

Application of a simple 2D-LC-MS/MS system to control ion suppression effects from DBS samples



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- **Background: Limitations with discovery generic sampling**
- **“Generic” 2D LC setup**
- **Data and results**
- **Summary**



- **FTA Elute proved to be the best generic performer**
 - 91% of compounds obtained 1ng/mL.
 - 91% of compounds had A&P within 20% of nominal concentration.
 - 92% average extraction recovery.
- **Majority of matrix effects observed originated from the paper and not the blood**
 - High concentration of chemical on paper can smear through column and suppress signal in early part of chromatogram.
 - More polar the compound greater the suppression.
- **Development of 2D-HPLC systems improved assay performance and modified matrix effects to acceptable levels**
 - uHPLC trapping system* and adaptation of Turboflow systems.

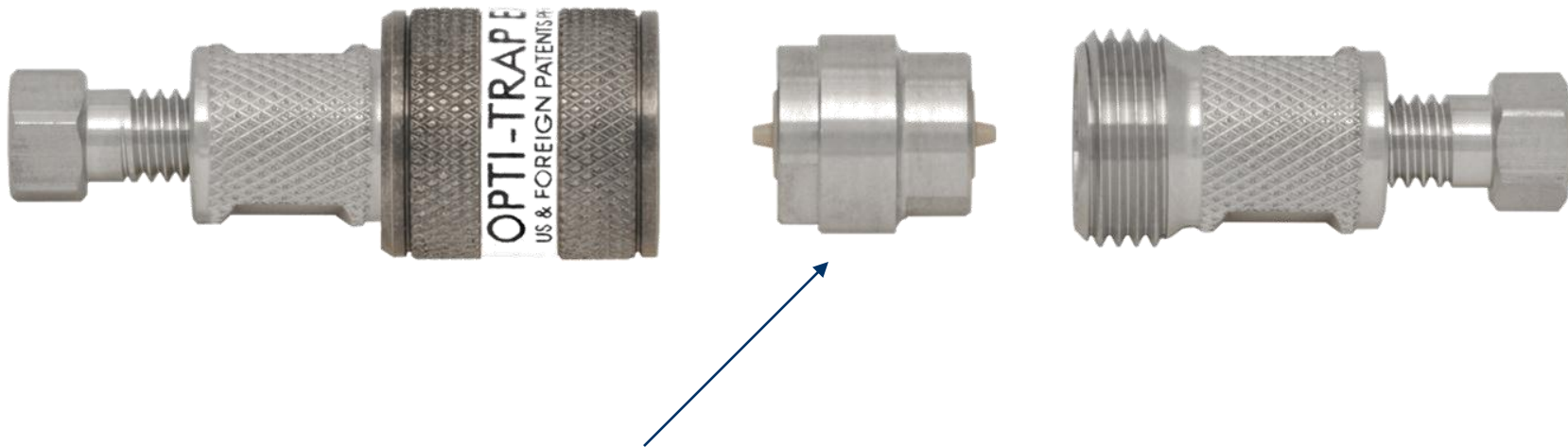


* Graeme T Clark, Julian J Haynes, Mark A J Bayliss, Lisa Burrows (2010).
Bioanalysis, Vol. 2, No. 8, 1477-1488.

High Pressure Uniform Particles Trap (Halo)



(U.S. and Foreign Patents Pending)

