

# Novel Approach for Screening of Drug Absorption via an Automated System

Application Note 218

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## Introduction

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It is imperative in drug discovery and ADMET to have a handle on the membrane permeability of drug compounds and small molecules. Membrane permeability, in turn, predicts the clinical absorption of these compounds/drugs. Acceptable methods for estimating drug permeability include liposome assays, Caco-2 cell line culture, or intestinal tissue. A basic issue with these methods lies in the fact that they can be costly and extremely labor intensive. An alternative to these methods involves Immobilized Artificial Membrane™ (IAM) chromatography phases, which mimic the lipid environment found in cell membranes. IAM Fast-Screen Mini Columns (1 cm x 3 mm) determine drug capacity factor ( $k'_{IAM}$ ), which correlates well with drug permeability in Caco-2 cells. The nature of the IAM.PC.DD 2 Drug Discovery column is based on IAM chromatography columns. The major phospholipid found in cell membranes is 1,2 Phosphatidylcholine (PC), and the IAM columns prepared from PC analogs are, therefore, models of cell membranes. IAMs can be used to measure the partitioning of drugs relative to fluid membranes. Similar to fluid membranes, a drug will exhibit an equilibrium constant ( $K_{IAM}$ ) to the immobilized membrane. The capacity factor  $k'_{IAM}$  is linearly related to  $K_{IAM}$  (i.e., measuring  $k'_{IAM}$  is effectively the same as measuring  $K_{IAM}$  for the purpose of drug discovery). The IAM.PC.DD 2 Fast-Screen Mini Column is designed for precise and reproducible measurements needed for large-scale drug absorption screening.

Implementing the IAM Fast-Screen columns in a HTS lab to determine drug permeability would be extremely advantageous, allowing for the screening of 100s to 1000s of possible drug candidates in a fraction of the time and at a substantially reduced cost. The concepts of employing chromatographic models to predict drug absorption are extremely efficient and cost effective relative to cell culture and animal models. The IAM.PC.DD 2 Drug Discovery chromatographic column, first developed by Charles Pidgeon (1) and later enhanced by Regis Technologies, is extremely effective in modeling the hydrophobic and hydrophilic characteristics associated with drug partitioning.

The automated system has the capabilities of incorporating from one up to as many as four HPLC systems to increase throughput for the analysis. The systems run independent of one another, which is extremely important since the capacity factor  $k'$  is measured and cannot be affected by flow splitting. The software is capable of generating a report that determines the capacity factor  $k'$  on the fly. The system is capable of solubilizing dry compounds prior to injection. The system can transfer the "hits" to a "hit" plate (reformatting) for further analysis or storage.

Gilson 215 Liquid Handler, equipped with: 175-mm arm, 10-mL syringe, and septum-piercing probe

Gilson 819 Injection Module (2), equipped with: Rheodyne® 7010 valves and 10- $\mu$ L sample loops

Gilson 32X HPLC Pump (2)

Gilson 15X UV/VIS Dual-wavelength Detector (2), equipped with: analytical flow cell, 5-mm path length, 0.1 sensitivity

