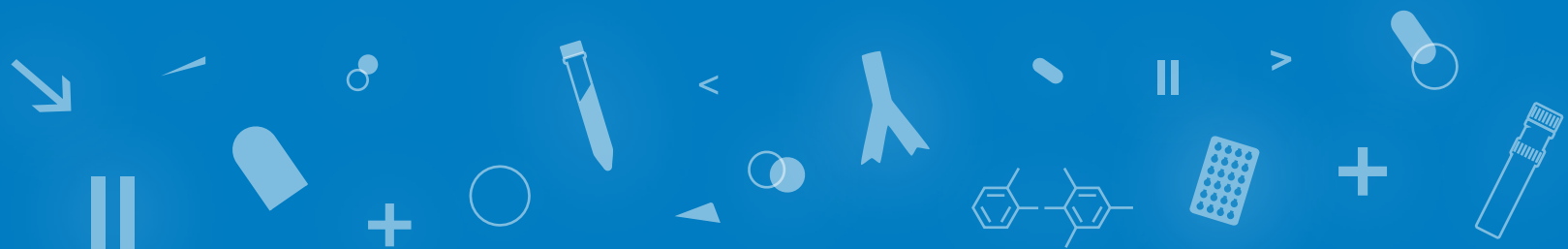
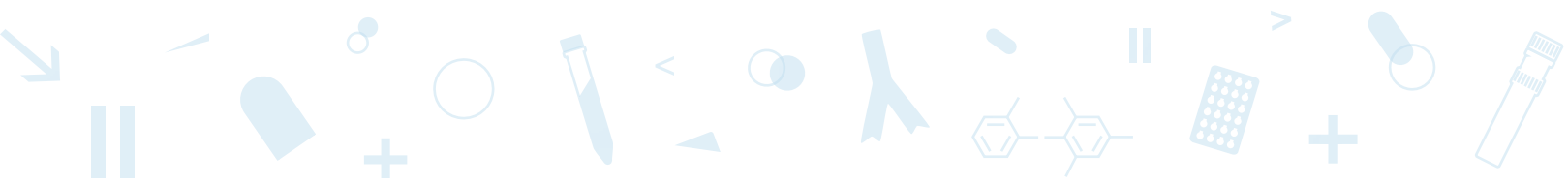




Helping all people
live healthy lives

BD GentestSM Contract Research Services





Partners in the search for new drugs

Introduction

BD GentestSM Contract Research Services

BD Gentest Contract Research Services has over 15 years experience developing *in vitro* services to support pharmaceutical drug discovery and development programs in the early ADME/Tox phase. Our Study Directors are highly skilled scientists with in-depth knowledge of absorption and transport, metabolism, and toxicity. This expertise gives BD Biosciences Study Directors the ability to partner with you to develop and deliver a broad range of *in vitro* ADME/Tox studies to meet your discovery and development project needs. We ensure the highest level of quality standards and adhere to current regulatory requirements and applicable FDA-sponsored guidance documents.

Utilizing state-of-the art techniques and equipment, BD Biosciences is able to assist our clients in screening for viable drug candidates during drug discovery or to prepare regulatory agency submission-quality reports for your drug development compounds. Let our team of experts take you to the next level with studies designed to predict drug-drug interactions and human pharmacokinetics using BD Gentest's innovative *in vitro* products, cell models, and methodologies.

Acronyms

7-BQ: 7-Benzoyloxyquinoline	BzRes: 7-Benzoyloxyresorufin	OCT: Organic cation transporter
7-MFC: 7-Methoxy-4-trifluoro-methyl-Coumarin	CEC: 3-Cyano-7-ethoxycoumarin	OMF: 3-O-Methylfluorescein
ABC: ABC-binding cassette	EFC: 7-Ethoxy-4-trifluoro-methyl-Coumarin	PB: Phenobarbital
ACE: Angiotensin converting enzyme	GLP: Good Laboratory Practice	P-gp: P-glycoprotein
AMMC: 3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methyl-Coumarin	HLM: Human liver microsome	PEPT: Proton oligopeptide co-transporter
AZA: Azamulin	KTZ: Ketoconazole	RIT: Ritonavir
AZT: Azidothymidine	MAMC: 7-Methoxy-4-amino methyl-Coumarin	RT-PCR: Real-time reverse-transcription polymerase chain reaction
BCRP: Breast cancer resistance protein	MRP: Multidrug resistance-associated protein	SLC: Solute-linked carrier
BCS: Biopharmaceutics Classification System	NCE: new chemical entity	TEA: Tetraethylammonium
BFC: 7-Benzoyloxy-4-trifluoro-methyl-Coumarin	NTCP: Sodium taurocholate co-transport protein	TDI: Time-dependent inhibition
BSEP: Bile salt export pump	OAT: Organic anion transporter	UGT: UDP-glucuronosyl transferases
	OATP: Organic anion transporting polypeptide	

