

# **Quantitative analysis of a Glyburide Analogue, a potential NLRP3 Inhibitor, using micro-sampling, hybrid solid phase extraction and Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)**

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## **Introduction:**

Recently, dried blood spot (DBS) sampling method has turned out to be a promising microsampling technique for use in preclinical studies of animals and humans in order to obtain matrix samples. With this technique, small-molecule PK characterization with LC-MS/MS has become simpler and faster. Blood sample volumes as low as 15-20 ul containing the drug are collected and applied to specialty paper, dried and then punched for analyte extraction for LC-MS/MS quantification. In comparison with the standard blood collection methods used for bioanalysis, DBS sampling method has low sample volume requirements in addition to easier sample collection, handling, and storage (1). A LC-MS/MS method using DBS as sampling technique is under way in our lab for quantification of (5-chloro-2-methoxy-N-[2-(4-sulfamoylphenyl) ethyl] benzamide, a novel small-molecule therapeutic drug candidate for inhibition of the NLRP3 inflammasome.

## **Purpose:**

Liquid chromatography tandem mass spectrometry (LC-MS/MS) has become the gold-standard for measurement of small molecules in biological matrices in a clinical laboratory with advantages such as lower run time, increased sensitivity and selectivity. The purpose this study was to quantify and provide pharmacokinetic data of recently discovered novel pharmacologic inhibitor of the NLRP3 inflammasome that limits myocardial injury after ischemic and non-ischemic injury in mice (2).

## **Methods:**

Plasma was collected at 1, 4, and 24 hours from Glyburide Analog (5-chloro-2-methoxy-N-[2-(4-sulfamoylphenyl) ethyl] benzamide treated mice after a single injection of 100 mg/kg and stored at -20 °C. Standard stock solutions of the reference analyte and glipizide (IS) were made in ethanol. The calibration curve (1.00 to 1000 ng/ml) and controls were made in fresh CD-1 mouse plasma

(sodium heparin) using standard stock solutions. Plasma samples, calibration standards, and controls were extracted using 96 well plate (HybridSPE, 30 mg/ml). 20 uL of sample was added to the HybridSPE plate bed to 50 uL of water. After that 50 uL of 25 ng/mL IS (glipizide) was added followed by 250 uL of 1% formic acid. The samples were mixed and collected onto a collection plate using Tomtec vacuum manifold and evaporated to dryness using N<sub>2</sub> gas. The samples were reconstituted using 50:50 mobile phase A (95:5 H<sub>2</sub>O/ACN in 0.5% formic acid) and mobile phase B (ACN in 0.5% formic acid). 25 ul sample was submitted to LC-MS system after mixing. Separation was achieved using Aquasil C18 column by Thermo Scientific (50 X 2.1 mm, 3.0 um). Linear gradient conditions shown in Table 1 were used for mobile phase. The mass spectrometer was operated under positive electrospray ionization mode and selected reaction monitoring (SRM) of the glyburide analogue (369→169) and structural analogue internal standard, glipizide (445→166) to quantify the analyte of interest, the calibration standards and the controls.

Table 1. Linear gradient conditions used for mobile phase

Time (min)	A %	B %	Flow (ml/min)
0.00	75	25	0.3
0.20	90	10	0.3
0.50	70	30	0.3
4.50	5	95	0.3

## Results:

Linearity was achieved over 1-1000 ng/ml in 25- $\mu$ L mouse plasma, with reverse predicted residuals within  $\pm 10\%$ . A post-column infusion experiment was employed to reveal negligible matrix effects at the retention times of the glyburide analogue and its structural analogue glipizide. The precision and accuracy of the method was within  $\pm 15\%$  RSD. Fig. 1 shows chromatograms of A) Glipizide (IS) and B) (5-chloro-2-methoxy-N-[2-(4-sulfamoylphenyl)]

ethyl] benzamide (glyburide analog) at 1 ng/ml (LLOQ).