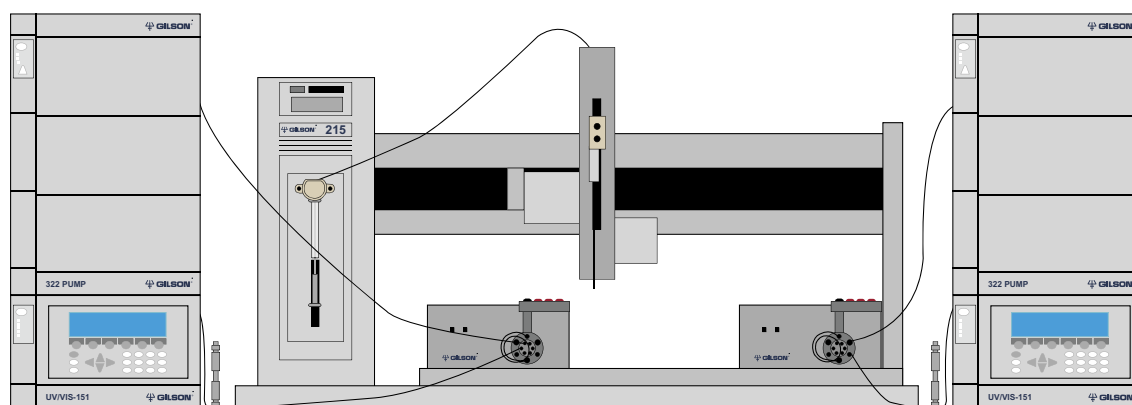
Orbital Shaker

Regis Technologies IAM.PC.DD 2 Fast-Screen Mini Columns (2), 1 cm x 3.0 mm ID

Gilson UniPoint™ LC System Software, Gilson 506C Interface

### Sample Preparation

- Samples (0.5 mg/mL) were manually placed into 20-mL scintillation vials with septum tops.
- Citric acid was used to determine ( $t_0$ ); 50 mg/mL solution in mobile phase.
- The 215 Liquid Handler was used to pipet 10 mL of reservoir solution (methanol) into each of the vials via UniPoint Software.
- The shaker was activated; the speed and time for agitation are set by the user through UniPoint software.
- The dual system was also controlled through UniPoint Software; two independent HPLC systems shared a common 215 Liquid Handler for injection via bi-directional communication between the two systems, which was accomplished by running two operation lists simultaneously (see Figure 1 below).
- Data for each system was independently stored in unique folders for interpretation purposes.



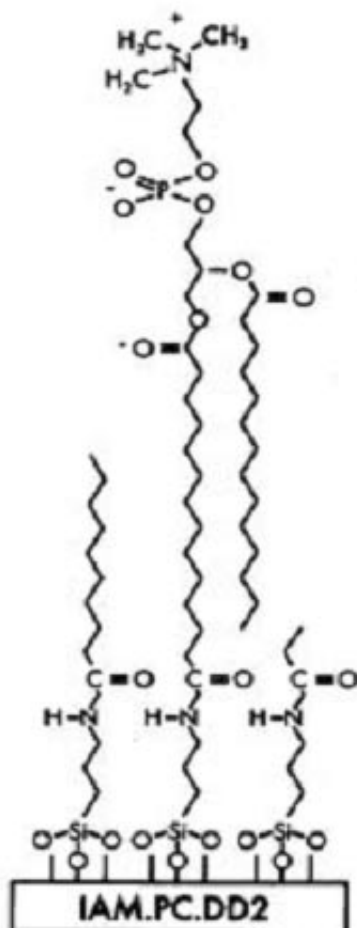
**Figure 1.** Automated Drug Permeability System. The dual HPLC system shown here uses one 215 Liquid Handler to increase the analysis throughput of absorption for drugs/small molecules. The system also employs an orbital shaker to facilitate the solubilizing of the compounds prior to analysis via the IAM.PC.DD 2 Fast-Screen Mini Columns.

## Sample Methodology

- Condition the column by allowing approximately 10 to 15 column volumes (10–15 mL for the Fast-Screen Mini Columns)
- Repeat injections of one compound with identical retention times to ensure the system is equilibrated
- Calculate the capacity factor
- Obtain K IAM by measuring the IAM capacity factor k'IAM
- Calculate capacity factor from the retention time (tr) and the column void volume time (t0) based on the following equation:

