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Next generation plasma collection technology for the clinical analysis of temozolomide by HILIC/MS/MS

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Alan J. Barnes[1], Carrie-Anne Mellor[2],
Adam McMahon[2], Neil Loftus[1]
[1]Shimadzu, Manchester, UK
[2]2WMIC, University of Manchester, UK

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Introduction

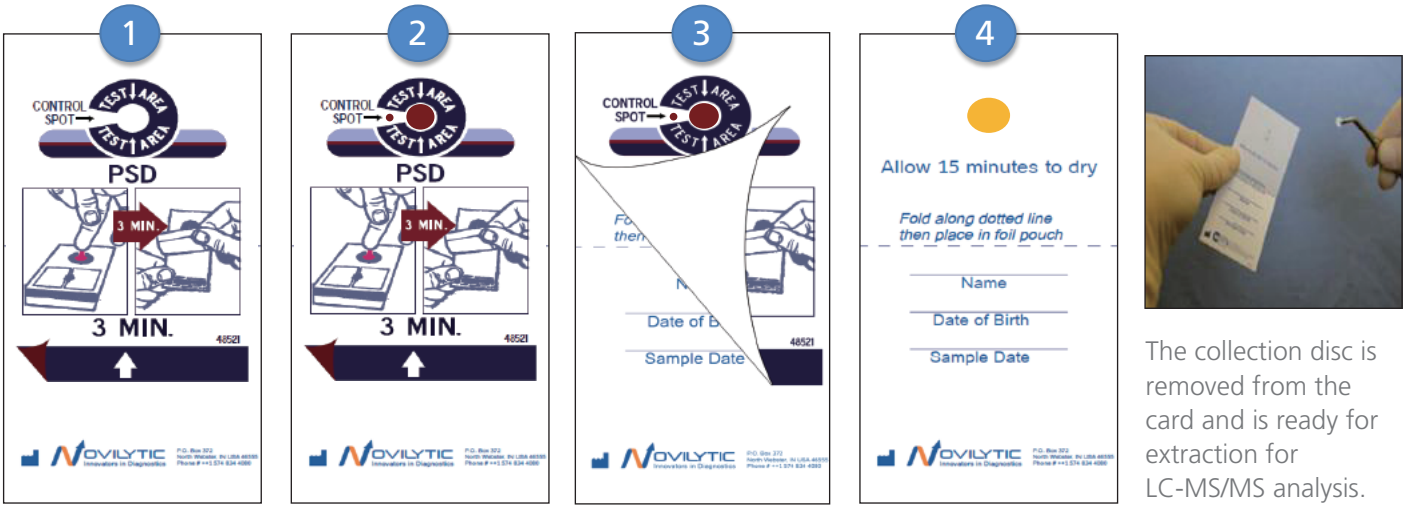
Plasma extraction technology is a novel technique achieved by applying a blood sample to a laminated membrane stack which allows plasma to flow through the asymmetric filter whilst retaining the cellular components of the blood sample.
Plasma separation card technology was applied to the quantitative analysis of temozolomide (TMZ); an oral imidazotetrazine alkylating agent used for the treatment of Grade IV astrocytoma, an aggressive form of brain tumour.

Under physiological conditions TMZ is rapidly converted to 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) which in-turn degrades by hydrolysis to 5-aminoimidazole-4-carboxamide (AIC). Storage of plasma has previously shown that both at -70C and 4C degradation still occurs. In these experiments, whole blood containing TMZ standard was applied to NoviPlex plasma separation cards (PSC). The aim was to develop a robust LC/MS/MS quantitative method for TMZ.

Materials and Methods

Plasma separation

TMZ spiked human blood calibration standards (50uL) were applied to the PSC as described below in figure 1.



A NoviPlex card is removed from foil packaging.

Approximately 50uL of whole blood is added to the test area.

After 3 minutes, the top layer is completely removed (peeled back).

The collection disc contains 2.5uL of plasma. Card is air dried for 15 minutes.

The collection disc is removed from the card and is ready for extraction for LC-MS/MS analysis.

