[APPLICATION NOTE]

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Separation of Diastereomeric Chiral Metabolites Using UPC² MS/MS

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APPLICATION BENEFITS

The combination of UPC² and tandem quadrupole mass spectrometry provides for a means to produce fast, high resolution separations of chiral compounds in the DMPK laboratory.

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ACQUITY UPC²™ System

Xevo® TQ-S MS

MassLynx® Software

KEY WORDS

UPC², tandem quadrupole MS, chiral, bioanalysis, drug metabolism, DMPK

INTRODUCTION

The separation and detection of GSK1322322, a novel antibacterial agent, and three associated stereoisomer metabolites was investigated utilizing UltraPerformance Convergence Chromatography™ (UPC²®) coupled with tandem quadrupole mass spectrometry. This separation was attempted on various other platforms with no success (UV, chiral HPLC, mass spec).

There have been numerous examples of metabolism creating new centers of chirality in new chemical entities (NCEs). This chiral inversion can have pharmacokinetic, pharmacodynamics, and safety consequences.[1-3] Therefore because of the heightened awareness of stereoselective metabolism, there are growing government requirements and regulations in chiral metabolite identification and quantification during drug development.

Recent advances have potentially opened the door for the use of UPC²/MS/MS in routine bioanalysis with chiral entities, in a regulated environment. UPC² applies the performance advantages of UPLC to supercritical fluid chromatography, using supercritical carbon dioxide as the major mobile phase. The results presented here explore the robustness of this technique and its application to clinical study samples in the quest to investigate *in vivo* chiral inversion.

EXPERIMENTAL

Chromatography conditions

System:	ACQUITY UPC^2
Column:	Chiral Pak AD-H, 5-µm, 4.6 x 150 mm
ABPR pressure:	2750 PSI
Column temp.:	40 °C
Sample temp.:	Ambient
Injection vol.:	5 µL
Flow rate:	3 mL/min
Mobile phase:	CO_2/isopropanol with 0.4% diethylamine (80/20)
Run time:	10 minutes

MS conditions

MS system:	Xevo TQ-S
Ionization mode:	ESI +
Acquisition mode:	MRM
Capillary voltage:	4 kV
Collision energy:	30 V
Cone voltage:	25 V

Data management

MassLynx 4.1 Software

Sample preparation

GSK1322322, GSK1343981, GSK1785312, and GSK1784667 were extracted from 100 µL human plasma by protein precipitation using acetonitrile containing [$^2H_2$ $^{13}C_2$] –GSK1322322 as an internal standard, followed by derivatization with camphanic chloride (1 mg/mL in acetontrile) for 15 minutes at 37 °C. Extracts were analyzed by UPC^2/MS/MS using an electrospray interface and multiple reaction monitoring (MRM).

RESULTS AND DISCUSSION

As stated in the introduction, the separation of GSK1322322 and associated stereoisomer metabolites was attempted on a variety of other platforms. One such method that was produced was a 45-minute chiral HPLC-UV method using heptane/ethanol/DEA and formic acid. However this method was neither compatible with nor ideal in a mass spectrometer-based DMPK bioanalytical environment due to the long run time and the organic mobile phase solvents required for the separation. The separation further produced wide peak widths on the order of 2- 5 minutes at the peak base. Figure 1 illustrates the separation that was produced by this method.

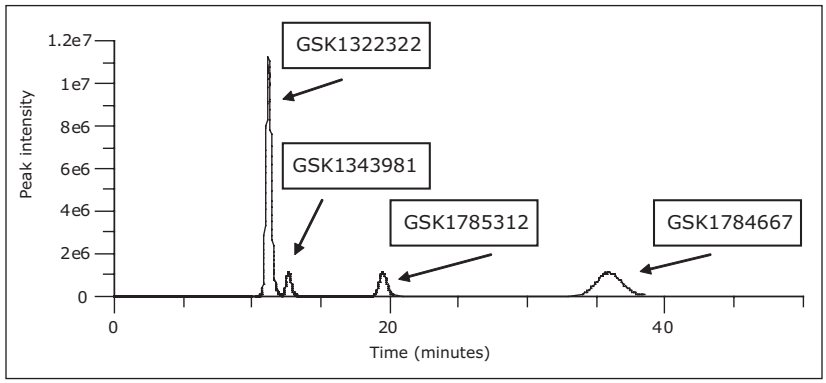

Figure 1. Normal-phase HPLC method of compound GSK 1322322 and three associated stereoisomer metabolites.

Due to the undesirable attributes of the normal-phase separation and the inability for reversed-phase to resolve all of the components, the analytes were then analyzed by UPC^2/MS/MS. As can be seen in Figure 2, the UPC^2/MS/MS produced a separation with reduced analysis time with much improved peak shape with near baseline resolution of all analytes.

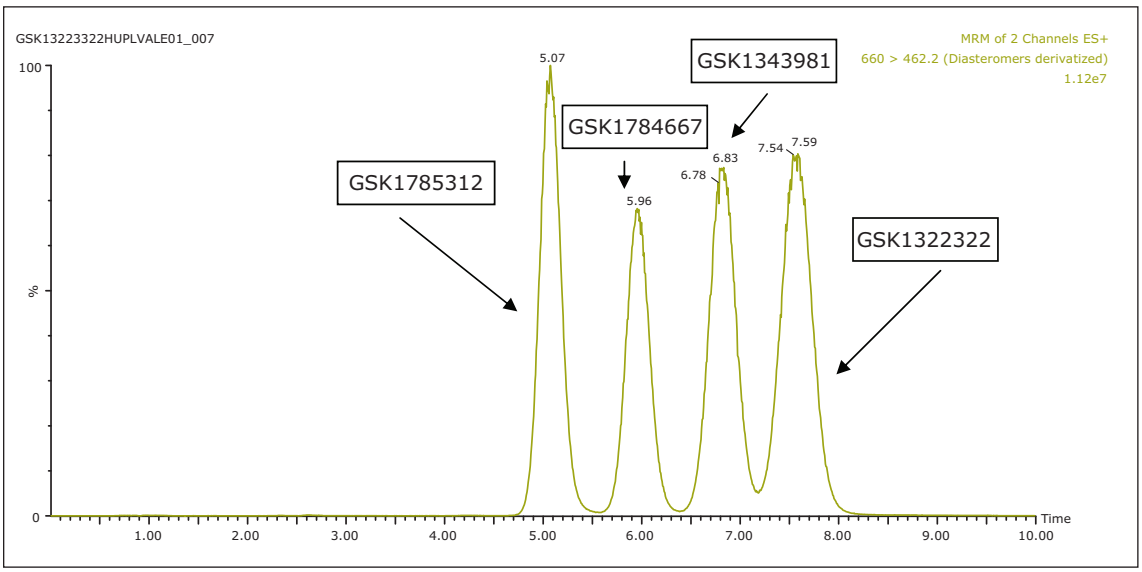

Figure 2. UPC^2/MS/MS separation of parent drug GSK1322322 and associated stereoisomer metabolites.

Samples from pooled preclinical and clinical plasma samples where then analyzed using the UPC^2/MS/MS method over a concentration range of 5 to 5000 ng/mL. Figure 3 shows the plasma blank along with the LLOQ and ULOQ for all of the four analytes under investigation. A three-day validation study was then performed to assess the precision and accuracy of the method. Table 1 shows the results of the validation study. The results from assay validation show that the method is rugged, precise, accurate, and well-suited to support the analysis of plasma samples.

