# QUANTITATIVE MEASUREMENT OF DRIED BLOOD SPOT GUANIDINOACETATE AND CREATINE FOR CLINICAL RESEARCH

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## INTRODUCTION

**Guanidinoacetate methyltransferase (GAMT) deficiency, a cerebral creatine (CRE) deficiency syndrome, was added to the Recommended Uniform Screening Panel for newborns in 2023. Patients with GAMT are unable to breakdown guanidinoacetate (GUAC) formed in the initial step of CRE synthesis. No test systems are on market in the USA that include GUAC and CRE screening, however, successful newborn screening programs have developed multi-tiered screening strategies that universally begin with flow-injection-tandem mass spectrometric (FIA-MS/MS) quantitative measurement of GUAC and CRE from routinely collected dried blood spots (DBS) for screening of inborn errors of metabolism. We have demonstrated the analytical performance of these biomarkers in contrived DBS using the Waters FIA-MS/MS system for clinical research.**

## METHODS

### Materials

Stable isotope labelled GUAC and CRE were purchased from Cambridge Isotope Laboratories, Inc. Unlabeled GUAC and CRE were from Merck. LC-MS/MS grade reagents water, methanol, acetonitrile and formic acid were from ROMIL.

### DBS Samples

In-house preparation of DBS material followed similar methodology reported by the Centre of Disease Control (CDC). The hematocrit was adjusted to reflect the endogenous concentrations of GUAC and CRE (L1), L9 was enriched to target concentrations of the analytes to the upper limits of linearity. L1 to L9 were proportionally mixed to construct the in-house calibrators, and spotted onto Whatman 903 filter paper, air dried and stored at -20°C (Kilgore et al., 2016). External DBS linearity samples (Lot 20211-20219) were sourced from the CDC.

### Method Procedure

A non-derivatized method was used to extract GUAC and CRE from a single 3.2mm DBS punch. DBS were placed into a 96-well plate and incubated at room temperature with an internal standard (IS) working solution containing GUAC-d2 and CRE-15N3, final concentration of 10 and 250 µmol/L respectively. The DBS was removed from the incubated extract and the 96 well plate was transferred to the FIA-MS/MS system, 10 µL was injected for analysis.

### MS/MS Method

The following m/z transitions for the analytes and the isotopically labelled internal standards used were: **GUAC** (118.1 > 76), **GUAC IS** (m/z 120.1 > 78), **CRE** (132.1 > 90) and **CRE IS** (135.1 > 91.1). The MS/MS system was operated in ESI positive polarity and MRM mode.

### Instrumentation

All samples were analyzed using the Waters FIA-MS/MS System (**Figure 1**). The system consists of the MassTrak™ ACQUITY™ UPLC™ I-Class PLUS system, FL Sample Manager and the Xevo™ TQD Mass Spectrometer. All data was collated using Analyse-IT™ software for Microsoft™ Excel™ spreadsheet.

## RESULTS

### Precision and Recovery

Using the Waters non-derivatized in-house method and in-house DBS linearity set (L1-L9), precision was assessed according to CLSI NBS04 ED2:2017 Guidelines. Four replicates of each linearity level were randomized and measured in a single FIA-MS/MS run. As shown in **Table 1**, all linearity levels for both metabolites had an imprecision <20%. The average recovery was 108% (GUAC) and 91% (CRE). Additionally, CDC Linearity (2021) material was compared for % accuracy to reported values (**Table 2**). The average % accuracy compared to CDC reported values was 96% (GUAC) and 105% (CRE), imprecision based on 4 replicates per level was <20%.

**The Waters MassTrak Xevo TQD System can be used in clinical research for the measurement of the two biochemical markers Guanidinoacetate and Creatine**

