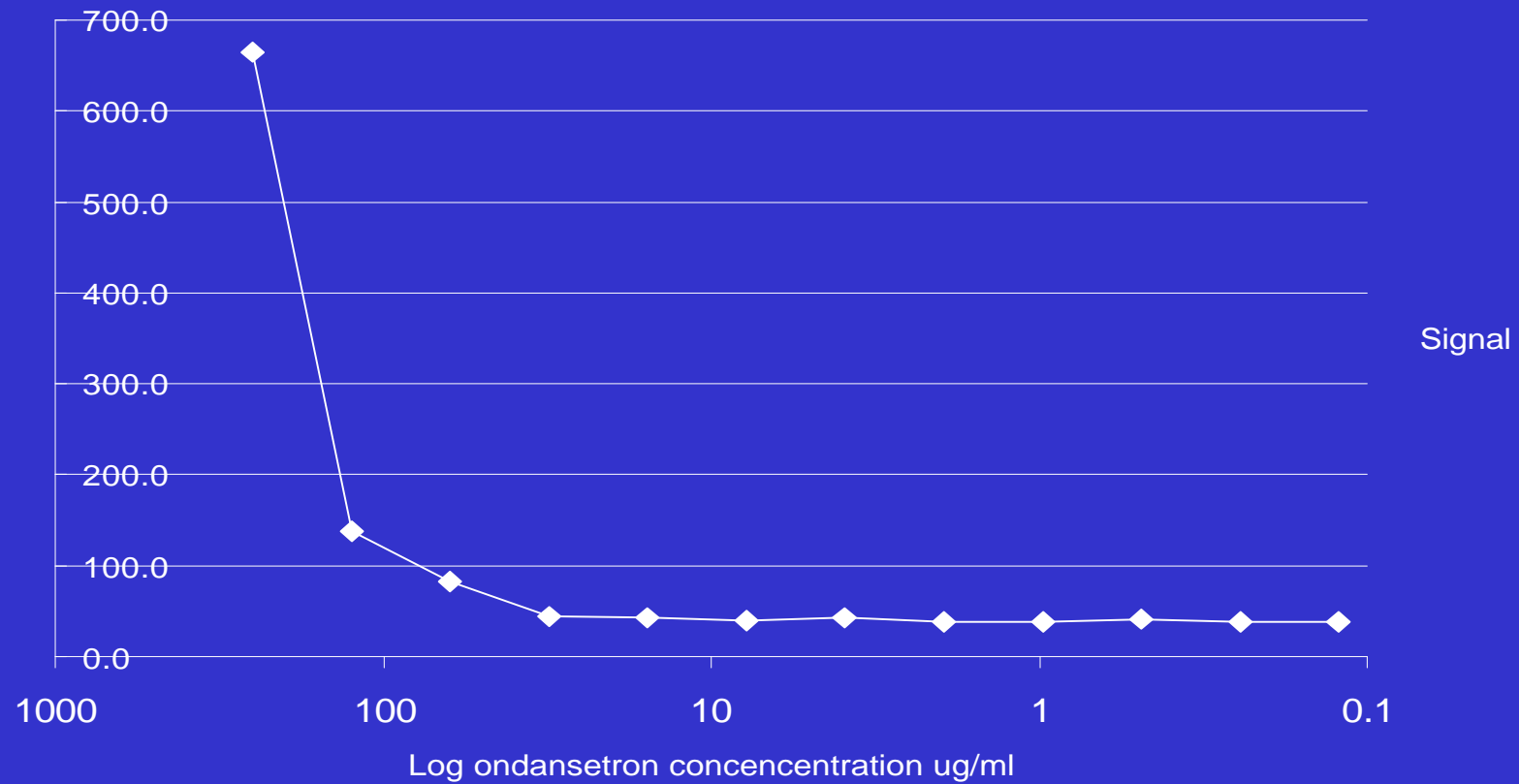
Silica LOD

- A significant change occurs after 27ug/ml
- For a drug (MW 500) equivalent to 54uM