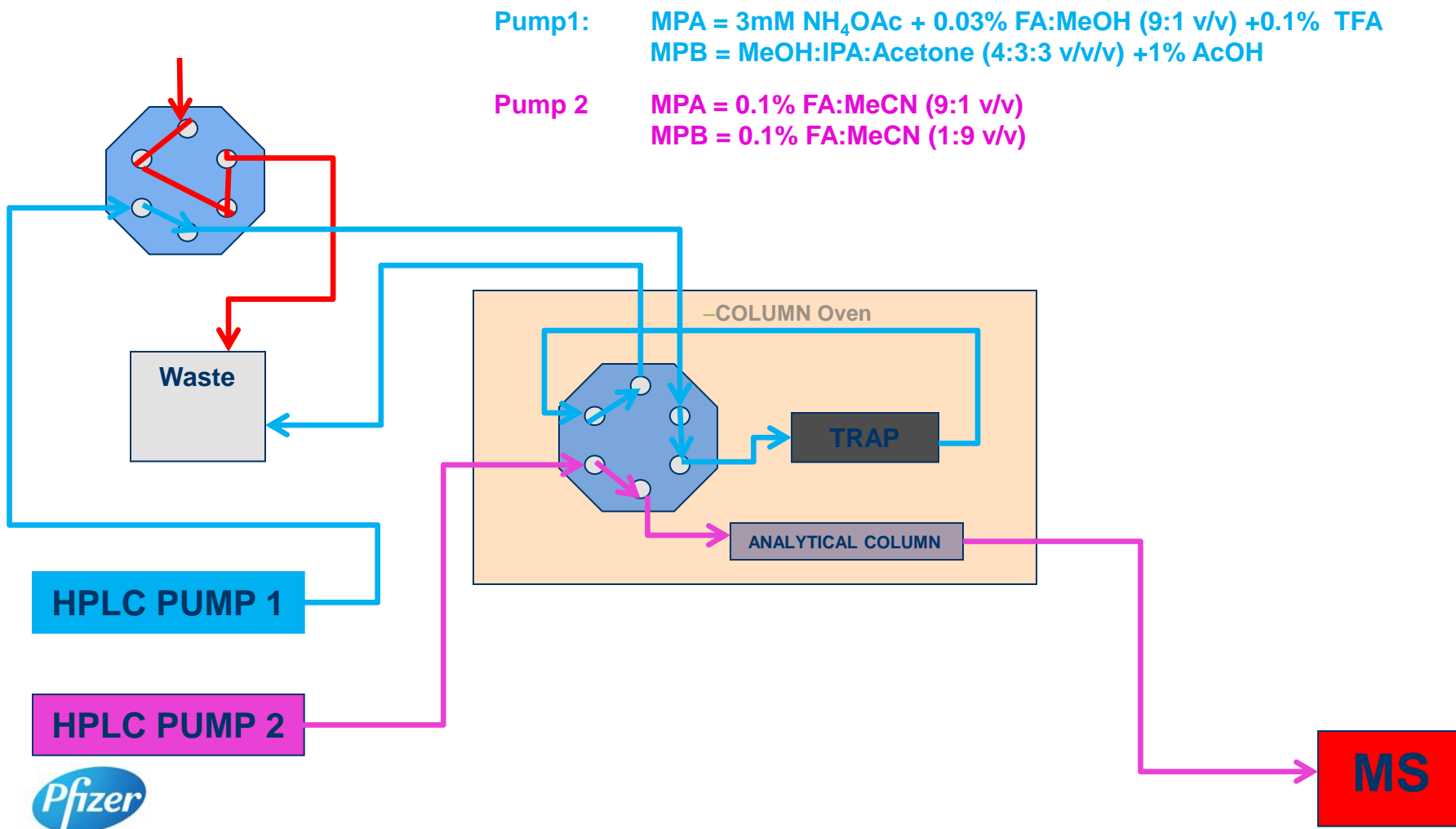


4.6 x 5 mm Halo™ C18 trap cartridge



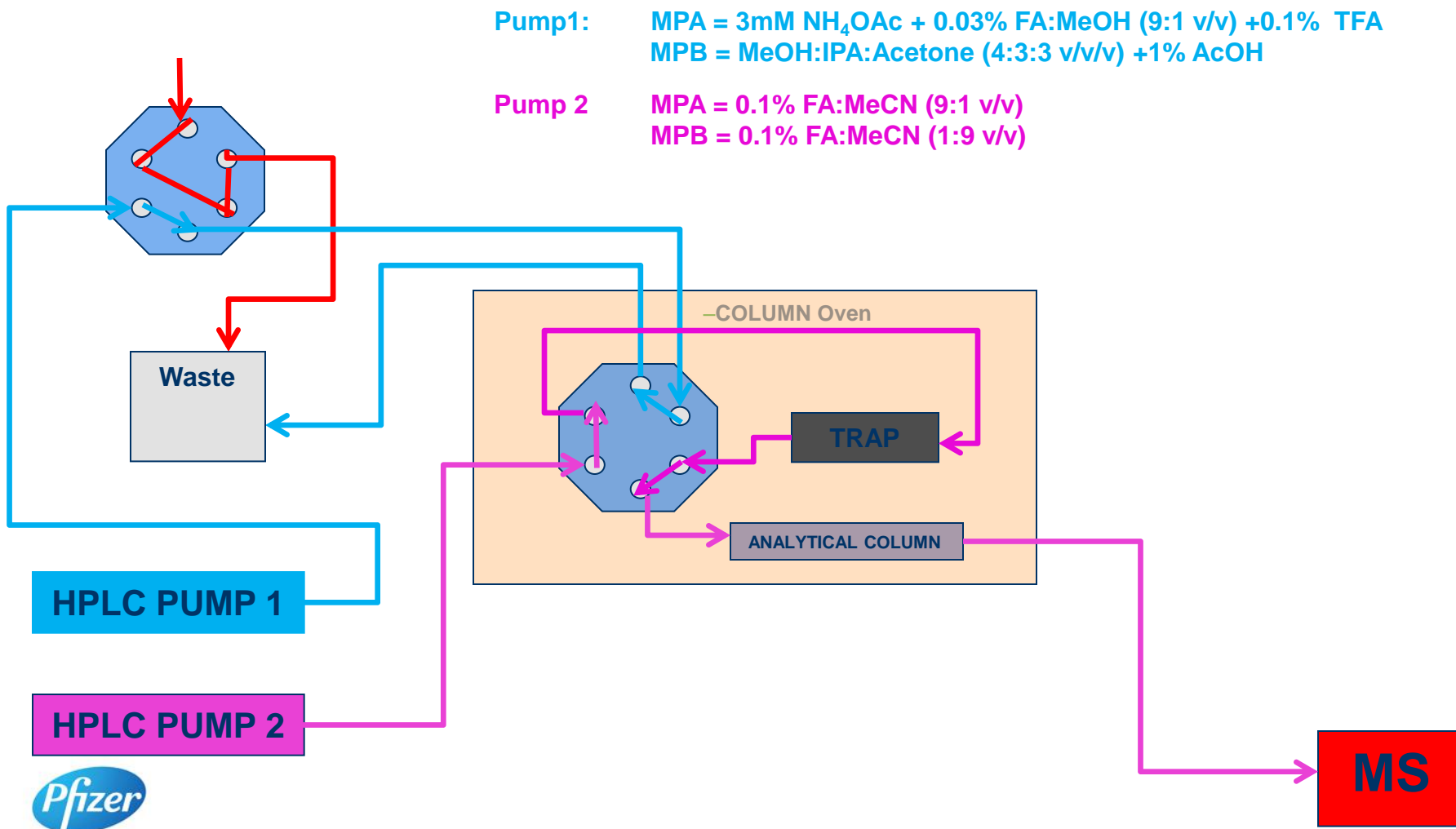


2D LC Trap Loading Configuration





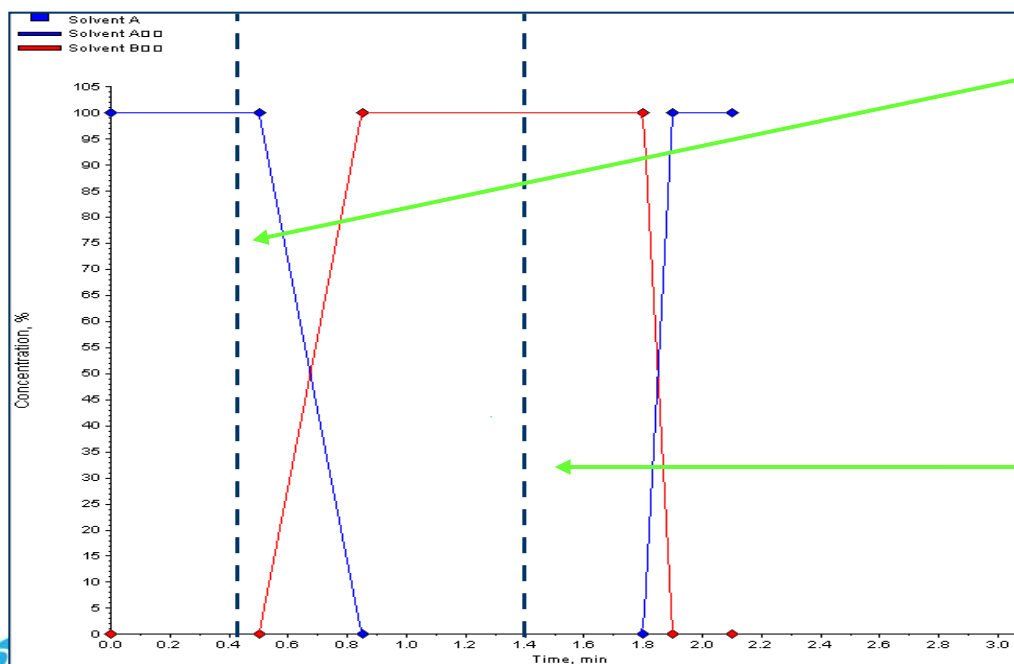
2D LC Trap Eluting Configuration





LC Gradient and Trap Alignment

- All the analytics were performed on a Fused Core C18 (or XB-C18) 3 x 50 mm, 1.8 μ column. Either directly or back flushed from a Halo trap onto this column
- A “Generic” rapid gradient (from 10% Acetonitrile (mobile phase A) to 90 % Acetonitrile (mobile Phase B)) was run through the analytical column to elute the analytes of interest as shown below.

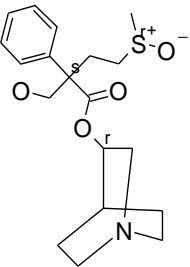
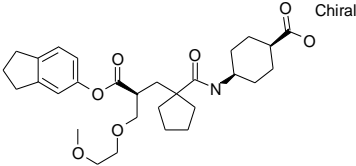
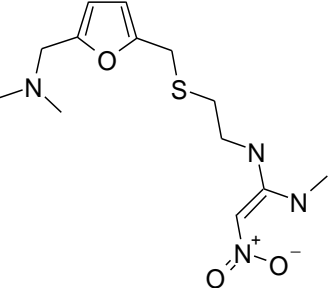
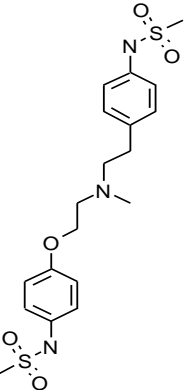
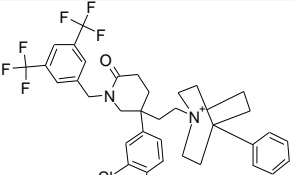
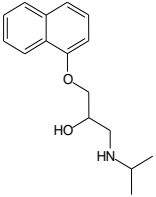
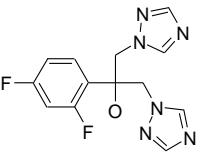
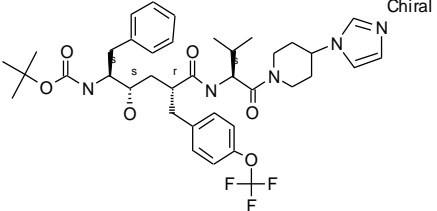
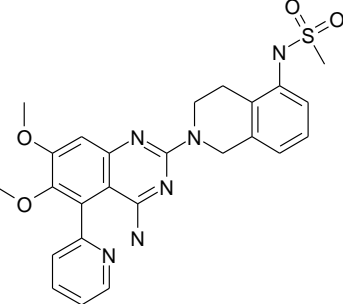
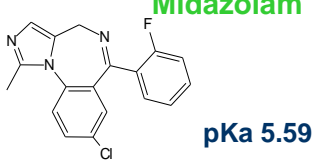
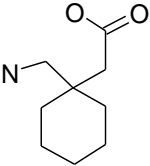


