

# High Sensitivity Bioanalysis for Small Drug-Like Compounds in Human Plasma using Microflow LC and High Resolution Mass Spectrometry

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## Abstract

**Introduction.** Bioanalysis to support PK-PD studies and clinical trials can be challenging especially when quantifying highly potent compounds. In this study, quantitation attributes were determined for a set of drug-like compounds in human plasma using microflow LC and high resolution mass spectrometry (HRMS) with ionKey/MS and the Xevo G2-XS Time of Flight (ToF) mass spectrometer. The iKey is a microflow separation device which is compatible with Waters Tandem and QToF platforms. The Xevo-G2XS QToF is the latest QToF instrument with enhanced resolution and sensitivity compared to its predecessor. The combined use of both technologies yields an extremely sensitive LC/HRMS instrument. Addition of a trap-and-elute configuration enables the system to handle analytical scale sample volume injection while maintaining excellent peak shape, which further enhances the system's sensitivity.

**Methods.** Test compounds, including buspirone, propranolol, verapamil, and clopidogrel were prepared in human plasma. The LC/MS system used was the AQUITY M-Class UPLC system, coupled with the Xevo G2-XS QToF mass spectrometer and the ionKey/MS source. The iKey tile used was the HSS T3 1.8  $\mu$  150  $\mu$  m x 50 mm at 45°C. The trap column used was the HSS T3 1.8  $\mu$  300  $\mu$  m x 50 mm. A generic, linear gradient from 5-60 B% in 3.5 minutes at flow rate of 3  $\mu$ L/min was used (mobile phase A was water + 0.1% formic acid; mobile phase B was acetonitrile + 0.1% formic acid (v/v)).

**Results.** Serially diluted samples of test compounds in human plasma were quantified via direct injection onto the LC/MS system using ToF-MRM mode of acquisition. Results showed excellent linearity ranging from log = 3.6 to 4.2 for verapamil and clopidogrel, respectively. LLOQs ranged from 0.8 fg to 3.0 fg on column. The signal/noise ratio at LLOQ ranged from 9 to 79. These attributes suggest the system is well-suited to meet the needs of routine bioanalysis. In the second set of experiments, a trap valve manager was installed, and the system was configured for trap-and-elution. Increasing injection volumes from 1 to 5, 10, and 20  $\mu$ L showed excellent peak shape and peak resolution. A linear response with  $R^2 = 0.9987$  was observed, indicating complete sample recovery using the trapping column. For a 20  $\mu$ L injection of samples containing 20% acetonitrile, which is equivalent to a 4 mL injection at analytical scale using a 50 x 2.1 mm column, polar compounds such as buspirone and propranolol were well-retained and showed no peak distortion. These data suggest the microflow LC/MS system is well-suited to support routine bioanalytical sensitivity requirements. Additional benefits for using the system include ease-of-use and a 90% reduction in solvent usage compared with analytical LC.

**Novel Aspect** Novel aspects of this paper include the use of microflow LC/HRMS and dual pump trap-and-elute for high sensitivity small molecule quantitation in human plasma at analytical scale injection volumes.

