

# Analytical performance evaluation of the Waters AA/AC RUO kit for the measurement of biological metabolites in dried blood spots, by FIA-MS/MS

Waters™

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## INTRODUCTION

The Waters AA/AC RUO Kit is a new product for the extraction and analysis of metabolites from dried blood spot (DBS) samples using flow injection analysis, tandem mass spectrometry (FIA-MS/MS). Analytes which are assessed in the provided DBS quality control (QC) material include guanidinoacetate (GuAc), creatine (Cre), succinylacetone (SuAc), amino acids (AA), carnitine and acylcarnitines (AC), C26:0-lysophosphatidylcholine (C26:0-LPC), adenosine (Ado), argininosuccinic acid (Asa), and anhydrides of Asa.

The analytical performance characteristics were evaluated on the Waters ACQUITY™ UPLC™ I-Class PLUS System with Xevo™ TQD IVD Mass Spectrometer. This research poster highlights robustness and imprecision through the ongoing analysis QC material, linearity of instrument response using materials prepared specifically for this purpose, and recovery compared with enriched values using an independent external source of DBS material.

## EXPERIMENTAL

Samples were prepared using a two-step protocol. Briefly, analytes were eluted from a 3.2mm DBS punch in a filtration plate and collected into a receiver plate. In a subsequent step, SUAC derivatization reagent was added to the punch, and, after a period of extraction and incubation, the sample was combined with the first-stage eluate through the filtration plate. The prepared sample was analyzed by positive electrospray ionization with multiple reaction monitoring using FIA-MS/MS (Figure 1). All data was collated using Analyse-IT™ software for Microsoft™ Excel™ spreadsheet.

## RESULTS

### Imprecision and Recovery versus Certificate of Analysis

Analytical method imprecision and accuracy were assessed over 28 testing days using the Waters AA/AC RUO Kit, with bi-level, Low and High concentration QC materials provided with the kit. A total of 190 replicates were analyzed per QC level. Each day, 7 replicates per QC level were evenly distributed among 550 whole blood spot-extracted samples. In total, 15,400 whole blood extracts were injected throughout the study. The overall imprecision was less than 20% CV for all analytes, except for Asa in the Low Concentration QC, which showed an imprecision of 33.9% CV. Mean recovery compared with Kit Certificate of Analysis (COA) assigned values are shown in Table 1. All mean analyte recoveries for both QC Low and QC High were within  $\pm 3$  standard deviation range stated on the COA. Additionally, analytical method imprecision was evaluated over 5 days using MSMS1 DBS QC materials (NSQAP program, Centers for Disease Control and Prevention, Lot 2415, Levels I to F), with 5 replicates per day per level (N = 25). The total imprecision was <20% for all analytes (Figure 2).

Table 1: Analytical imprecision of the Waters AA/AC RUO Kit QC Low and High analytes (%CV), mean recovery compared with values stated on the kit COA and Linear range of in-house prepared DBS material spanning 9-11 concentration levels and calculated  $R^2$  values compared to enriched values.