Ondansetron LOD

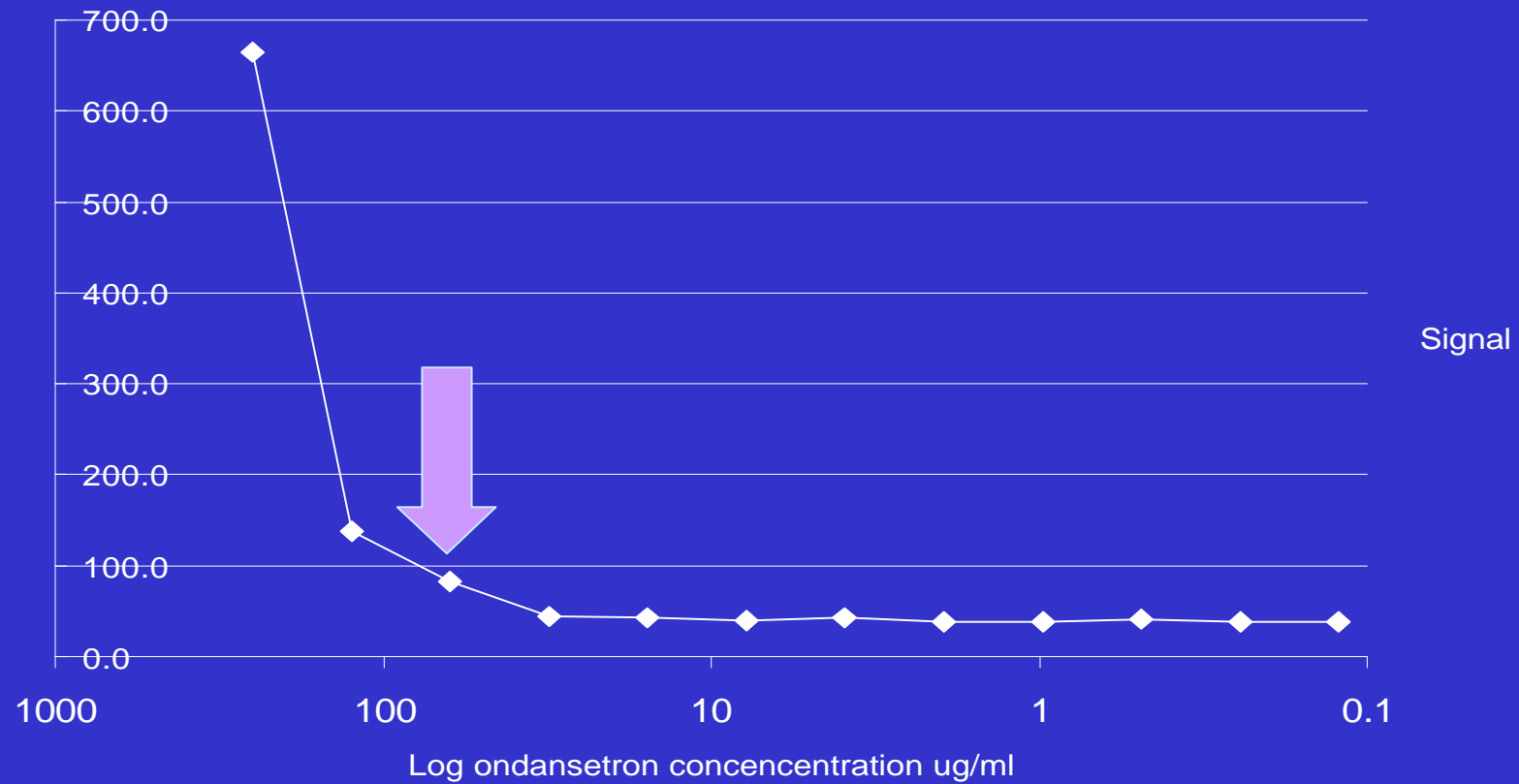
- Ondansetron is a stable, high-purity drug. Its solubility lies in the range of interest. A 5mg/ml solution in DMSO was prepared
- A series of 10 double dilutions with 5% DMSO/PBS (8 replicates)