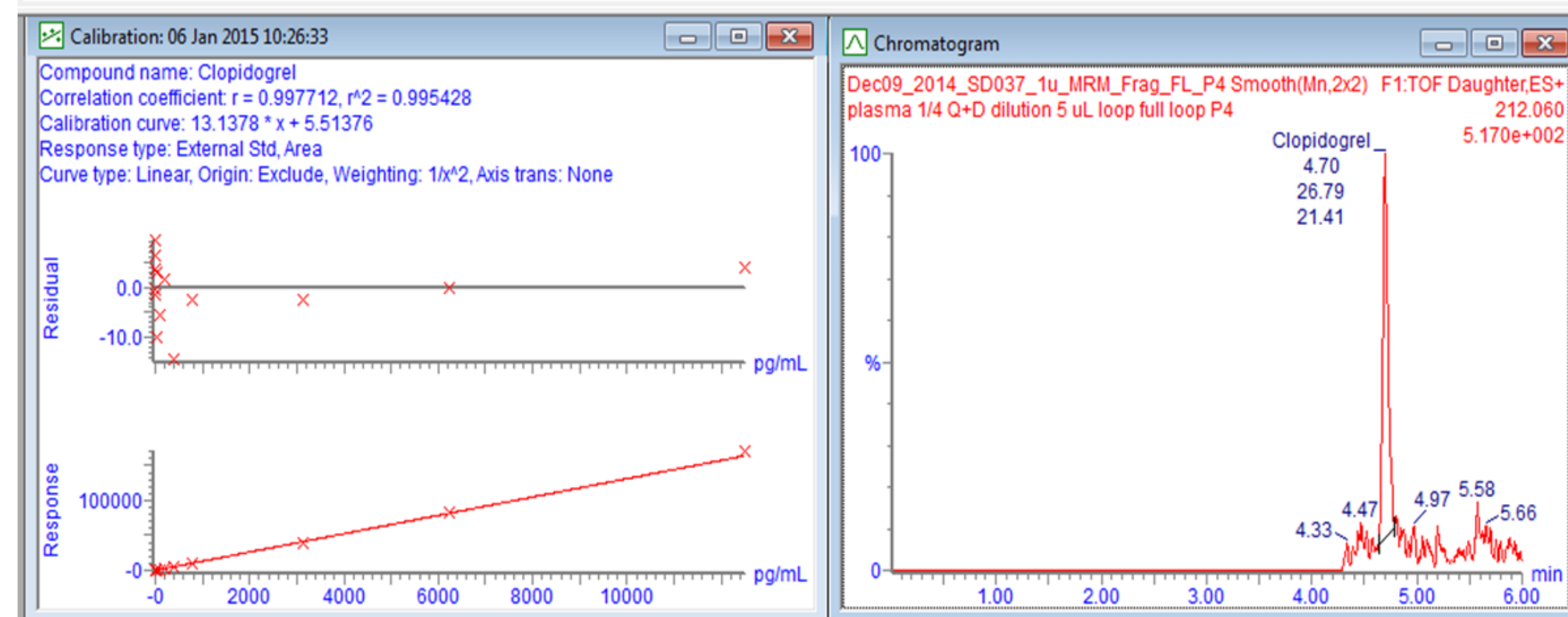
ionKey/MS is an integrated plug and play microfluidics LC/MS system designed for ease of use, robustness, and at both reduced sample and solvent usage. When coupled with the most sensitive QToF, Xevo G2-XS HRMS, the system has potential to deliver low quantification limit while taking advantage of high resolution power of QToF HRMS. The system can also be flexibly configured to perform either direct injection or using trapping column to enable trap-and-elute. The use of trap-and-elute for reduced sample run time and enabling larger analytical scale injection volume are described in more detail here.

## Quantitation in Human Plasma Matrix

Compound	CE Ramp	Transition	Linear range [Log] (pg/mL)	R <sup>2</sup>	LOQ (pg/mL)	LOQ (fg) amount on-column	S/N @LOQ
Buspirone	30	386 > 122	1.5-12,500 [3.9]	0.991	1.5	1.5	78
Clopidogrel	16	322 > 212	0.8-12,500 [4.2]	0.995	0.8	0.8	9
Verapamil	36	455 > 165	3.1-12,500 [3.6]	0.996	3.1	3.1	38