Analyte	Abbreviation	Total Imprecision (%CV)		Mean % Recovery vs COA	Measured Linear Range ( $\mu$ M)		$R^2$
		QC Low	QC High		Lower	Upper	
Glycine	Gly	8.9%	8.80%	105.8%	180.30	2666.25	0.9866
Alanine	Ala	9.2%	8.9%	106.0%	90.05	1743.50	0.9855
Proline	Pro	9.6%	9.1%	106.5%	5.02	1637.25	0.9910
Valine	Val	8.4%	8.0%	109.8%	45.02	1460.25	0.9916
Leucine	Leu	9.1%	8.6%	112.0%	25.67	1891.00	0.9909
Glutamine	Gln	8.8%	8.7%	103.2%	26.91	2431.25	0.9804
Methionine	Met	9.7%	8.2%	106.2%	0.56	959.18	0.9923
Succinylacetone	SuAc	17.1%	18.5%	108.5%	0.44	152.93	0.9938
Phenylalanine	Phe	8.6%	8.4%	114.5%	0.75	1963.25	0.9946
Tyrosine	Tyr	8.5%	8.4%	114.7%	11.03	1689.00	0.9875
Guanidinoacetic Acid	GuAc	14.1%	11.3%	103.9%	1.17	26.42	0.9917
Argininosuccinic Acid	Asa	33.9%	15.6%	92.1%	3.11	204.03	0.9884
Creatine	Cre	9.3%	9.4%	108.4%	92.57	1275.75	0.9920
Ornithine	Orn	9.7%	11.0%	111.5%	35.68	1421.25	0.9967
Arginine	Arg	9.8%	10.1%	108.1%	5.47	589.28	0.9968
Citrulline	Cit	10.7%	10.2%	109.7%	7.97	1383.5	0.9996
Adenosine	Ado	10.8%	8.4%	110.4%	0.46	56.10	0.9961
Free Carnitine	C0	10.7%	11.5%	107.1%	6.34	587.28	0.9913
Acetyl carnitine	C2	9.3%	9.6%	108.0%	2.16	173.53	0.9857
Propionyl carnitine	C3	10.2%	9.3%	115.6%	0.29	91.46	0.9975
Butyrylcarnitine	C4	10.8%	9.5%	119.2%	0.06	12.85	0.9766
Isovalerylcarnitine	C5	10.3%	10.0%	110.0%	0.08	23.95	0.9850
Hexanoylcarnitine	C6	11.6%	10.3%	103.4%	0.06	13.46	0.9909
3-Hydroxyvalerylcarnitine	C5OH	11.0%	10.0%	108.8%	0.03	10.03	0.9961
Glutaryl carnitine	C5DC	14.0%	11.5%	106.9%	0.06	11.32	0.9936
Octanoylcarnitine	C8	10.7%	10.4%	103.5%	0.05	62.34	0.9919
Decanoylcarnitine	C10	12.4%	12.8%	98.2%	0.15	11.50	0.9854
Dodecanoylcarnitine	C12	12.1%	11.6%	100.2%	0.07	12.43	0.9878
Myristoylcarnitine	C14	11.3%	10.5%	100.0%	0.03	15.63	0.9926
Palmitoylcarnitine	C16	10.0%	9.2%	113.3%	0.19	68.98	0.9920
3-Hydroxyplamitoylcarnitine	C16OH	12.3%	11.1%	106.0%	0.07	7.73	0.9918
Stearoylcarnitine	C18	9.9%	8.8%	107.3%	0.32	17.75	0.9937
C26:0-Lysophosphatidylcholine	C26:0-LPC	18.4%	16.9%	125.6%	0.08	10.30	0.9994