Figure 1. Noviplex plasma separation card workflow

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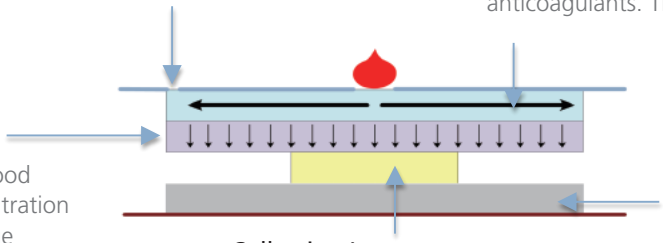
Control Spot:
[Determines whether enough blood was placed on the card].

Spreading Layer
[Lateral spreading layer rapidly spreads blood so it will enter the filtration layer as a front while adding buffers and anticoagulants. The lateral spreading rate is 150um/sec].



Filtration Layer
[Filtration layer captures blood cells by a combination of filtration and adsorption. The average linear vertical migration rate is approximately 1um/sec].

Isolation Screen
[Precludes lateral wicking along the card surface].

Collection Layer
[Loads with a specific aliquot of plasma onto a 6.35mm disc]. Although flow through the filtration membrane is unlikely to be constant throughout the plasma extraction process, the average loading rate of the Collection Disc was 13 nL/sec. This corresponds to a volumetric flow rate into the Collection Disc of 400 pL/mm^2/sec.

Figure 1. Noviplex plasma separation card workflow (Cont'd)

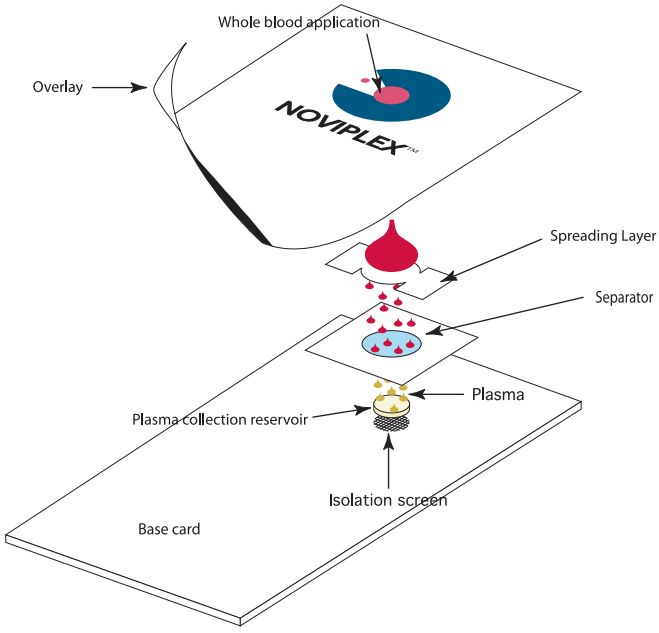


Figure 2. Applying a blood sample, either as a finger prick or by accurately measuring the blood volume, to the laminated membrane stack retains red cells and allows a plasma sample to be collected. The red cells are retained by a combination of adsorption and filtration whilst plasma advances through the membrane stack by capillary action. After approximately three minutes the plasma Collection Disc was saturated with an aliquot of plasma and was ready for LC/MS/MS analysis.

Sample preparation

TMZ was extracted from the dried plasma collection discs by adding 40uL acetonitrile + 0.1% formic acid, followed by centrifugation 16,000g for 5 min. 30uL supernatant was added directly to the LC/MS/MS sample vial for analysis.

As a control, conventional plasma samples were prepared by centrifuging the human blood calibration standards at 1000g for 10min. TMZ was extracted from 2.5uL of plasma using the same extraction protocol as applied for PSC.

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LC/MS/MS analysis

Ionisation	: Electrospray, positive mode MRM 195.05 >138.05 CE -10
Desolvation line	: 300ºC
Drying/Nebulising gas	: 10L/min, 2L/min
Heating block	: 400ºC

HPLC	: HILIC Nexera UHPLC system	Reverse Phase Nexera UHPLC system
Flow rate	: 0.5mL/min (0-7min), 1.8mL/min (7.5min-17.5min)	0.4mL/min
Mobile phase	: A= Water + 0.1% formic acid B= Acetonitrile + 0.1% formic acid	A= Water + 0.1% formic acid B= methanol + 0.1% formic acid
Gradient	: 95% B – 30%% B (6.5 min), 30% B (7.5 min), 95% B (18 min)	5% B – 100%% B (3 min), 100% B (7 min), 5% B (10 min)
Analytical column	: ZIC HILIC 150 x 4.6mm 5um 200ª	Phenomenex Kinetex XB C18 100 x 2.1mm 1.7um 100A
Column temperature	: 40ºC	50ºC
Injection volume	: 10uL	2µL

Results

HILIC LC/MS/MS

Temozolomide is known to be unstable under physiological conditions and is converted to 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) by a nonenzymatic, chemical degradation process. Previous studies have used HILIC to analyze the polar compound and to avoid degradation in aqueous solutions.