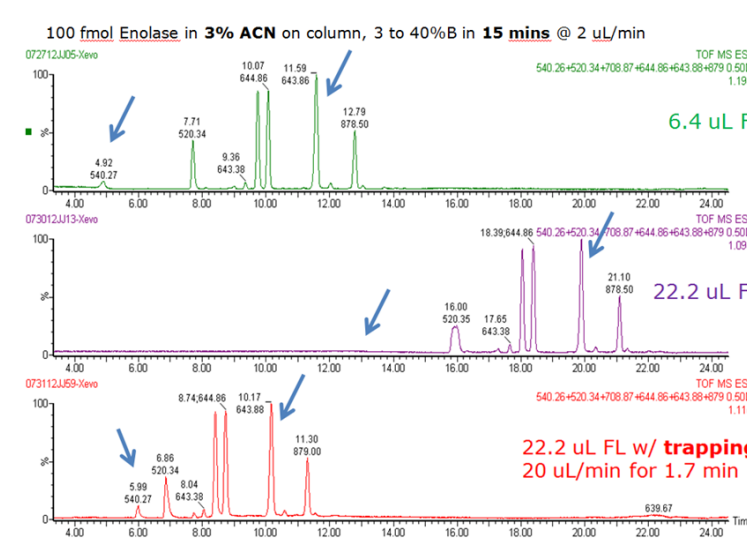
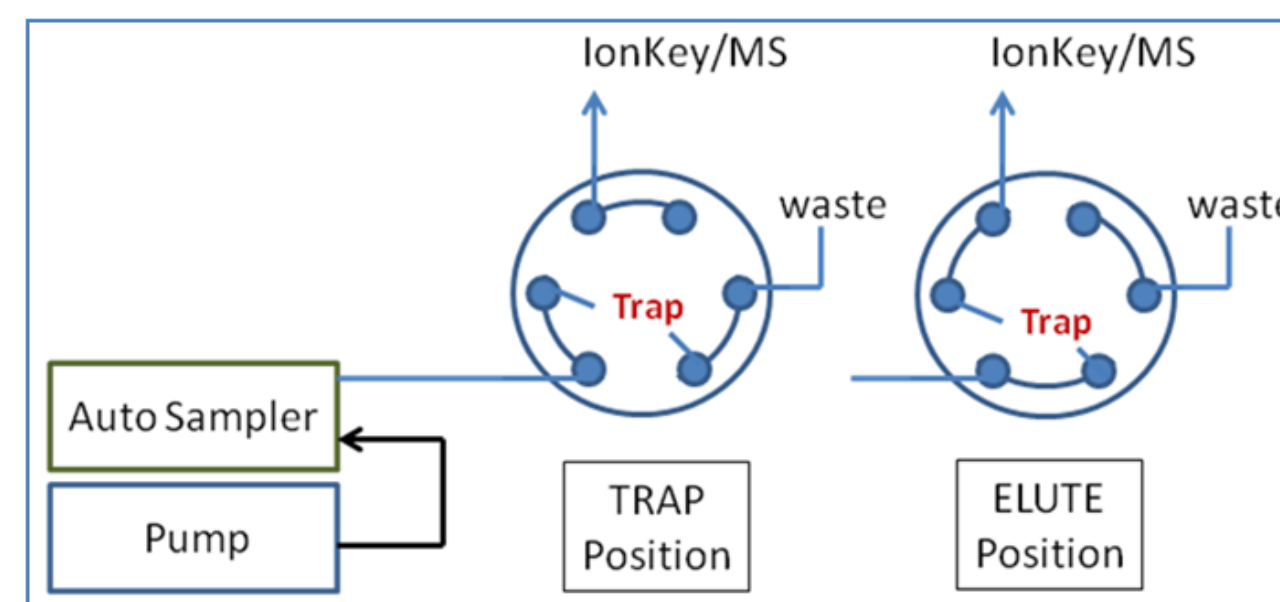
Table 1. Summary of MRM transition, linear dynamic range, and quantification limit. The data was acquired using 1  $\mu$ L direct injection.