## KEY HIGHLIGHTS

### Robust and streamlined clinical research analysis of 53\* analytes in DBS using the Waters AA/AC RUO Kit

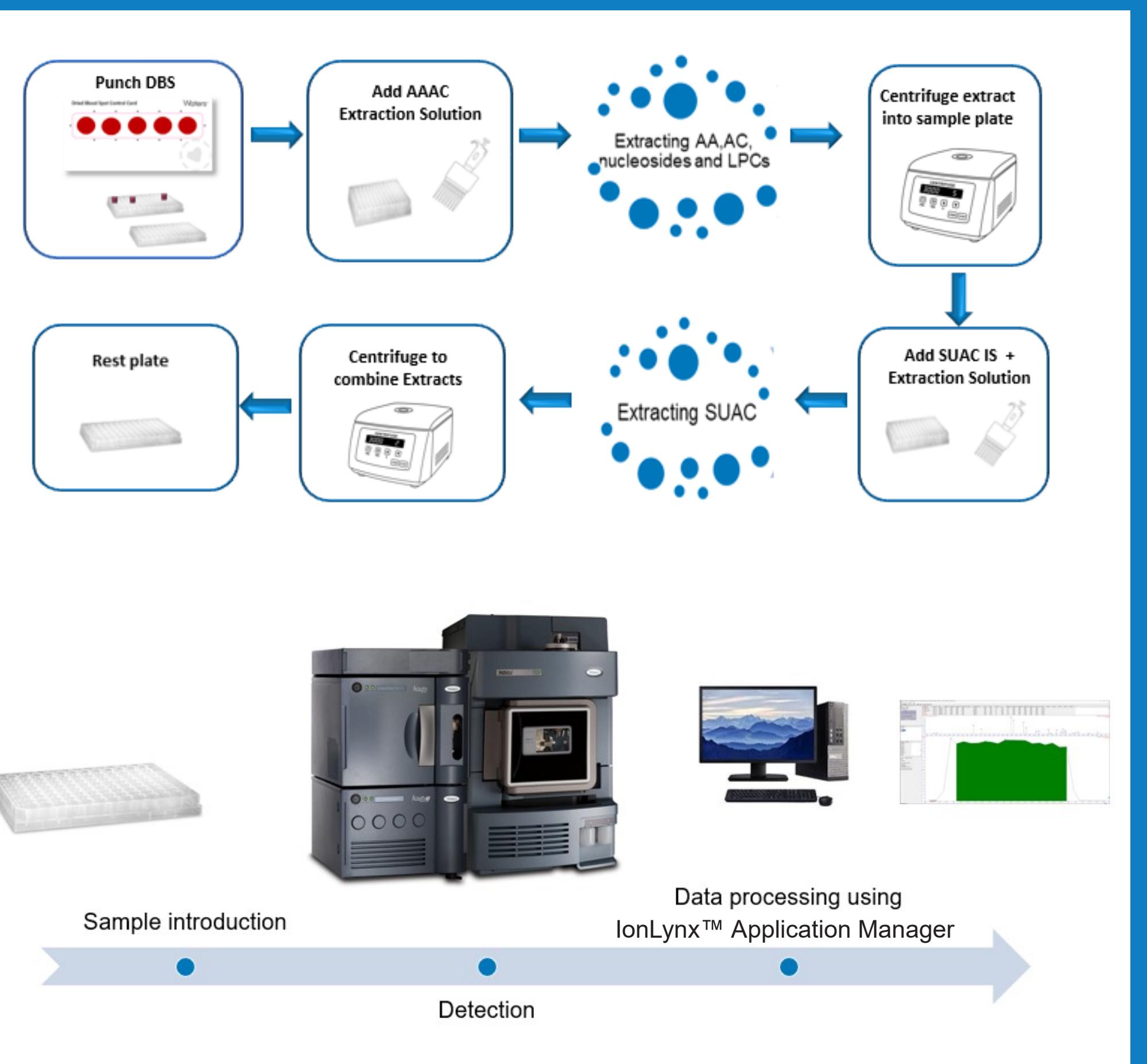


Figure 1: Waters AA/AC RUO Kit two-step workflow for the extraction and measurement of amino acids, acylcarnitines, nucleosides, ketone and lysophospholipids from a single 3.2mm DBS punch.

\* not including isomeric/isobaric species.

