



Application Note 301

Direct Plasma Injection for DMPK Using MDLC MS/MS

A high throughput multi-dimensional HPLC system was used for direct injection of plasma samples on to a denaturing size exclusion column (DSEC) to remove all of the high MW matrix components which normally interfere with MS analyses. The MW range from 200-2000 containing the drugs and metabolites of interest was heart cut from the DSEC column, desalted and then analyzed by ballistic gradient RPLC using SRM on a Triple Quad MS/MS for both qualitative and quantitative analyses. With a total cycle time of 2.5 minutes per assay, up to 576 (6x96) DMPK samples can be run per day.

Introduction

Although SRM MS allows very selective analysis of drugs, sample matrix interferences can clog the chromatography column and/or contaminate the MS source (impacting throughput and robustness), as well as causing signal suppression (impacting quantitation). In physiological fluid samples, the major interferences are molecules over 2000 MW (biomolecules) and less than 200 MW (salts). In DSEC, the mobile phase helps solubilize sample components, dissociates protein-drug interactions, eliminates secondary interactions (hydrophobicity, charge) and provides true separation by MW.

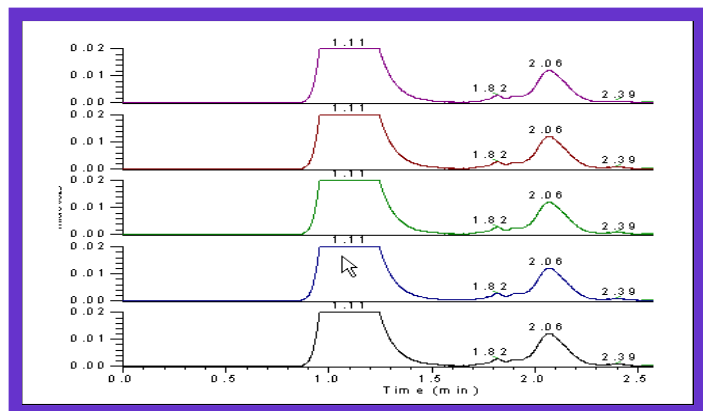


Figure 1: DSEC UV (280nm) traces of five 100ul spiked plasma samples run at 4 hour intervals, with eluant from 1.9-2.3 min heartcut to SM trap column for desalt prior to D2 RPLC SRM MS.

Results

Using this 2DLC direct injection method, six 96 well plates of samples can be analyzed in 24 hours. Figure 1 shows the DSEC UV (280nm) traces of spiked plasma samples from five of the plates and confirms excellent reproducibility over 24 hours. The heart cut small molecule MW range of interest (200-2000) is then analyzed by RP ballistic gradient LC MS/MS and the resulting SRM traces for a spiked plasma sample (10 ng/ml) run using this 2DLC direct injection method is shown in Figure 2.

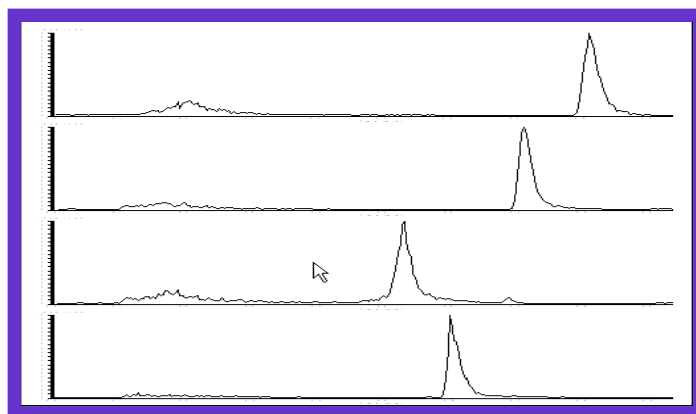


Figure 2: RPLC SRM MS traces of a spiked plasma sample (10 ng/ml) showing the parent drug candidate (top trace), two metabolites (middle traces) and internal standard (bottom trace) separated by RP ballistic gradient LC in 2 minutes.

Conclusions

The Paradigm HTC PAL and HT MDLC coupled with a triple quad MS is a powerful system for scientists working in ADMET or DMPK at pharmaceutical companies or contract labs. The Paradigm HTC PAL allows rapid injection of up to 576 (6x96) plasma samples which can then be separated on the Paradigm HT MDLC prior to SRM MS analysis. This MDLC method for direct plasma injection is ideal for scientists who need high sensitivity, high throughput and highly reproducible results for both preclinical and clinical applications.

Hardware Used in This Application

<u>Description</u>	<u>Part Number</u>
Paradigm HTC PAL Autosampler	AS3/00000/00
Paradigm MS4B High Throughput MDLC	MS4/00000/03
Paradigm 1 λ Micro/Anal UV-Vis Detector	MD1/00000/00
Triple Quadrapole Mass Spectrometer	
2DLC (DSEC-RP) Micro (20-100ul) DMPK LC/MS Kit	AK1/00002/03
Michrom General Purpose Guard Column	
5u 200A Magic DSEC Column (2.0 x 150 mm)	
DSEC Buffer Concentrate (100 ml)	
2x Small Molecule Micro Traps and Holders	
5u 200A Magic C18 Micro Magic Bullet and Holder	
AK1/00002/03 Plumbing/Fittings/Instruction Kit	
2DLC (DSEC-RP) Macro (100-500 ul) DMPK LC/MS Kit	AK1/00002/04
Michrom General Purpose Guard Column	
5u 200A Magic DSEC Column (4.6 x 150 mm)	
DSEC Buffer Concentrate (100 ml)	
2x Small Molecule Macro Traps and Holders	
5u 200A Magic C18 Macro Magic Bullet and Holder	
AK1/00002/04 Plumbing/Fittings/Instruction Kit	

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