#	Name	Type	Std. Conc	RT	Area	IS Area	Response	Primar.	pg/mL	%Dev	S/N
3	Dec09_2014_SD037_1u_MRM_Frag_FL_P3	Standard	0.763	4.71	15.476		15.476	MM	0.8	-0.6	9
4	Dec09_2014_SD037_1u_MRM_Frag_FL_P4	Standard	1.526	4.70	26.792		26.792	bb	1.6	6.1	21
5	Dec09_2014_SD037_1u_MRM_Frag_FL_P5	Standard	3.052	4.70	49.339		49.339	bb	3.3	9.3	19
6	Dec09_2014_SD037_1u_MRM_Frag_FL_P6	Standard	6.104	4.70	93.076		93.076	bb	6.7	9.2	57
7	Dec09_2014_SD037_1u_MRM_Frag_FL_P7	Standard	12.207	4.71	171.784		171.784	bb	12.7	3.7	86
8	Dec09_2014_SD037_1u_MRM_Frag_FL_P8	Standard	24.414	4.70	294.832		294.832	bb	22.0	-9.8	163
9	Dec09_2014_SD037_1u_MRM_Frag_FL_P9	Standard	48.828	4.70	665.921		665.921	bb	50.3	2.9	298
10	Dec09_2014_SD037_1u_MRM_Frag_FL_P10	Standard	97.656	4.70	1217.343		1217.343	bb	92.2	-5.5	693
11	Dec09_2014_SD037_1u_MRM_Frag_FL_P11	Standard	195.313	4.70	2608.253		2608.253	bb	198.1	1.4	1699
12	Dec09_2014_SD037_1u_MRM_Frag_FL_P12	Standard	390.625	4.70	4404.746		4404.746	bb	334.9	-14.3	1956
13	Dec09_2014_SD037_1u_MRM_Frag_FL_P13	Standard	781.250	4.70	10025.908		10025.908	bb	762.7	-2.4	3839
14	Dec09_2014_SD037_1u_MRM_Frag_FL_P15	Standard	3125.000	4.70	40057.633		40057.633	bb	3048.8	-2.4	3427
15	Dec09_2014_SD037_1u_MRM_Frag_FL_P16	Standard	6250.000	4.70	82000.500		82000.500	bb	6241.2	-0.1	8642
16	Dec09_2014_SD037_1u_MRM_Frag_FL_P17	Standard	12500.000	4.70	170698.719		170698.719	bb	12992.6	3.9	14604

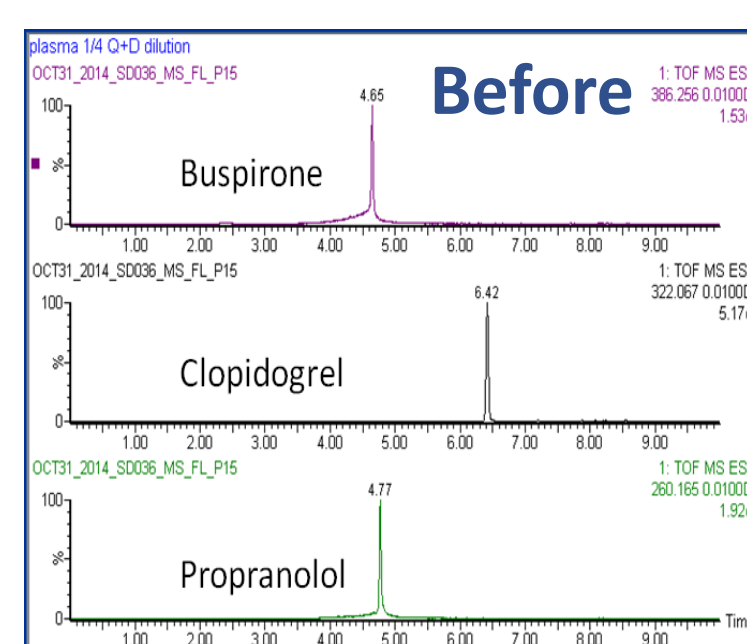


TargetLynx results for clopidogrel. Lower right is chromatogram at 1.5 fg on column.

