A COMPARISON OF LC-MS AND A PROTOTYPE MICROFLUIDICS-MS DEVICE IN THE METABOLITE IDENTIFICATION OF IN-VITRO AND IN-VIVO SAMPLES

IN-VITRO および IN-VIVO サンプル代謝物同定における LC-MS とプロトタイプマイクロ流路デバイスの比較

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SUMMARY

DMPK departments have frequently pushed for analytical tools that enable higher sensitivity analyses with lower detection limits for both quantitative and qualitative applications. The use of narrower bore chromatographic systems has been an approach utilized in obtaining better sensitivity evidenced by the shift over recent years from using 4.6 mm id columns to 2.1 mm id columns. Improved sensitivity would result from use of even narrower id columns, though the use of sub mm id columns in the past has been a barrier to routine use in DMPK environments.

The present study is designed to test the suitability of a prototype integrated capillary LC -MS system for the analysis of pharmaceutical small molecules.

INSTRUMENTAL PARAMETERS

Acquity System	Prototype System
2.1 x 50 mm C18 BEH	150 µm x 50 mm C18 BEH
1.7 µm particle size	1.7 µm particle size
600 µL/min	3 µL/min
1 µL injected	1 µL injected
1 column volume approx 80 µL	1 column volume approx 0.5 µL
Capillary voltage used 1 kV	Capillary voltage used 2.6 - 3.5 kV

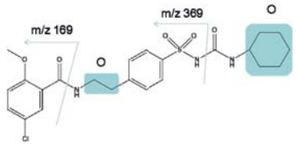
MS System used

Xevo G2-S QTof

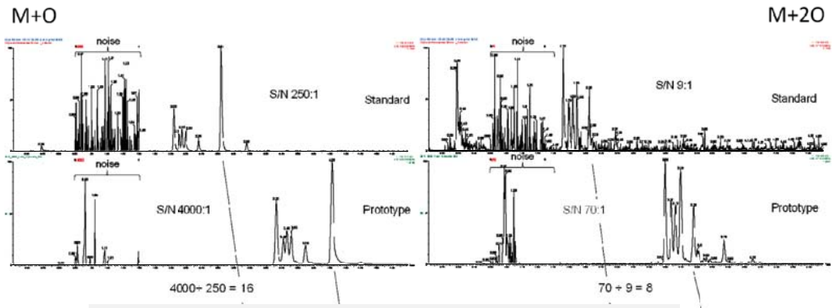
EXPERIMENTAL

Two compounds, glyburide, and nefazodone are included in this study. Samples of glyburide were prepared from 90 minute human liver microsome incubates. The nefazodone samples were obtained from a bile duct cannulated rat study dosed IV. Protein precipitation (1 volume acetonitrile) was carried out, followed by centrifugation and the supernatant used.

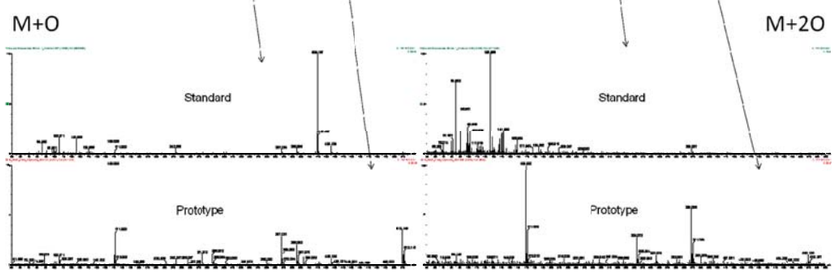
The flow rate was 3 µL/min. Gradient program: 0 - 4.5 min 10 - 70% acetonitrile (0.1%formic acid) held at 95% ACN for 2 min, with a total cycle time of 10 min.

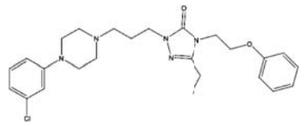


Study 1 : Comparison of M+O and M+2O metabolites of Glyburide from human liver microsomes

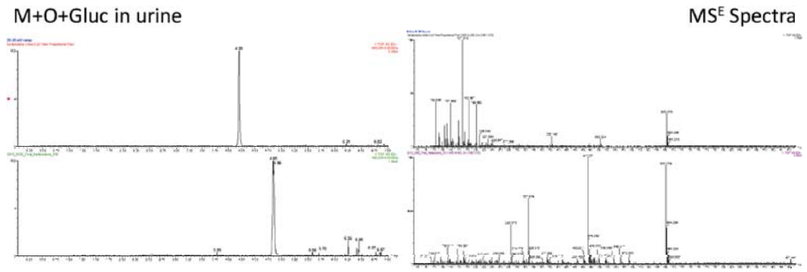


Study 2 : Comparison of MS^E spectra quality from M+O and M+2O metabolites of Glyburide





Study 3 : Comparison of the Nefazodone and its M+O+Gluc metabolite observed in rat urine and the MS^E spectra quality



RESULTS / CONCLUSIONS

In study 1 the MS response for the M+O and M+2O metabolites were compared between the prototype microfluidics-MS device and a standard ion source using the same G2-S Q-Tof mass spectrometer. When the signal-to-noise is compared the prototype affords a 16-fold increase (M+O) and 8-fold increase (M+2O) over the standard source. Study 2 compared the Mse data quality obtained from the same samples and in both instances the prototype data was easier to interpret.

Study 3 was a comparison, of the Nefazodone M+O+glucuronide metabolite in urine, between the prototype and standard ion source. The chromatographic data appears to be very similar between the two. The MSE spectra quality is also similar, indicating that the level of enhanced sensitivity observed in the in-vitro samples is not observed in these in-vivo samples.

The prototype affords a marked (>10-fold) increase in MS response for in-vitro samples, but no increase was observed for in-vivo samples. The standard sample preparation approaches may need to be modified to obtain a sensitivity increase for in-vivo samples.

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