Trap switched in line

Trap switched back out of line

Test Compound Mix for Matrix Evaluation

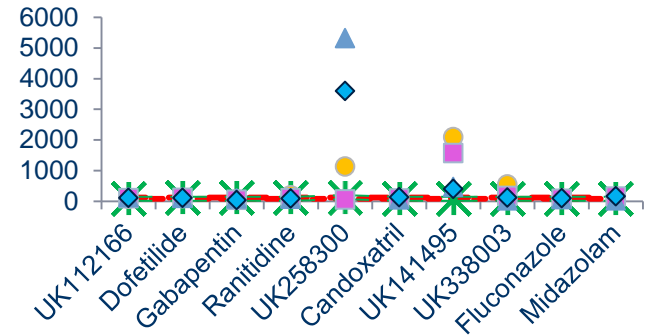
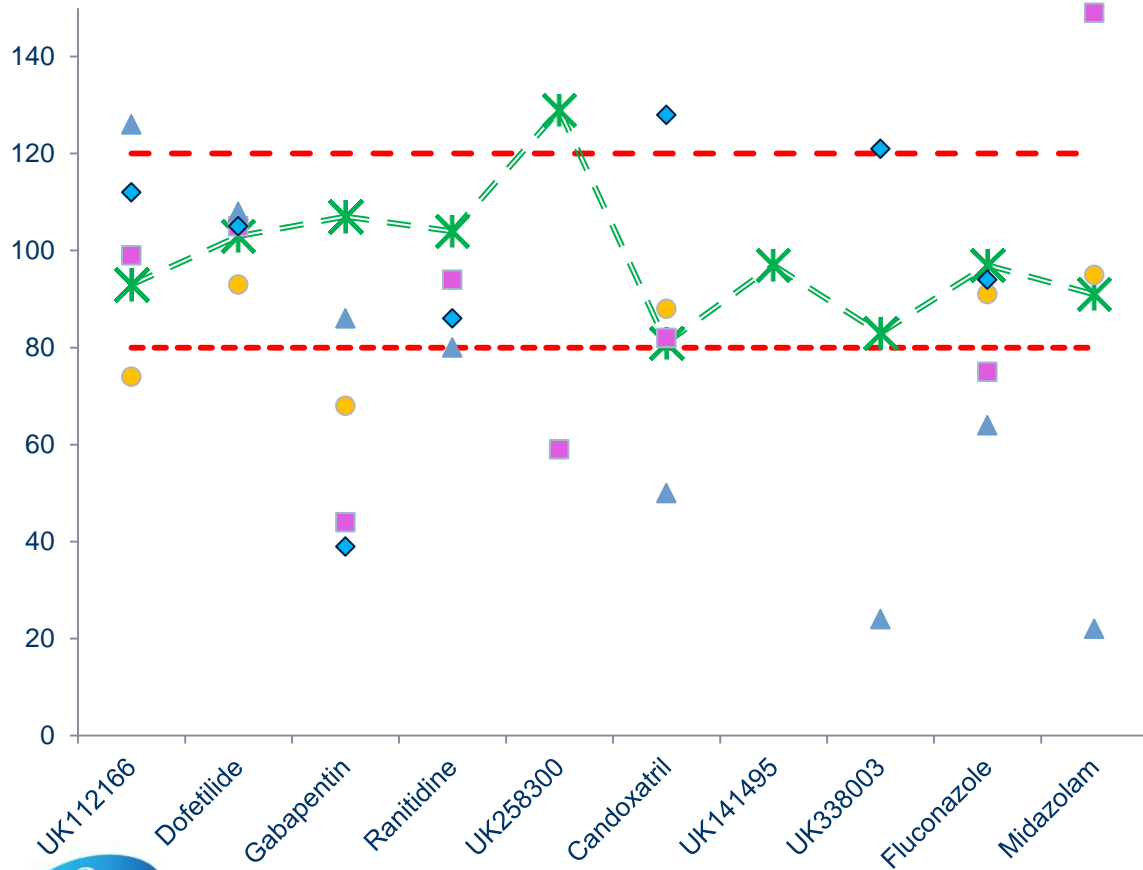


 <p>UK-112166 pKa 8.6/4.9 cLogP -0.05 Mw 365</p>	 <p>Candoxatril pKa 4.5 cLogP 2.94 Mw 515</p>	 <p>Ranitidine pKa 8.18/2.28 cLogP 1.28 Mw 314</p>
 <p>Dofetilide pKa 7 / 9.2 cLogP 1.56 Mw 441</p>	 <p>UK-258300 pKa NA, Mw 682 cLogP 4.2</p>	 <p>Propranolol (IS) pKa 13.84/9.14 cLogP 3.1 Mw 259</p>
 <p>Fluconazole pKa 4.5 / 5.3 cLogP -0.11 Mw 306</p>	 <p>UK-141495 pKa 6.1 cLogP 3.94 Mw 730</p>	 <p>UK-338003 pKa 10.32/9.29 cLogP 0.69 Mw 506</p>
 <p>Midazolam pKa 5.59 cLogP 3.93, Mw 325</p>	 <p>Gabapentin pKa 3.7 cLogP 1.19 Mw 171</p>	