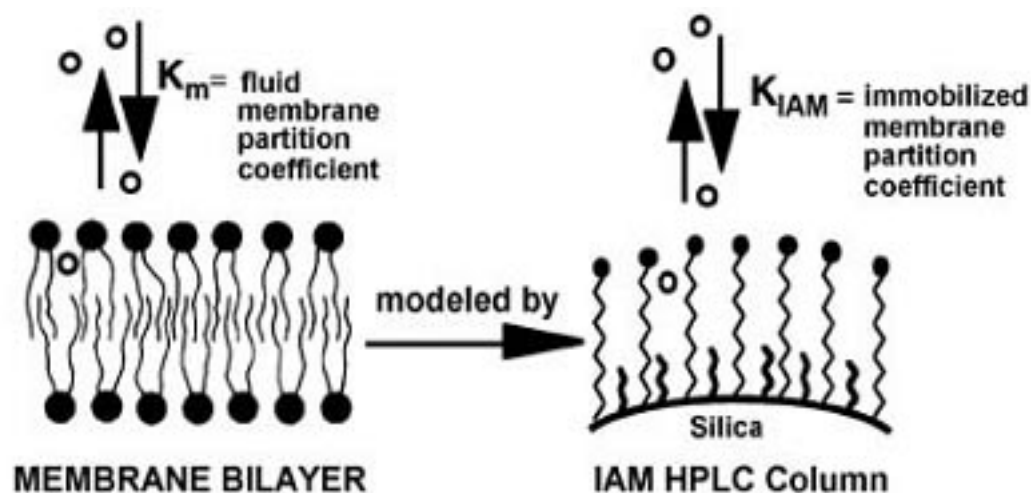
$$k' \text{ IAM} = \frac{t_r - t_0}{t_0}$$

- Using the retention values observed for the compounds (tr) and the (t0) for the system, calculate the capacity factor for each of the compounds under evaluation using the above formula
- Determine correlation coefficients by plotting log % absorbance of inverted rat intestine vs. log k' IAM



**Figure 2.** IAM.PC.DD.2. The ester bonding of the IAM.PC.DD 2 packing offers more hydrophobicity, thus giving longer retentions to compounds not well retained on the IAM.PC.DD packing. Retentions are typically double on the IAM.PC.DD 2 packing, but still exhibit excellent correlation for groups of compounds. (Image courtesy of Regis Technologies, [www.registech.com](http://www.registech.com)).

## Predicting Drug Membrane Permeability



**Figure 3.**  $K_{IAM}$ . The equilibrium constant describing the relative concentrations of drug in the membrane and in the external fluid is analogous to the  $k'_{IAM}$ . (Image courtesy of Regis Technologies, [www.registech.com](http://www.registech.com)).

IAM more closely mimics the interaction of analytes with biological membranes where a combination of hydrophobic, ion pairing, and hydrogen bonding interactions are possible. The combination of interactions measured by the IAM column is known as “phospholipophilicity”.

The two systems (System 1 and System 2, see Figures 4 & 5) both use the 215 Liquid Handler for injection. Communication is uniquely accomplished through control methods by writing to the display of the 215 and requesting the software to read the display prior to the action that is being controlled within two operations lists.

Control Method [system 1.gcl]				
	Time	Device(s)	Command	Comment
1	0.01	Solvent A 2 / Solvent B 2	0.3 (ml/min); 50% Solvent A 2, 50% Solvent B 2	
2	0.50	Injector	Write to Display "BUSY"	System 1 Controlling 215
3	0.60	SYSTEM 1 INJECTION	<start> 6, SAMPLE, 60	
4	1.34	Detector 16	Autozero Channels	
5	1.35	System Controller	Synchronize	
6	1.36	Data Channels	Start Chromatogram Channels	
7	1.50	Solvent A 2 / Solvent B 2	0.3 (ml/min); 50% Solvent A 2, 50% Solvent B 2	
8	3.00	Injector	Write to Display "READY"	System 1 Releases 215
9	8.00	Data Channels	Stop Chromatogram Channels	
10	8.10	Solvent A 2 / Solvent B 2	0.3 (ml/min); 50% Solvent A 2, 50% Solvent B 2	