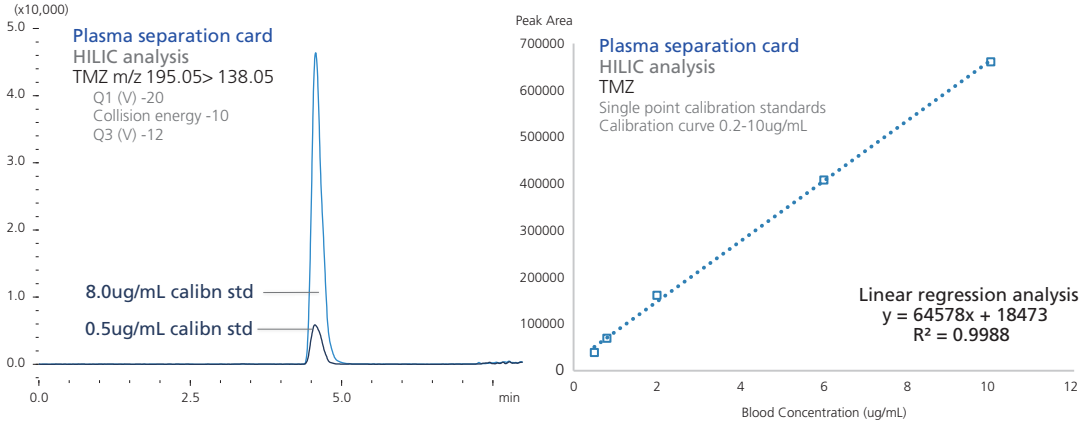

Figure 3. HILIC LC/MS/MS chromatograms for PSC TMZ analysis at 0.5 and 8ug/mL. The PSC calibration curve was linear between 0.2-10ug/mL (r2>0.99). HILIC was considered in response to previous published data and to minimize potential stability issues. However, to reduce sample cycle times a reverse phase method was also developed.

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Reversed Phase LC/MS/MS

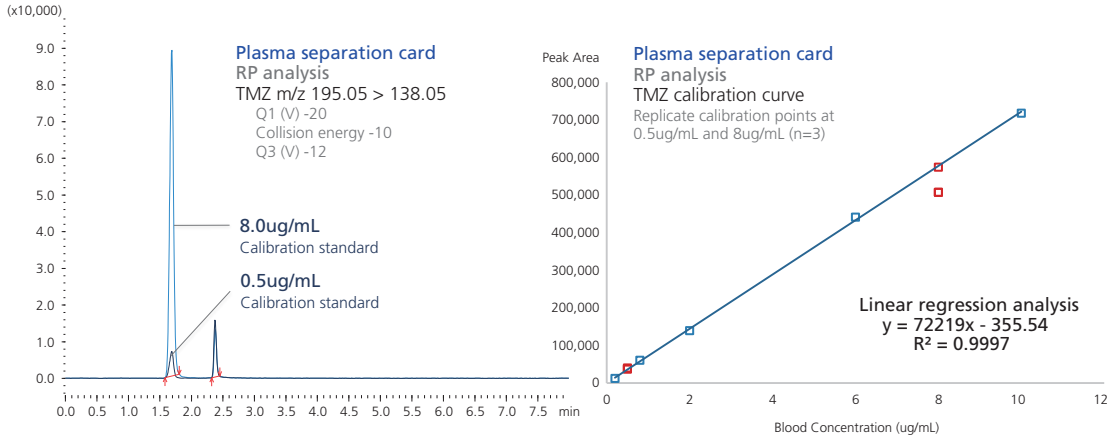

Figure 4. Reverse phase LC/MS/MS chromatograms for PSC TMZ analysis at 0.5 and 8ug/mL. The PSC calibration curve was linear between 0.2-10ug/mL (r2>0.99; replicate samples were included in the liner regression analysis at 0.5 and 8ug/mL; n=3).