Ondansetron LOD



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Ondansetron LOD



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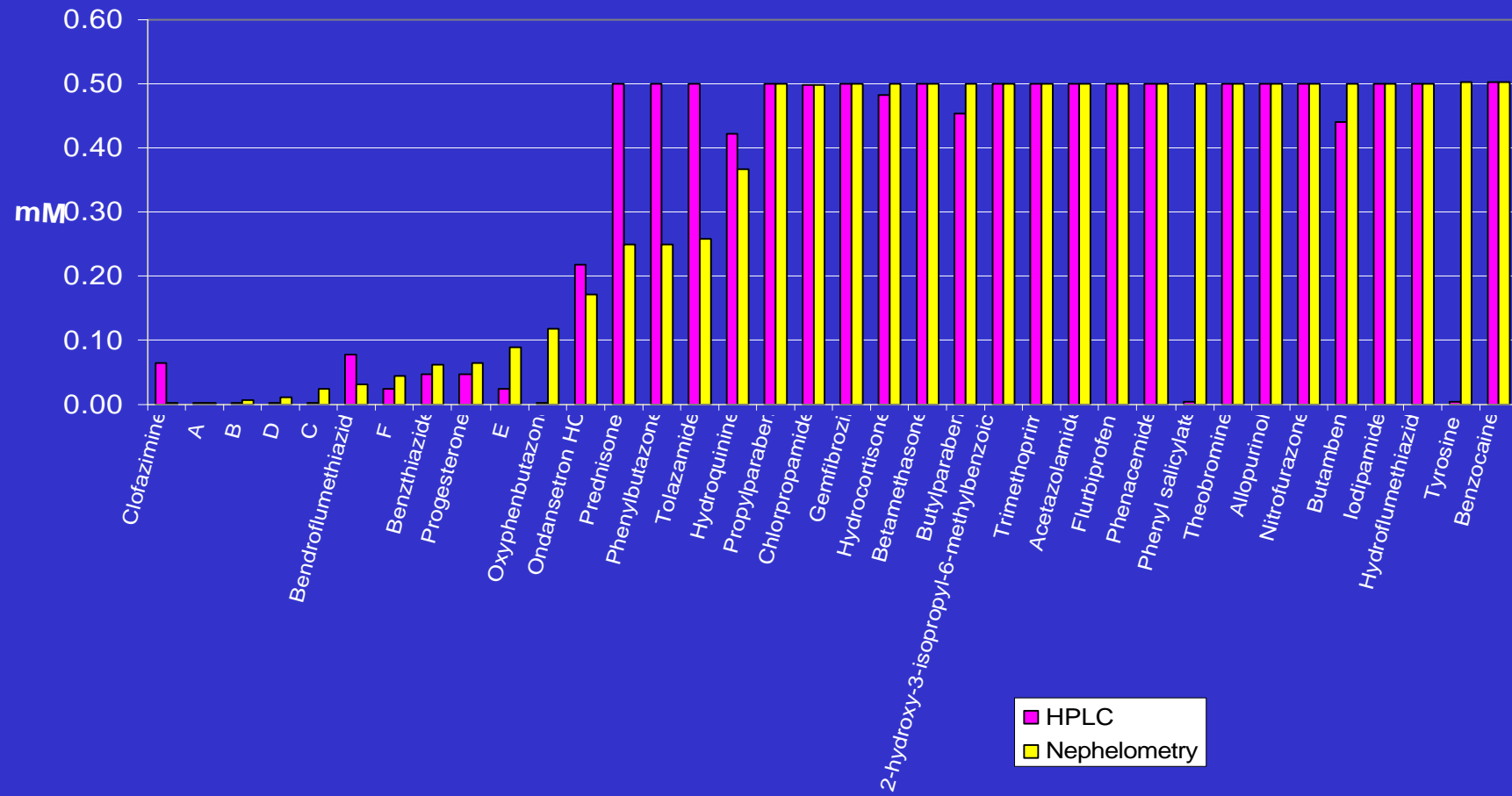
Ondansetron LOD

- A significant change occurs after 63ug/ml
- Solubility by HPLC is typically 160ug/ml
- Nephelometry would put ondansetron in the same range as the HPLC result
 - sparingly (less than 10ug/ml), partially soluble (10-100ug/ml), soluble (greater than 100ug/ml)

Solubility determined by HPLC and nephelometry

- A set of commercially available compounds, for which the solubility had been determined by a 'from solids' and a from 'DMSO solution' by HPLC
- A set of 6 'sparingly soluble' compounds (A-F) from a development project were added

Solubility determined by HPLC and nephelometry



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Solubility determined by HPLC and nephelometry

- The results by nephelometry are generally in the same category as those determined by HPLC
 - sparingly (less than 10ug/ml), partially soluble (10-100ug/ml), soluble (greater than 100ug/ml)

Conclusion

- Detect turbidity at levels below those detectable by eye in transparent and coloured solutions.
- Determine drug solubility a hundred times more quickly than by HPLC.

Conclusion

- Access a solubility range typically from 1 to 1000 $\mu\text{g}/\text{ml}$ (~ 3 to $\sim 500\mu\text{M}$) with a precision and range that compares with HPLC
- Concentration of DMSO is kept constant (unlike previously published methods)

Conclusion

- However, this method is insensitive to purity/stability/identity of compound unlike the relatively slow HPLC-UV and HPLC-MS methods
- We were able to very quickly deduce the solubility of compounds in 5% DMSO/buffer

Conclusion

- The method is non-destructive, simple and has been integrated into our high throughput drug screening process.

Acknowledgements and reference

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