Ordering Information

United States

BD GentestSM Contract Research Services

To discuss and order BD Gentest Contract Research Services, contact BD Biosciences at:

tel: 888.334.5229 x2246 or
781.935.5115 x2246

Technical Support

Contact a BD Biosciences Technical Support Representative at:

tel: 877.232.8995 or 978.901.7389

Monday through Friday

9:00 a.m. – 6:00 p.m. Eastern Time

fax: 978.901.7491

e-mail: admetox@bd.com

International

Orders for BD GentestSM Contract Research Services, should be placed with your regional BD Biosciences office or contact BDCustomerService@bd.com for further details.

Solubility and Aggregation Studies

Introduction

Aqueous solubility is a key profiling property for predicting the oral bioavailability of drug candidates. It is essential that oral drugs be absorbed through the gastrointestinal tract and remain in solution to reach the intended therapeutic target. BD Biosciences provides *in vitro* assays to help assess your drug candidate's solubility performance and efficacy.

Traditional Shake Flask Solubility

BD Biosciences offers traditional equilibrium solubility using a 24-hour shake flask method in an aqueous phase to ensure equilibrium. Centrifugation or filtration is used to recover equilibrated aqueous phase. Liquid chromatography and UV absorbance are used to detect and accurately measure the amount of compound in aqueous versus solvent phase.

Solubility Testing by BD Gentest™ Solubility Scanner

In addition to traditional shake flask or thermodynamic methodology, we also provide evaluation of compound precipitation patterns via a flow cytometry-based detection method using the BD Gentest Solubility Scanner. In addition to drug delivery, adequate aqueous solubility is required to obtain accurate activity data in *in vitro* screens. The BD Gentest Solubility Scanner method is specifically designed to mimic the *in vitro* screening environment and provide assay relevant solubility data.

Aggregate Detection and Particle Sizing

When drug compounds aggregate, they often inhibit enzymes in a non-specific manner, creating false positive behavior.¹ The BD Gentest Solubility Scanner is specifically optimized for highly sensitive, reproducible scatter detection of compound aggregates and precipitates, providing flexibility to rule out false positive leads from your screens early in the drug discovery process.

Sample Data

Five compounds known to form aggregates—Econazole, Miconazole, Sulconazole, Nicardipine, and tetraiodophenolphthalein (TIPP)^{1,2}—are shown. The BD Gentest™ Solubility Scanner can detect and count individual aggregate particle events, enabling rapid evaluation of compounds at low concentrations. In **Figure A**, the five compounds were run in a serial dilution prepared from DMSO stocks (10-30 mM) and diluted into aqueous buffers with a residual DMSO concentration of 1%. Samples were read at 30 minutes, 1 hour, and 2 hours after the aqueous mix (1 hour data are shown). Compound concentration is plotted on the (x) axis and the total events per well are shown on the (y) axis. Each concentration point represents a well where the number of aggregate particles is individually evaluated.

In addition to detection and counting, a detailed particle intensity distribution histogram for each sample enables advanced and definitive studies of aggregation behavior. For the compound Nicardipine, aggregate particle histograms for well concentrations 75 μ M and 150 μ M are shown in **Figures B and C**. The numbers of aggregate particle events are plotted on the (y) axis and particle intensity is plotted on the (x) axis.