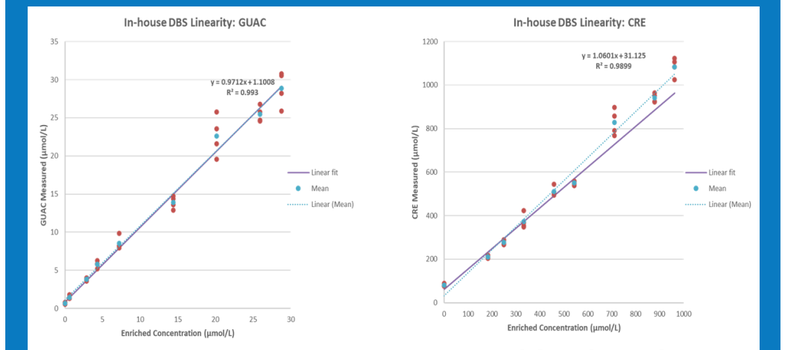


**Figure 1:** In-house enriched linearity results of GUAC and CRE measurements

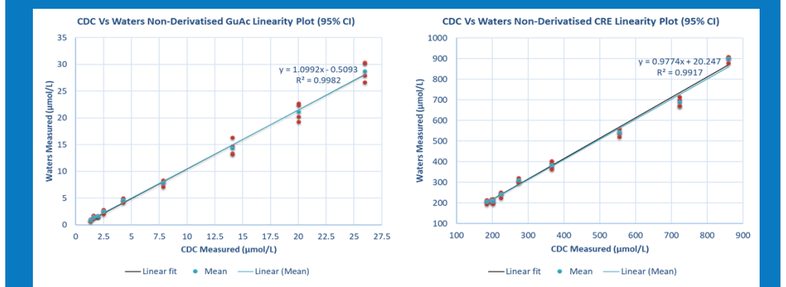


**Figure 2:** Results summary of in-house linearity DBS samples enriched with GUAC and CRE

| In-house DBS Linearity set L1-9: GUAC | | | | |
|---|---|---|---|---|
| | Waters Enriched Value (µM) | Waters Measured Value (µM) | %CV | %Recovery |
| WAT L1 | 0 | 0.68 | 18.1% | NA |
| WAT L2 | 0.58 | 1.47 | 14.6% | 137% |
| WAT L3 | 2.88 | 3.81 | 4.7% | 109% |
| WAT L4 | 4.32 | 5.78 | 7.4% | 118% |
| WAT L5 | 7.20 | 8.56 | 10.3% | 109% |
| WAT L6 | 14.40 | 13.93 | 6.0% | 92% |
| WAT L7 | 20.16 | 22.63 | 11.8% | 109% |
| WAT L8 | 25.92 | 25.45 | 4.1% | 95% |
| WAT L9 | 28.80 | 28.86 | 7.9% | 98% |
| | | | **Average** | **108%** |

| In-house DBS Linearity set L1-9: CRE | | | | |
|---|---|---|---|---|
| | Waters Enriched Value (µM) | Waters Measured Value (µM) | %CV | %Recovery |
| WAT L1 | 0 | 79.95 | 7.5% | NA |
| WAT L2 | 182.09 | 211.05 | 3.1% | 72% |
| WAT L3 | 249.21 | 278.00 | 4.2% | 79% |
| WAT L4 | 333.12 | 373.15 | 9.2% | 88% |
| WAT L5 | 458.97 | 511.90 | 4.5% | 94% |
| WAT L6 | 542.88 | 550.15 | 1.9% | 87% |
| WAT L7 | 710.68 | 828.38 | 7.2% | 105% |
| WAT L8 | 878.49 | 940.75 | 2.2% | 98% |
| WAT L9 | 962.40 | 1084.25 | 3.9% | 104% |
| | | | **Average** | **91%** |

**Table 1:** Results summary of in-house linearity DBS samples enriched with GUAC and CRE

### Linearity and Method Comparison

Linearity of GUAC and CRE measurements was demonstrated with in-house prepared multilevel DBS samples following CLSI EP06-ED2:2020 guidelines. **Figure 2** shows good linearity, $r^2$ of 0.99 for both analytes. The allowable %deviation (ADL) from the linear line was assessed across the 9 linearity intervals for GUAC and CRE and was <20%. Additionally, CDC Linearity (20211-20219) material was extracted and compared against CDC reported values; the correlation between the Waters in-house non-derivatized method and CDC Linearity reports $r^2$ of ≥ 0.99 for both analytes.

| CDC 2021 Linearity Set: GUAC | | | | |
|---|---|---|---|---|
| | CDC Measured Value (µM) | Waters Measured Value (µM) | %CV | %Accuracy to CDC |
| CDC L1 | 1.33 | 0.84 | 16.0% | 64% |
| CDC L2 | 1.61 | 1.48 | 16.3% | 92% |
| CDC L3 | 1.99 | 1.58 | 3.4% | 79% |
| CDC L4 | 2.5 | 2.50 | 14.7% | 100% |
| CDC L5 | 4.29 | 4.63 | 7.9% | 108% |
| CDC L6 | 7.88 | 7.83 | 6.5% | 99% |
| CDC L7 | 14.09 | 14.37 | 10.0% | 102% |
| CDC L8 | 20.05 | 21.09 | 7.9% | 105% |
| CDC L9 | 25.99 | 28.73 | 6.3% | 111% |
| | | | **Average** | **96%** |

| CDC 2021 Linearity Set: CRE | | | | |
|---|---|---|---|---|
| | CDC Measured Value (µM) | Waters Measured Value (µM) | %CV | %Accuracy to CDC |
| CDC L1 | 184.87 | 206.80 | 4.3% | 112% |
| CDC L2 | 197.76 | 211.48 | 3.1% | 107% |
| CDC L3 | 202.07 | 207.70 | 4.8% | 103% |
| CDC L4 | 223.85 | 241.90 | 5.2% | 108% |
| CDC L5 | 274.15 | 308.78 | 3.1% | 113% |
| CDC L6 | 366.14 | 382.10 | 4.7% | 104% |
| CDC L7 | 554.53 | 540.90 | 2.8% | 98% |
| CDC L8 | 722.76 | 687.60 | 3.0% | 95% |
| CDC L9 | 859.54 | 899.48 | 1.7% | 105% |
| | | | **Average** | **105%** |

**Table 2:** Method Comparison summary of external CDC linearity material measurements versus Waters Measurements using Xevo TQD

## REFERENCES

- *Baby's First Test, 2022*. URL https://www.babysfirsttest.org/newborn-screening/conditions/guanidinoacetate-methyltransferase-deficiency Accessed: May2023.
- *Kilgore et al., 2023*. Development of a Universal Second-Tier Newborn Screening LC-MS/MS Method for Amino Acids, Lysophosphatidylcholines, and Organic Acids. Anal Chem. 95(6): 3187-3194.

## CONCLUSION

- Based on this preliminary study, ESI +ve mode MS/MS measurements of GUAC and CRE from in-house prepared DBS material shows good method precision, recovery and linearity using the MassTrak Xevo TQD MS.
- Correlation analysis of the external CDC material has demonstrated that the in-house DBS preparation and the Waters' non-derivatized methodology for detection of the biochemical markers GUAC and CRE results are equivalent to values reported by the CDC.
- Whilst the preliminary data has demonstrated the analytical performance of two metabolites using the MassTrak Xevo TQD MS, this system is capable of testing for many metabolites for clinical research.

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