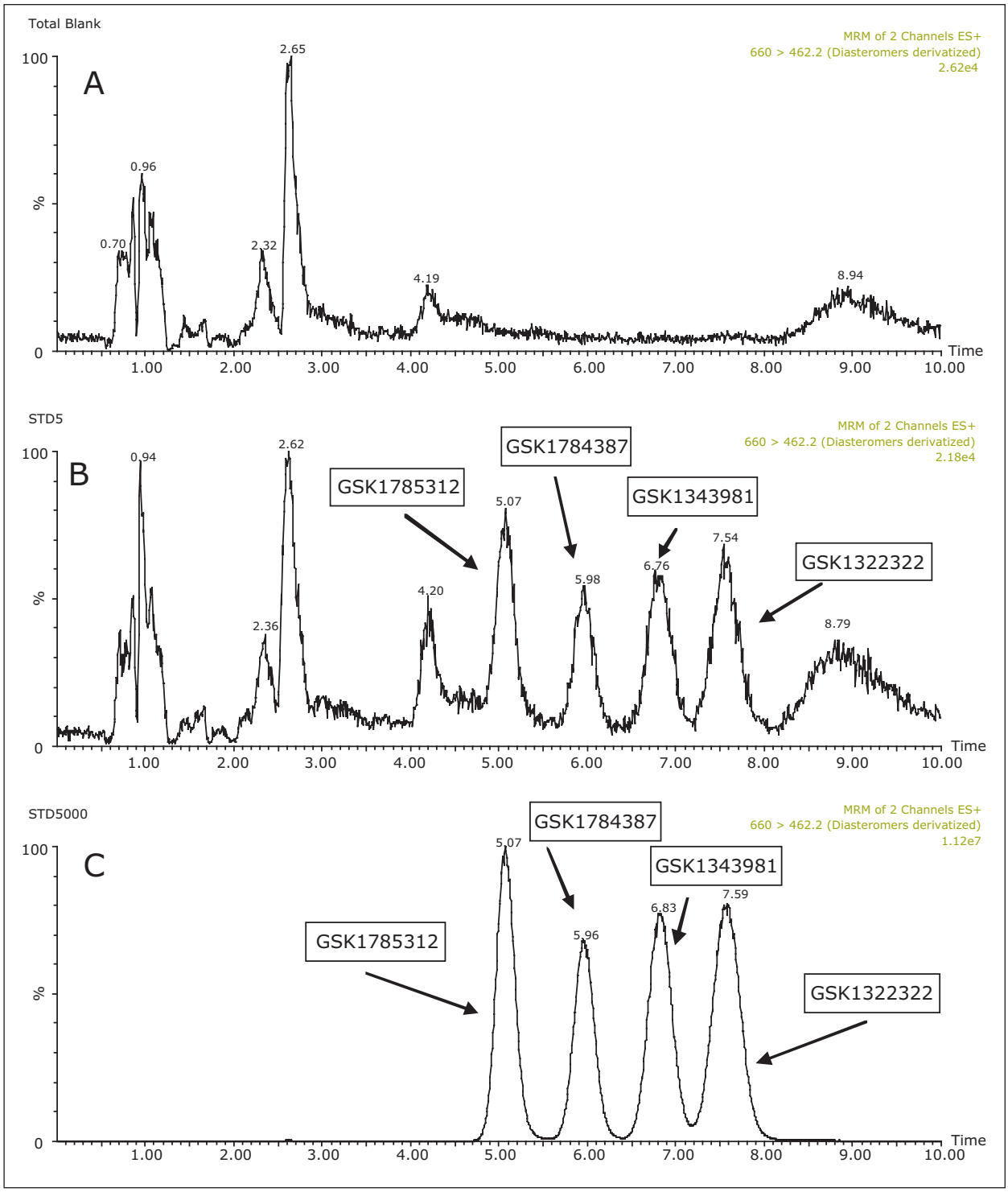

Figure 3. A) Plasma blank. B) LLOQ 5 ng/mL. C) ULOQ 5000 ng/mL.

			GSK1322322 (Parent Molecule)		
Mean (ng/mL) n=18	4.72	14.46	247.76	3956.5	4859.67
Precision (%CV)	5.6	4.7	2.4	1.3	1.2
Bias %	-5.6	-3.6	-0.9	-1.1	-2.8
Between-run Precision (%)	2.1	4.0	0.5	0.3	0.8
			GSK1343981 (Diasteriomer)		
Mean (ng/mL) n=18	4.63	14.98	247.17	3936.67	4843.79
Precision (%CV)	5.1	2.8	2.1	1.9	1.7
Bias %	-7.4	-0.1	-1.1	-1.6	-3.1
Between-run Precision (%)	3.9	Negligible	1.1	1.2	1.8
			GSK1785312 (Diasteriomer)		
Mean (ng/mL) n=18	4.86	15.27	246.72	3830.84	4697.48
Precision (%CV)	5.6	4.0	2.3	2.6	2.0
Bias %	-2.8	1.8	-1.3	-4.2	-6.1
Between-run Precision (%)	4.2	2.3	1	1.7	1.9
			GSK1784667 (Enantiomer)		
Mean (ng/mL) n=18	4.77	15.02	244.72	3883.63	4771.53
Precision (%CV)	3.6	2.9	2	2.0	0.9
Bias %	-4.7	0.2	-2.1	-2.9	-4.6
Between-run Precision (%)	Negligible	1.2	0.9	Negligible	Negligible

Table 1. Validation statistics for GSK1322322 and three associated stereoisomer metabolites.

CONCLUSIONS

UPC^2 coupled with tandem quadrupole mass spectrometry was successfully applied in the investigation of stereoselective metabolism in a DMPK environment.

The UPC^2/MS/MS method was successfully validated over a three-day period for the parent compound GSK1322322 and the three diastereomeric metabolites from pooled clinical and preclinical dog plasma samples.

References

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