References

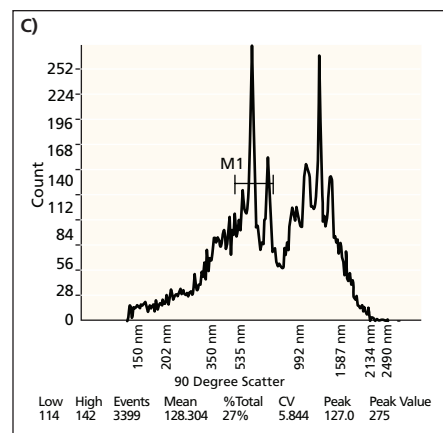
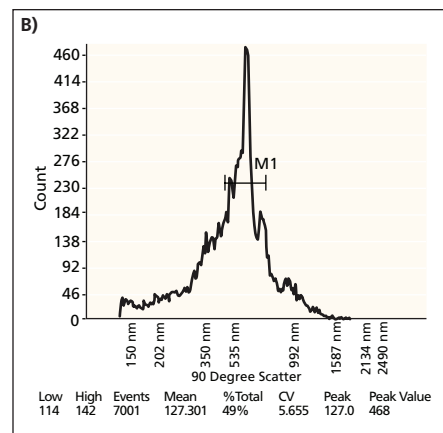
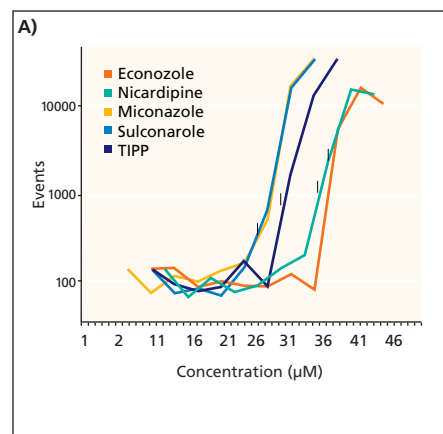
1. Seidler, J., et al. Identification and Prediction of Promiscuous Aggregating Inhibitors among Known Drugs. *J. Med. Chem.* **46**:4477 (2003).
2. McGovern, S.L., et al. *J. Med. Chem.* **45**:1712 (2002).
3. Goodwin, J.J. Rationale and benefit of using high throughput solubility screens in drug discovery. *Drug Discovery Today: Technologies* **3**(issue 1):67 (2006).

Solubility Services

- Shake flask
- Solubility testing by flow
- Aggregate detection and particle sizing

Custom Designed Studies

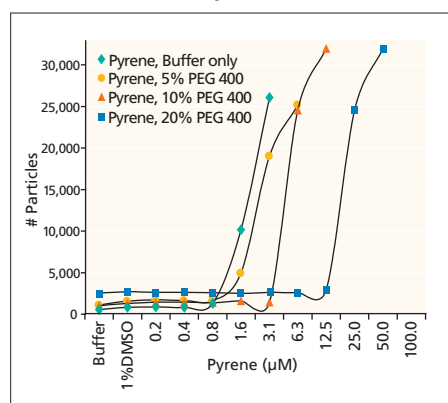
Aggregate Detection Multiple Compounds



Solubility in Assay Buffer

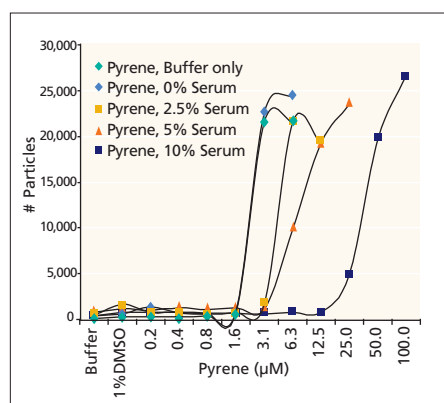
Some drug compounds show differing aqueous solubilities when dissolved in a variety of assay buffers, especially with serum or excipients. BD Biosciences can detect and measure aqueous solubility in a variety of common assay conditions using the BD Gentest™ Solubility Scanner. For example, hepatocyte media, transportocyte assay buffer, or other matrices can be tested.

Solubility of Pyrene in pH 7.4 Buffer with Increasing Amounts 5, 10, or 20% of PEG 400 in the Aqueous Phase



Flow cytometry-based particle size discrimination allows end users to reduce small particle background from excipient samples while maintaining high sensitivity. Above, the excipient PEG 400 was used to improve the solubility of Pyrene in an aqueous buffer. With higher concentrations of the excipient PEG 400, higher amounts of Pyrene can be added before it comes out of solution and is subsequently detected by the laser-based particle detector.

Solubility of Pyrene in Culture Medium with 0, 2.5, 5, or 10% FBS



The BD Gentest Solubility Scanner can also be used to measure differences in compound solubility as a function of serum concentration. Above, fewer Pyrene particles are detected by the BD Gentest Solubility Scanner as serum concentration increases, reflecting the decrease in Pyrene precipitation as serum is added.



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