**Figure 4.** Chromatographic conditions—System 1 Control method.

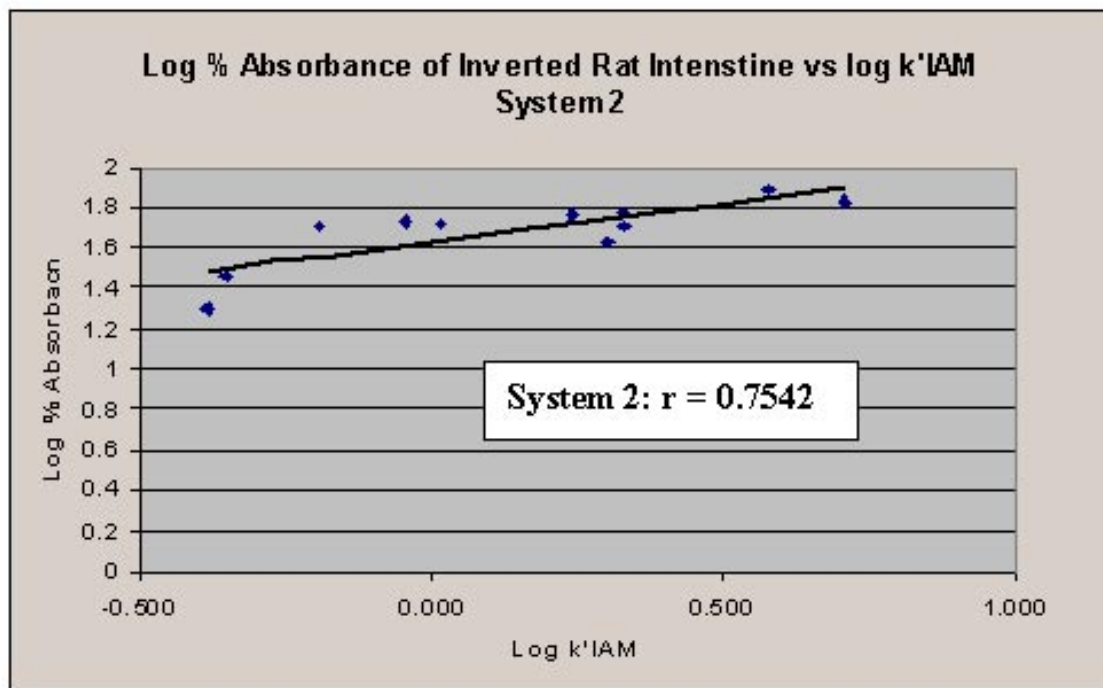
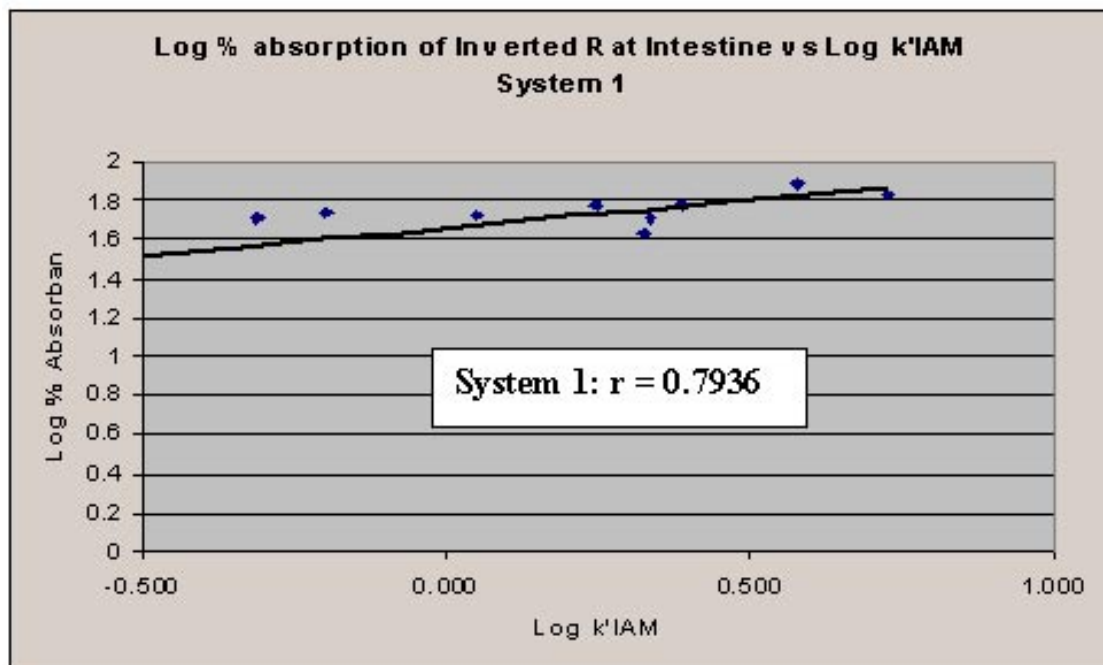
Control Method [system2.gct]				
	Time	Device(s)	Command	Comment
1	0.01	Solvent A 3/Solvent B 3	0.3 (ml/min); 50% Solvent A 3, 50% Solvent B 3	
2	0.50	Injector	Wait for Display Message "READY"	System 2 Waiting for 215
3	0.60	SYSTEM 2 INJECTION	<start> 6, SAMPLE, 60	
4	1.00	Solvent A 3/Solvent B 3	0.3 (ml/min); 50% Solvent A 3, 50% Solvent B 3	
5	1.34	Detector 18	Autozero Channels	
6	1.35	System Controller	Synchronize	
7	1.36	Data Channels	Start Chromatogram Channels	
8	8.00	Data Channels	Stop Chromatogram Channels	
9	8.10	Solvent A 3/Solvent B 3	0.3 (ml/min); 50% Solvent A 3, 50% Solvent B 3	