DBS Matrix Effects in 1 and 2D LC



% Matrix Effects From Dried Blood Spot (Focussed Scale)



- 1D XBC18 pH 3.0
- 1D XBC18 pH 8.0
- ▲ 1D C18 pH 3.0
- ◆ 1D C18 pH 8.0



System Suitability Table for Compound Assay



Collectively 8 of the 10 compounds could be run using the 1D generic C18 systems.

Optimisation across all 4 generic settings is required to obtain this and still leaves 2 failed and one with some minor matrix effects.

2D LC generates conditions on a single generic setup for all 10 compounds with only one having any minor matrix effects.

2D LC additionally negates any reconstitution solvent optimisation as even neat methanol can be injected with no detrimental peak performance*

Conditions	Chromatography System	1D LC-MS/MS				2D LC-MS/MS		
	Reconstitution Solvent	95/5 v/v H ₂ O/MeOH		80/20 v/v H ₂ O/MeCN		80/20 v/v H ₂ O/MeCN		
	Analytical Column	XB-C18		C18		XB-C18		
		pH 3.0	pH 8.0	pH 3.0	pH 8.0	pH 3.0	pH 8.0	
Compound	UK112166	✓	✓	✓	✓	✓	-	✓
	Dofetilide	✓	✓	✓	✓	✓	✓	✓
	Gabapentin	-	-	✓	-	-	-	✓
	Ranitidine	-	-	✓	✓	-	-	✓
	UK258300	-	-	-	-	-	-	✓
	Candoxatril	✓	✓	-	✓	✓	✓	✓
	UK141495	-	-	-	-	-	-	✓
	UK338003	-	-	-	✓	-	-	✓
	Fluconazole	✓	✓	-	✓	✓	✓	✓
	Midazolam	✓	-	-	-	-	-	✓



Matrix effects from both the paper and DBS are within ± 20% of nominal peak area (from solvent) and as such can be considered negligible



Matrix effects from both the paper and DBS are within ± 30% of nominal peak area (from solvent) and as such can be considered manageable

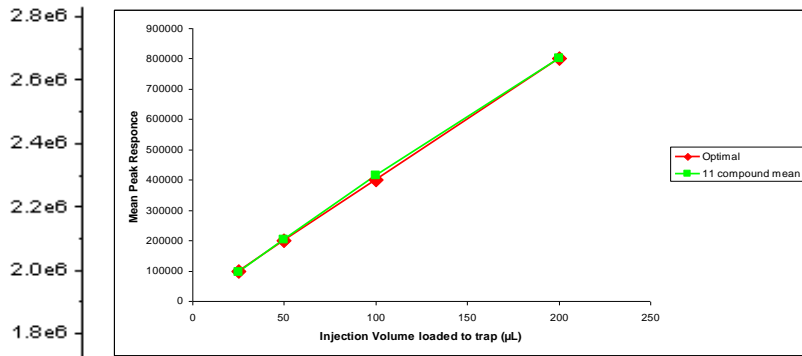
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Matrix effects from both the paper and DBS are > 30% of nominal peak area (from solvent)