Due to the relatively long cycle time (18 min), a faster reversed phase method was developed (10 min). Sample preparation was modified with PSC sample disk placed in 40uL methanol + 0.1% formic acid, followed by centrifugation 16,000g 5 min. 20uL supernatant was added directly to the LC/MS sample vial plus 80uL water + 0.1% formic acid. In addition to reversed phase being faster, the sample injection volume was reduced to just 2uL as a result of higher sensitivity from narrower peak width (reversed phase,13 sec; HILIC, 42 sec).

Comparison between PSC and plasma

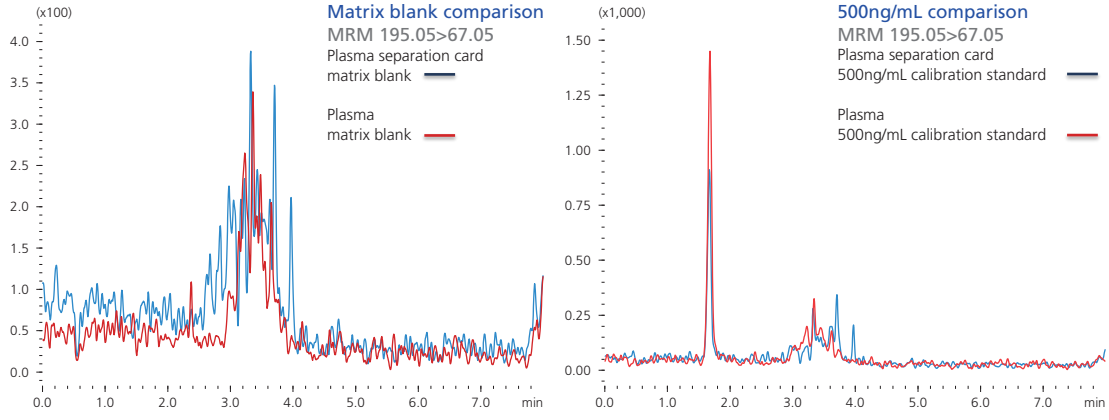

Figure 5. Human blood TMZ calibration standards were prepared using PSC and conventional plasma. Using the confirmatory ion transition 195.05>67.05 both the PSC and plasma sample are in broad agreement with regard to matrix ion signal distribution.

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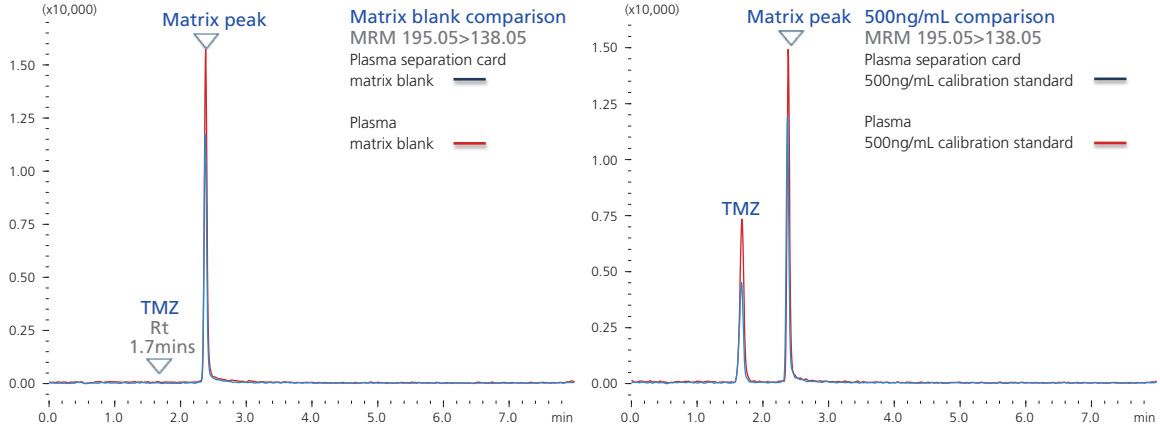

Figure 6. Human blood TMZ calibration standards were prepared using PSC and conventional plasma. Using the quantitation ion transition 195.05>138.05 both the PSC and plasma sample are in broad agreement in signal distribution and intensity including the presence of a matrix peak at 2.4mins.

Conclusions

This technology has the potential for a simplified clinical sample collection by the finger prick approach, with future work aimed to evaluate long term sample stability of PSC samples, stored at room temperature. Quantitation of drug metabolites MTIC and AIC also could help provide a measure of sample stability.

References

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