**Figure 5.** Chromatographic conditions—System 2 Control Method: column, IAM Fast-Screen Mini Columns, 1 cm x 3.0 mm ID; mobile phase, Dulbecco's phosphate buffered saline, pH 5.4; flow rate, 0.3 mL/min; sample volume, 10 µL; detection, UV 254 nm, 0.1 AUFS.

## Results

Compounds Used to Determine Drug Membrane Permeability	
#	Compound
1	m-Nitroaniline
2	p-Nitroaniline
3	Salicylic Acid
4	p-Toluidine
5	Aniline
6	m-Nitrobenzoic acid
7	Phenol
8	Benzoic Acid
9	Acetanilide
10	Theophylline
11	Acetylsalicylic acid

**Table 1.** Compounds used to determine drug membrane permeability. A series of small compounds were used to evaluate the usefulness of automating the analysis of drug membrane permeability via the IAM.PC.DD 2 Fast-Screen Mini Columns.



**Figures 6 & 7.** Correlating drug partitioning into IAM with rat intestinal drug absorption.  $r$  (correlation factor) is derived by plotting  $\log k'$  vs.  $\log \%$  absorption of inverted rat intestine.

### System 1

Compound	Time (sec.)	k'IAM	CV (%)	STD (%)
1	179.33	3.761	0.85	1.5
2	237.33	5.301	1.35	3.2
3	128.66	2.416	1.95	2.5
4	103.66	1.752	1.47	1.5
5	61.33	0.628	4.10	2.5
6	79.66	1.115	1.44	1.2
7	119.00	2.159	1.45	1.7
8	56.00	0.487	3.09	1.7
9	116.66	2.097	2.15	2.5
10	47.00	0.248	0.00	0.0
11	48.33	0.283	1.19	0.6