* Recovery from the trap can be lower in strong solvents for Polars

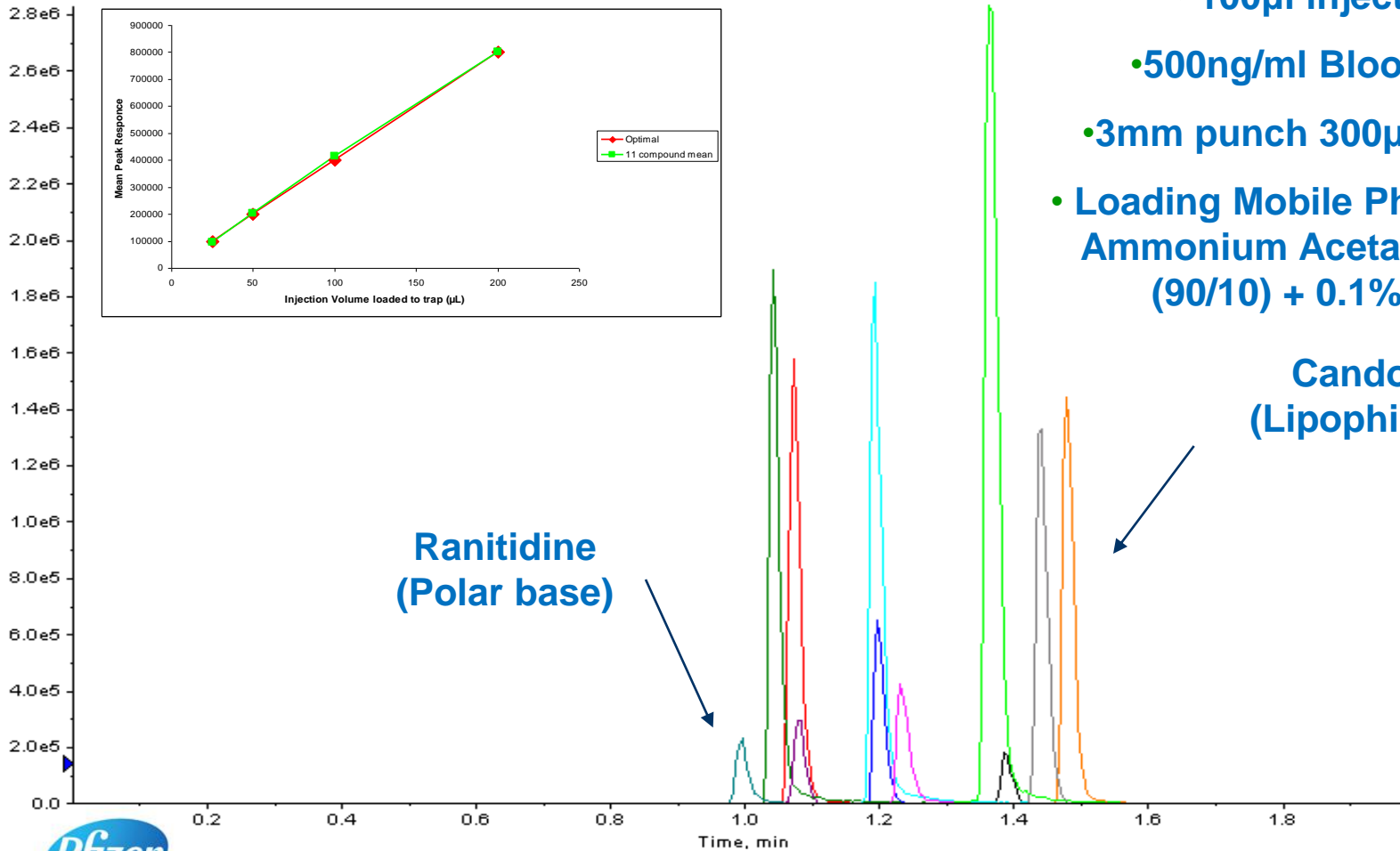
Example DBS Chromatogram



- 100µl injection
- 500ng/ml Bloodspot
- 3mm punch 300µL Recon
- Loading Mobile Phase: 2mM Ammonium Acetate/MeOH (90/10) + 0.1% TFA