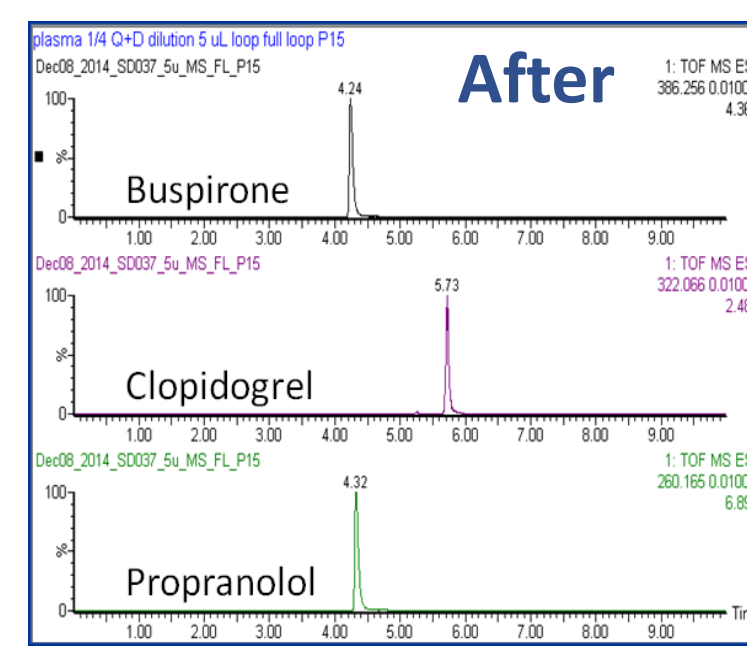
## Single Pump Trap-and-Elute Diagram



(Left) When using a large sample loop, the low flow of microfluidics will add long delay time (middle). With trap-and-elute (bottom chromatogram), the run time is reduced to similar to direct injection (top chromatogram).