## ACKNOWLEDGEMENTS

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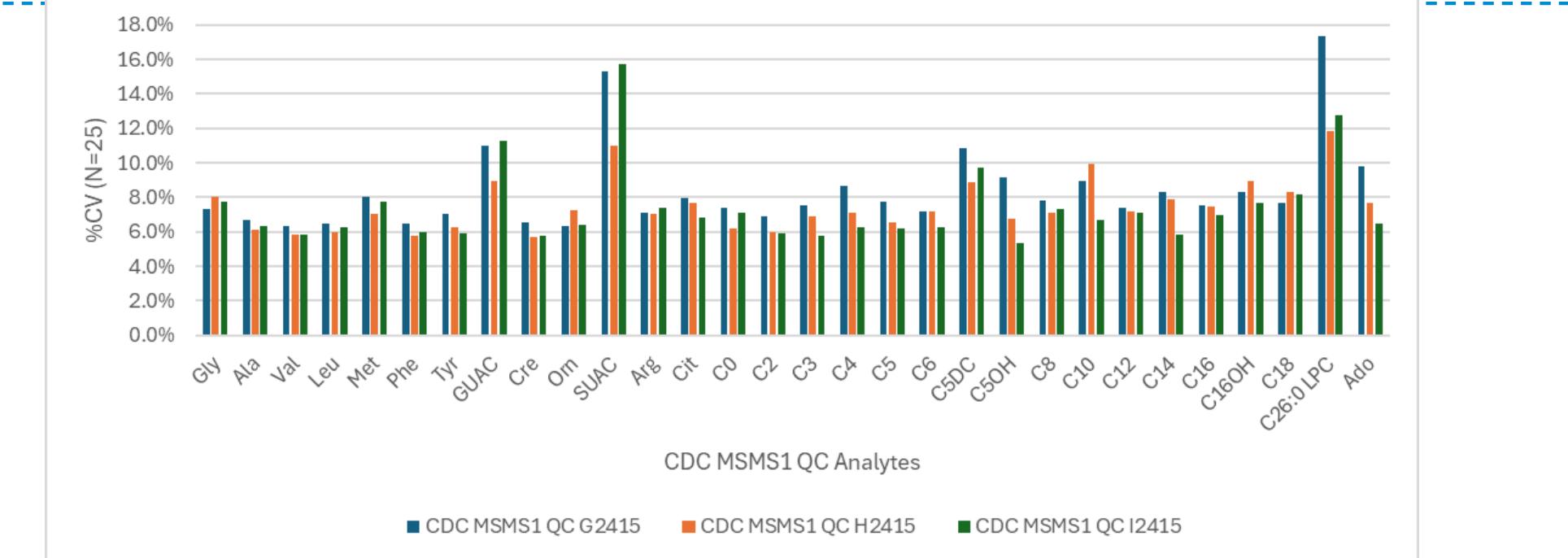


Figure 2: MSMS1 QC imprecision results (%CV) using the Waters AA/AC RUO Kit and Waters ACQUITY UPLC I-Class PLUS System with Xevo TQD IVD Mass Spectrometer.

## Recovery versus External Quality Control Material Enrichment Values

Percent recovery was also calculated as observed measurement relative to enrichment values of the MSMS1 QC material (N = 25; 5 days; 5 replicates per level). Observed values were adjusted by subtracting endogenous concentrations (NSQAP QC Lot F2415) from Levels G2415-I2415. All analytes demonstrated recovery within the range of 65–123%, with the exception of SuAc, which showed a mean recovery of 32%. Due to the absence of enriched Asa, Pro, and Gln in the external QCs, in-house prepared samples were assessed for recovery. 3 independent sample lots spanning 3 levels where measured in triplicate, mean % recovery 83.9, 81.2 and 60.8% respectively.

## Linearity

Linearity was evaluated for each analyte using samples prepared in quadruplicate, randomized, and injected in singlicate across six independent days. For each analyte, 9 to 11 measuring intervals were selected to adequately span the intended analytical range. Data analysis was performed with linear regression analysis, all analytes demonstrated linearity, with  $R^2$  values exceeding 0.97 (Table 1).

## Analytical Drift

Assay drift was evaluated over a continuous 17-hour period during the precision study. The relative deviation of IS intensity from the mean was monitored for each analyte in the 7 sets of QC samples (Figure 3A-B). The average drift in IS intensity over 17 hours was less than 10.5% for all analytes. GuAc and Cre are highlighted to illustrate absolute IS intensity at Time 0 (T0) and at 17 hours (T17) (Figure 3B-C), with no visible change observed.