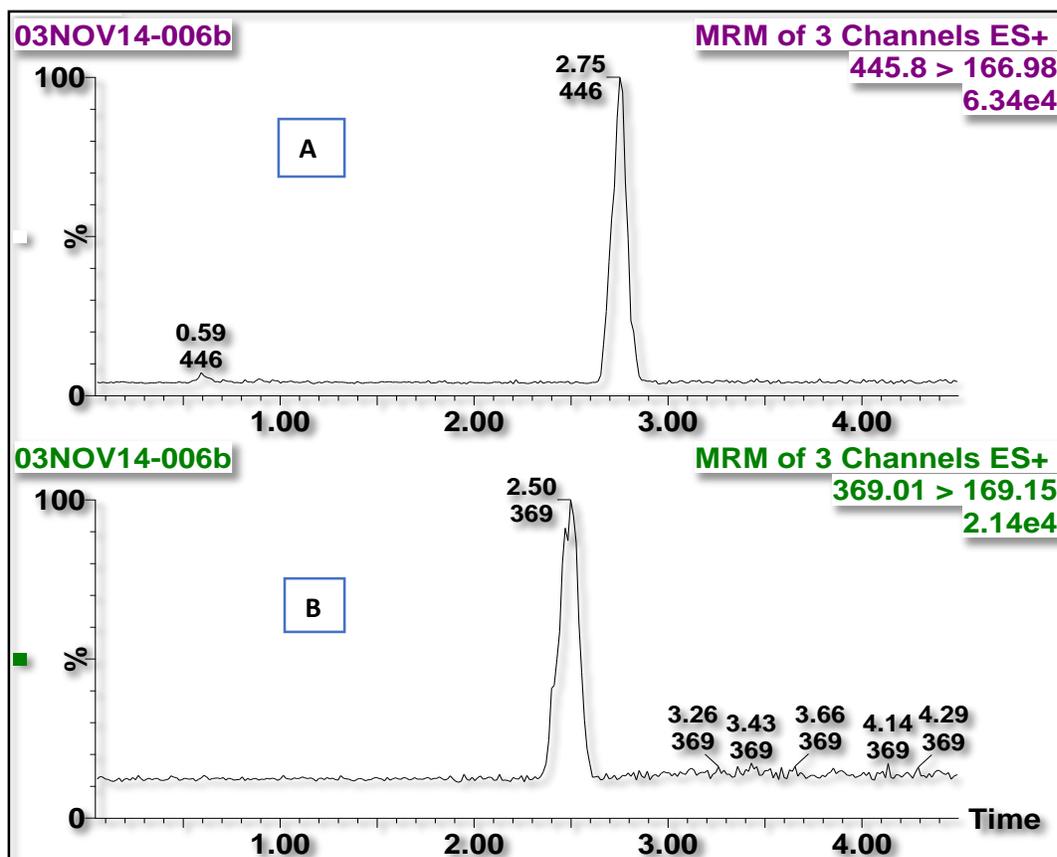


Fig. 1. Representative chromatogram of a plasma extracted lower limit of quantification (1 ng/mL). A) Structural analogue internal standard chromatogram, glipizide (25 ng/mL). B) Glyburide analogue.

### Conclusions:

An LC-MS/MS method was developed for the quantification of a glyburide analogue from mouse plasma using 96 well solid phase extraction technique that provides significant removal of major phospholipids. Furthermore, our work will be enhanced by cross-validating our current method in DBS, a microsampling technique which would render the need for higher blood volume (0.1 ml) for the toxicokinetic study. The cross-validated method with sample volumes as low as 25  $\mu$ L of dried blood spots enables us for a clean, faster and robust method for quantification of glyburide analogue.

**References:**

- (1) Soto M. Evaluation of matrix microsampling methods for therapeutic drug candidate quantification in discovery-stage rodent pharmacokinetic studies. *Bioanalysis* (2014) 6(16), 2135-2146.
- (2) Marchetti C et al., Pharmacologic inhibition of the NLRP3 inflammasome preserves cardiac function after ischemic and non-ischemic injury in the mouse. Manuscript in progress with a working title. Department of Pharmacotherapy and Outcomes Sciences. Virginia Commonwealth University.