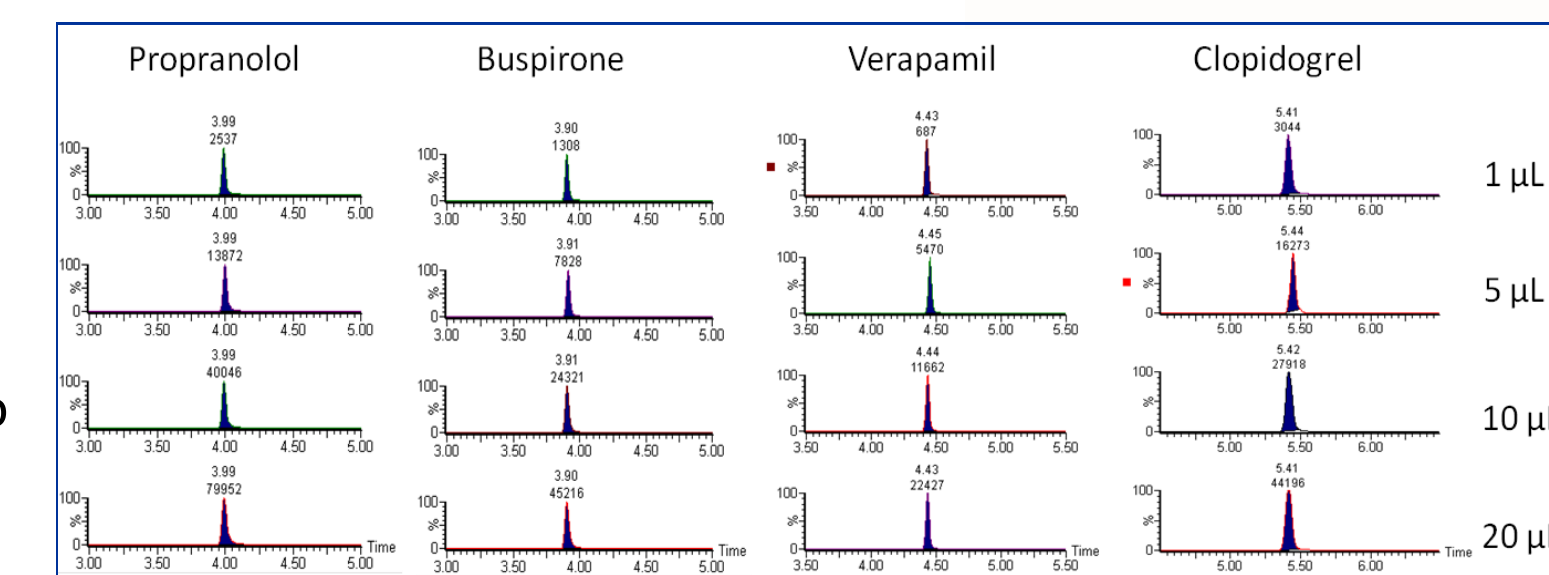
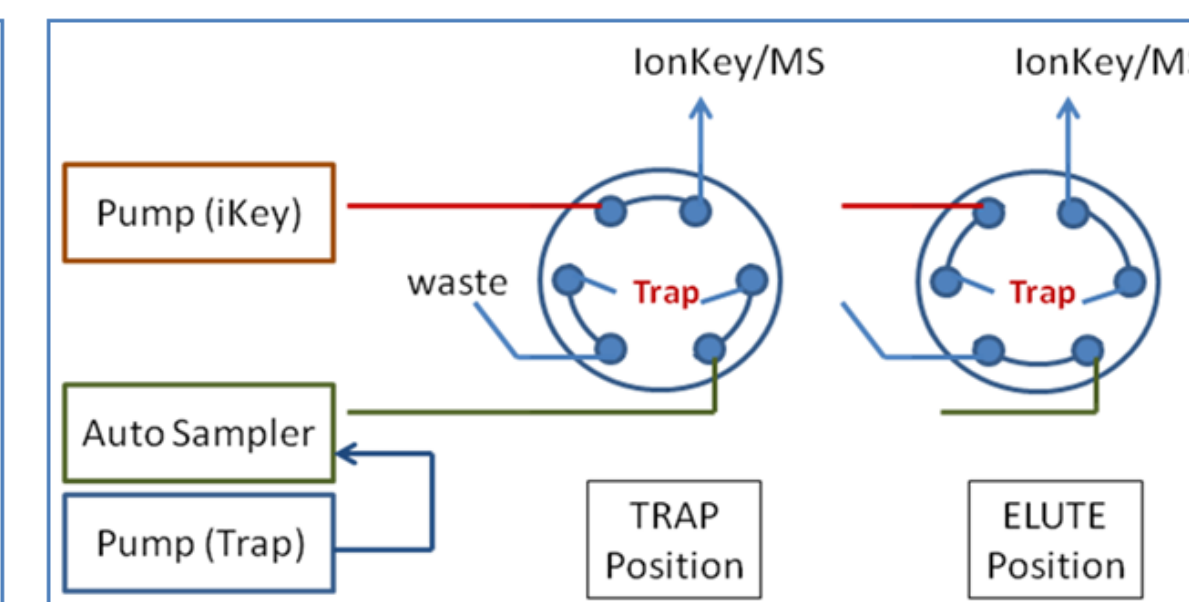


(Left) Injecting sample in relatively strong sample solvent can cause peak distortion due to low system volume of microfluidics to dilute sample solvent. In this example, injecting 5  $\mu$ L in human plasma, relative polar compound, propranolol and buspirone show peak fronting, while clopidogrel shows excellent peak shape.