### System 2

Compound	Time (sec.)	k'IAM	CV (%)	STD (%)
1	162.66	3.604	0.93	1.5
2	215.00	5.085	0.80	1.7
3	110.00	2.113	1.81	2.0
4	97.00	1.745	1.03	1.0
5	67.33	0.906	1.71	1.2
6	72.00	1.038	1.38	1.0
7	110.66	2.132	1.04	1.2
8	58.00	0.642	2.98	1.7
9	106.00	2.000	0.94	1.0
10	51.00	0.443	1.96	1.0
11	50.00	0.415	0.00	0.0

**Tables 2 & 3.** Correlation coefficient and standard deviation for the series of compounds evaluated on the IAM.PC.DD 2 Fast-Screen Mini Column Systems 1 and 2. The retention time is an average of three separate runs, two occurring on the same day and the third on a separate day. The CVs and STDs show excellent within-run and between-day correlations.

### Summary

The automated system used here provided a faster alternative to other screening methods.

High sample throughput in combinatorial chemistry provides an initial estimate of membrane permeability.

The dual automated system used here allows for hundreds of samples to be injected into a single IAM Fast-Screen Mini Column.

Measured values for k'IAM are highly reproducible, both from run to run and day to day.

IAM Fast-Screen Mini Columns have shown identical correlation factors ( $r$ ), original  $k'$ , and  $k'$  after 5000 column volumes.

The software is capable of generating the capacity factor  $k'$  on the fly, which drastically increases sample throughput and saves time over manual calculations.

The automated system used here provides an inexpensive analysis estimate of drug permeability for hundreds of drug candidates in a fraction of the time of conventional screening methods.

## Conclusion

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The IAM. PC.DD 2 Fast-Screen Mini Column is not designed as a separation tool, but rather as a tool for characterizing the capacity factor ( $k'$ ) of individual compounds/drugs. The measured  $k'$  of the compounds on these columns can then be used to estimate a value for drug permeability.

It has been proven and documented in several articles over the last few years that the use of IAM techniques to determine drug permeation is a viable rapid option for drug discovery and ADMET. The automated drug absorption system presented here proved to be stable and consistent for the analysis of drug membrane permeability for small compounds/drugs. Merging automation with the IAM.PC.DD 2 Fast-Screen Mini Columns offers an economical screening method for the high

throughput estimation of drug permeation. Therefore, the automated system offers a solution that can be used to characterize large libraries of compounds at a fraction of the cost and time of other techniques. Having a versatile Liquid Handler allows the preparation of solubility to be accomplished on the same instrument prior to analysis, in addition to reformatting of possible candidates for further evaluation.

Solubilizing the dry compounds on line prior to injection minimizes the preparation time for the samples. Volumes can be imported from text files for variable options/consistent concentrations. The 215 Liquid Handler is shared between two HPLC systems by a bi-directional communication that involves reading the display before moving. This is a much more consistent approach than contact closure, where the instrument may not be ready for the contact or the contact may not be in the correct state on start up or during an interruption.

The data presented shows excellent correlation for the IAM.PC.DD 2 Fast-Screen Mini Columns and % absorbance of inverted rat intestine;  $r = 0.794$  (system 1),  $r = 0.754$  (system 2). The IAM.PC.DD 2 columns showed remarkable consistency in regards to retention time of the individual compounds between runs within a given day, and between days, where two runs on the same day were compared to a run from another day. The CVs for the series of compounds did not exceed 4%, and the STD for the series was in the 1.5% range.

This automated drug absorption system offers a viable alternative to the classic techniques of drug absorption by allowing values for drug permeability to be estimated.

### Acknowledgements

The authors would like to thank Regis Technologies for supplying the IAM.PC.DD 2 Fast-Screen Mini Columns used in this application.

### References

- (1) C. Yang, S. Cai, H. Liu, and C. Pidgeon, "Immobilized Artificial Membranes—Screens for Drug Membrane Interactions," *Advanced Drug Delivery Reviews* (23), 229–265 (1996).