**Cadoxatril
(Lipophilic acid)**

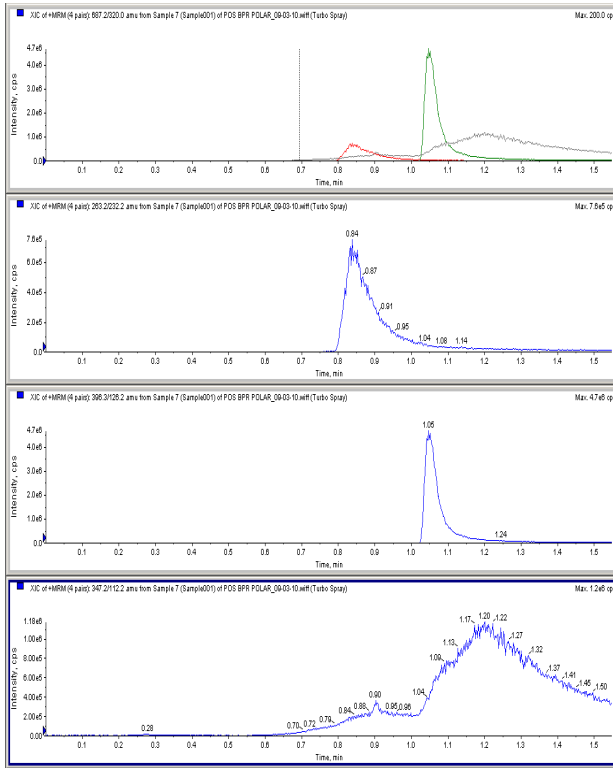
**Ranitidine
(Polar base)**



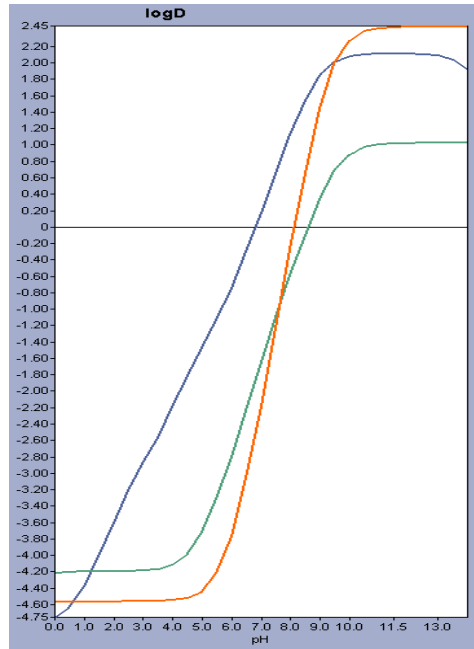
System Limits



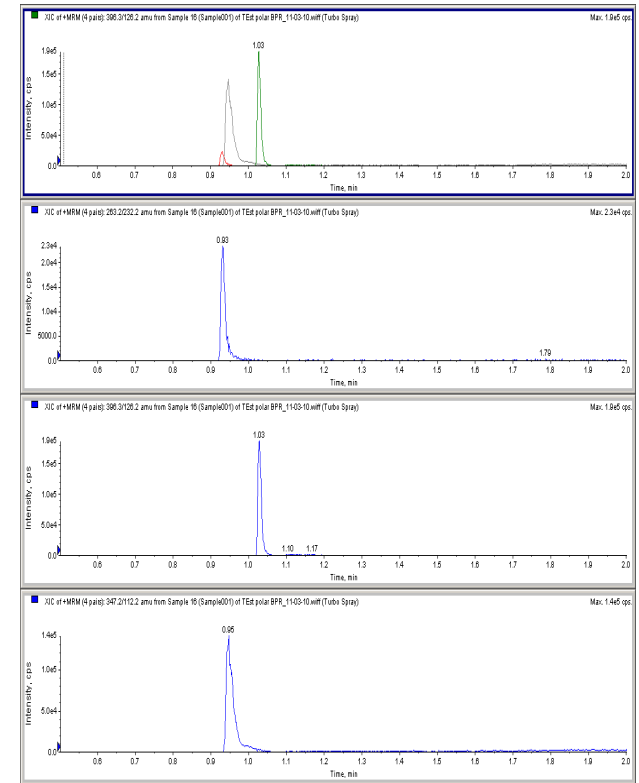
Direct injection in ~pH 3 mobile phase



Log D plot of trio of Polar compounds



2D LC injection in ~pH 3 mobile phase





- **Over 250 Studies analysed by DBS**

Regularly hitting 1 ng/mL with a 3mm cut from spot with quantitative recovery and negligible matrix effects

- **Over 2000 unique compounds Assayed in HT-ADME screening**

HT-ADME screening have been using this same system for the last year and in that timeframe less than 1% of all submissions have failed to adequately resolved with this generic 2D system

- **Chemical space Limitations**

$\text{clogD} \sim -4$ to 9.5 have all successfully been assayed (only real limits being extremely polars (sub -4 clogD) and very small polars)