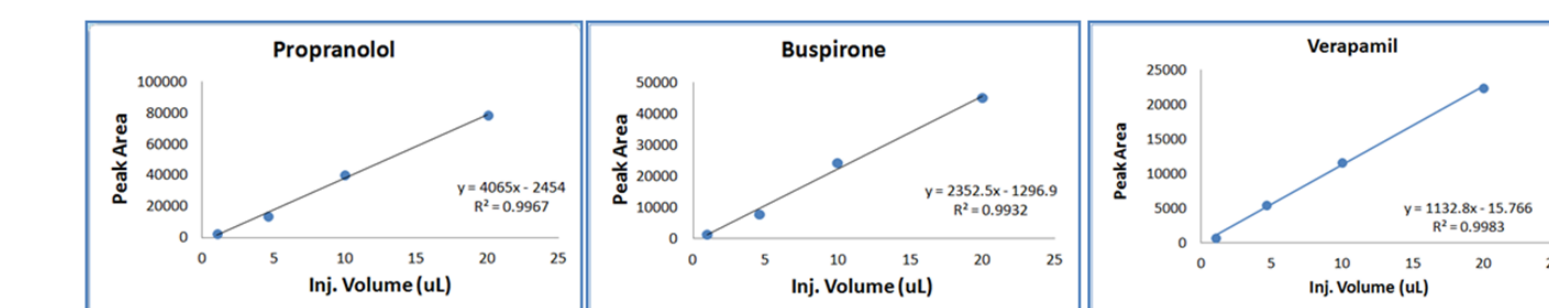


(Left) The same sample using single pump trap-and-elute. The peak shape of both early eluting buspirone and propranolol and late eluting clopidogrel are excellent.

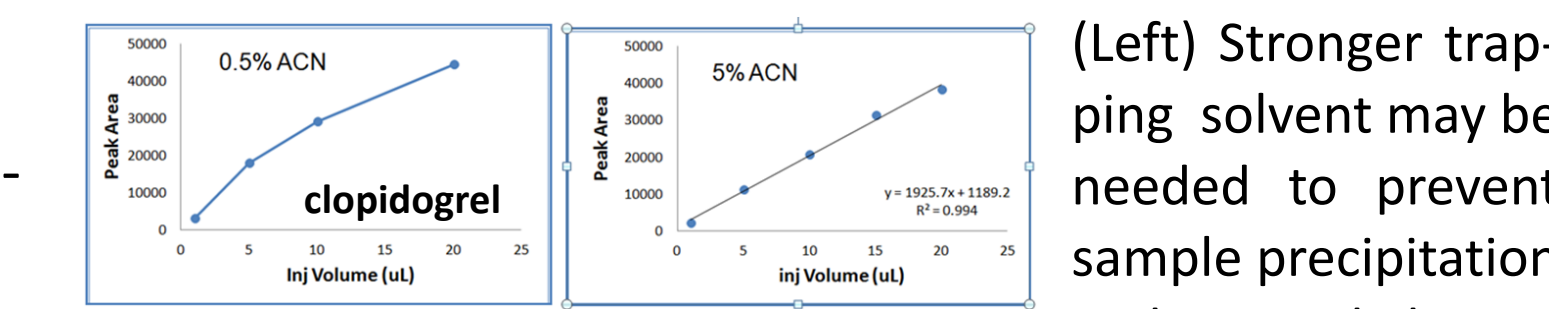
## Dual Pump Trap-and-Elute Diagram



(Above) Overlaid XIC of samples at 1, 5, 10, and 20  $\mu$ L injection. Peak resolution and peak shape were maintained for all volumes injected. The iKey can handle 20  $\mu$ L injection of human plasma sample with no adverse effect on peak shape or resolution. It should be noted that injection 20  $\mu$ L onto iKey is equivalent to injecting 4 mL onto a 2.1 mm i.d. x 50 mm analytical column!!



(Above) Plot of injection volume versus peak area. A linear response indicates complete sample recovery.



(Left) Stronger trapping solvent may be needed to prevent sample precipitation in the sample loop.

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