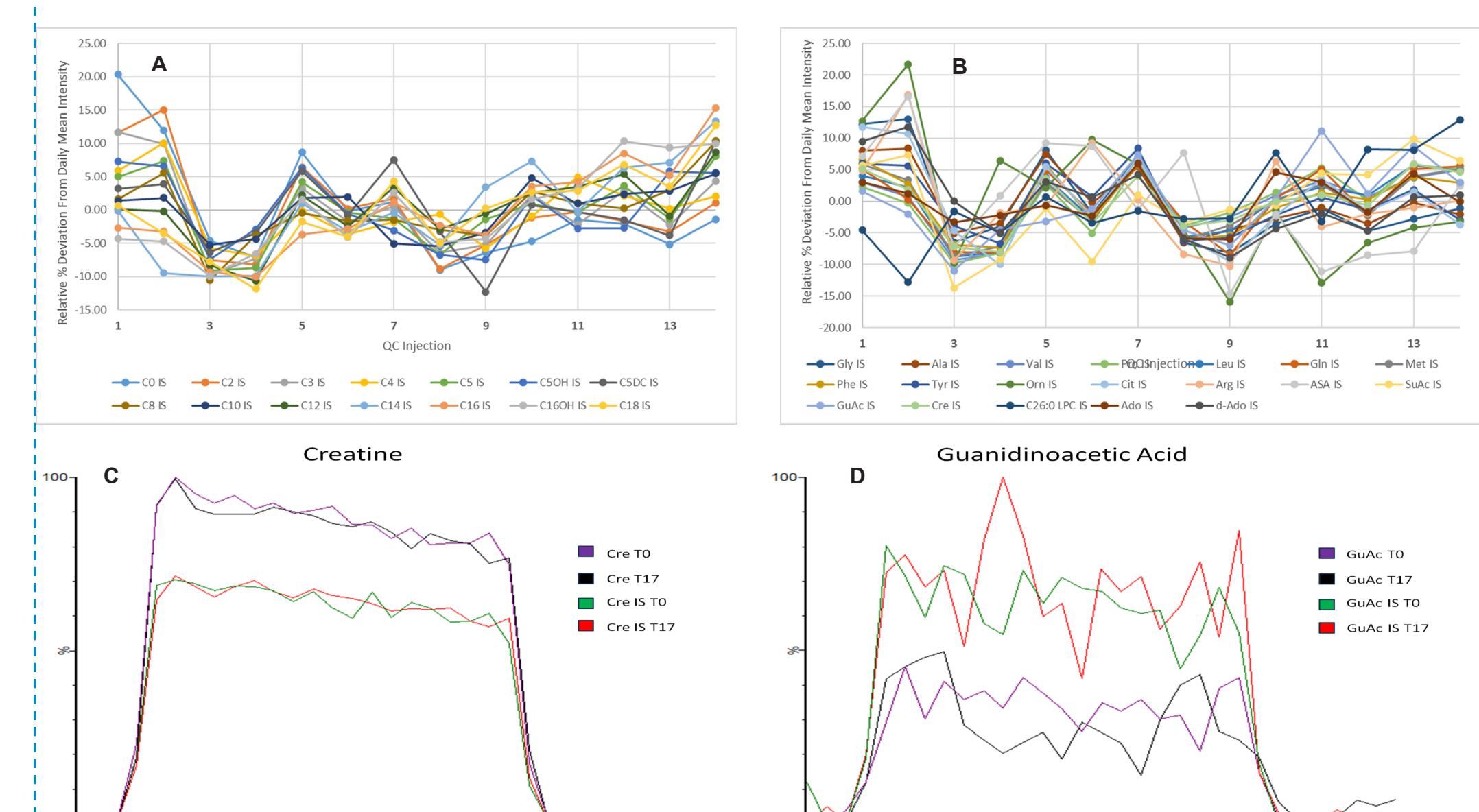


Figure 3: (A) Relative Deviation (%) of Free Carnitine and Acylcarnitines and (B) Amino Acids, Ketone, Lysophospholipid and Nucleosides IS intensities in Kit QC materials over 17 hours (N=14, 7 replicates of each level of QC material). QC Low and QC High intensity flow profile plots for (C) Cre and Cre IS and (D) GuAc and GuAc IS from T0 (QC injection 1) compared to T17 (QC injection 14), no significant change was observed.

## CONCLUSION

The Waters AA/AC RUO Kit demonstrates robust analytical performance across multiple metrics, including imprecision, recovery, and linearity. These results support its potential for reliable metabolite measurements for clinical research.