

PATsmart™ REBEL® XT Validation Study

Technical Note

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Introduction

The PATsmart™ REBEL® XT At-Line Media Analyzer from Repligen allows for fast, reliable, and accurate data acquisition, processing, and analysis of over 20 analytes in cell culture media. The purpose of this study is to illustrate the REBEL XT System's performance using a statistically significant data set that includes both biological and technical replicates in order to establish representative performance metrics for the device.



Figure 1. REBEL XT At-line Media Analyzer

Background

The REBEL XT System combines Capillary Electrophoresis (CE) based separation with High Pressure Mass Spectrometry (HPMS), and analyzes nanoliter-scale sample volumes to provide automated results in millimolar-scale concentrations. The REBEL XT System uses the REBEL SMARt® Kit to analyze a panel of all 20 essential and non-essential amino acids, plus Choline and alanyl-glutamine (GlutaMAX), in cell-free media samples. See Table 1 for a complete list of analytes.

This validation study used a third-party amino acid standard (AAS18, Figure 2) to establish expected performance metrics when analyzing cell culture media. The study described here can be used to inform end users' own validation procedures.

Total batch runtime for this validation study was 4 hours (6-minute average per injection) including automated Performance Qualification analyses performed at the beginning of the batch and every five samples thereafter.

Materials

- REBEL SMARt® Kit
- Millipore Sigma AAS18 Amino Acid Standard
- Calibrated Pipettes: 100 μ L, 1000 μ L, 5 mL
- HPLC-grade water (or equivalent i.e. RO/DI water)
- Vortex shaker
- Calibrated microbalance
- Weighboats
- 10 mL centrifuge tubes, qty. 5
- 2 mL autosampler vials and caps, qty. 10

Experimental Design and Procedure

To assess pipetting precision and accuracy, 50 μ L of HPLC-grade water was measured in ten weighboats and recorded. The measurements, along with the average, standard deviation, and %RSD are shown in Table 2. These quantities are used in the final error calculations.

Table 1. REBEL SMART Kit analytes

Analyte	Abbreviation
DL-alanyl-DL-glutamine	AQ
DL-Alanine	Ala
DL-Arginine	Arg
DL-Asparagine	Asn
DL-Aspartic Acid	Asp
Choline	Choline
DL-Glycine	Gly
DL-Histidine	His
DL-Isoleucine	Iso
DL-Leucine	Leu
DL-Lysine	Lys
DL-Methionine	Met
DL-Phenylalanine	Phe
DL-Proline	Pro
DL-Serine	Ser
DL-Threonine	Thr
DL-Tryptophan	Trp
DL-Tyrosine	Tyr
DL-Valine	Val
DL-Glutamine	Gln
DL-Glutamic Acid	Glu
Cystine	Cystine


Samples were prepared by creating five stock solutions of 5 mL each. The stock solutions were prepared by pipetting 50 µL of the AAS18 solution into 4950 µL of REBEL XT Diluent into each of the 10 mL centrifuge tubes, creating five 100x dilutions. Each tube was then mixed using the vortex shaker for 10 seconds. Two sample vials, 1000 µL each, were prepared from each stock solution; see Figure 3 for an illustration of the sample preparation method.

A batch run was set up on the REBEL XT System using the Repligen Batch Runsheet Template with four technical replicates per sample. In total, 40 replicates were analyzed in the validation study. Generally, one can improve the precision of the REBEL assay by taking additional replicates per measurement. The precision is expected to improve proportionally to the square-root of the number of replicates. Four replicates will improve the precision by approximately 50%.

Table 2. Pipetting Analysis

Measurement	Weight (mg)
1	49.5
2	49.9
3	49.8
4	49.6
5	49.3
6	49.9
7	49.6
8	49.9
9	49.7
10	49.7
Average	49.7
Standard Deviation	0.2
RSD	0.4%

Figure 2. AAS18 Specification Sheet



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Product Information

AMINO ACID STANDARD SOLUTION
For calibrating amino acid analyzers

Stock No. **AA-S-18**

Store at 2-8 °C

Amino acids and related compounds are in 0.1 N HCl at the indicated concentrations ± 4%. Molecular weights are listed to four significant figures.

COMPONENT	MOL. WT.	µMoles/mL
L-Alanine	89.09	2.50
Ammonium Chloride	53.49	2.50
L-Arginine	174.2	2.50
L-Aspartic Acid	133.1	2.50
L-Cystine	240.3	1.25
L-Glutamic Acid	147.1	2.50
Glycine	75.07	2.50
L-Histidine	155.2	2.50
L-Isoleucine	131.2	2.50
L-Leucine	131.2	2.50
L-Lysine	146.2	2.50
L-Methionine	149.2	2.50
L-Phenylalanine	165.2	2.50
L-Proline	115.1	2.50
L-Serine	105.1	2.50
L-Threonine	119.1	2.50
L-Tyrosine	181.2	2.50
L-Valine	117.2	2.50

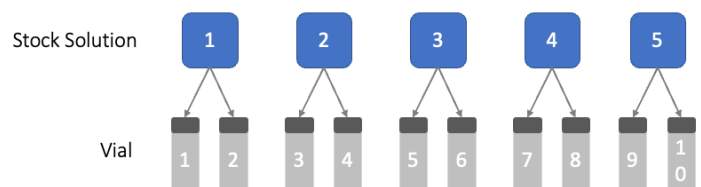
Storage
Store at 2-8 °C

For laboratory use only.

Not for drug, household or other uses.

Pcs 2/01

Figure 3. Sample preparation diagram



Results

Figure 4 illustrates the average concentration with standard deviation for all analytes tested. According to the specification sheet (Figure 2), all analytes present in AAS18 are expected to have concentrations of 2.50 mM ± 4%, with the exception of Cystine, which is specified at 1.25 mM ± 4%. All measurements fell within the expected ranges, and %RSD was low across the replicates, indicating good accuracy and repeatability of the method. Figure 5 illustrates the average error with standard deviation of the error across replicates (standard error). Low

standard errors indicate a high degree of confidence in the values when compared to the REBEL XT System calibration.

The validation study results are summarized in Tables 4 through 6. Overall accuracy and precision of the study were then determined using the guidelines in Table 3. Confidence limits for each analyte’s error and RSD were determined using standard *t* values and χ^2 values, respectively, for a 95% confidence interval with $n - 1 = 39$ degrees of freedom.

Figure 4. Mean concentrations of all analytes. Error bars indicate standard deviation for the analyte.

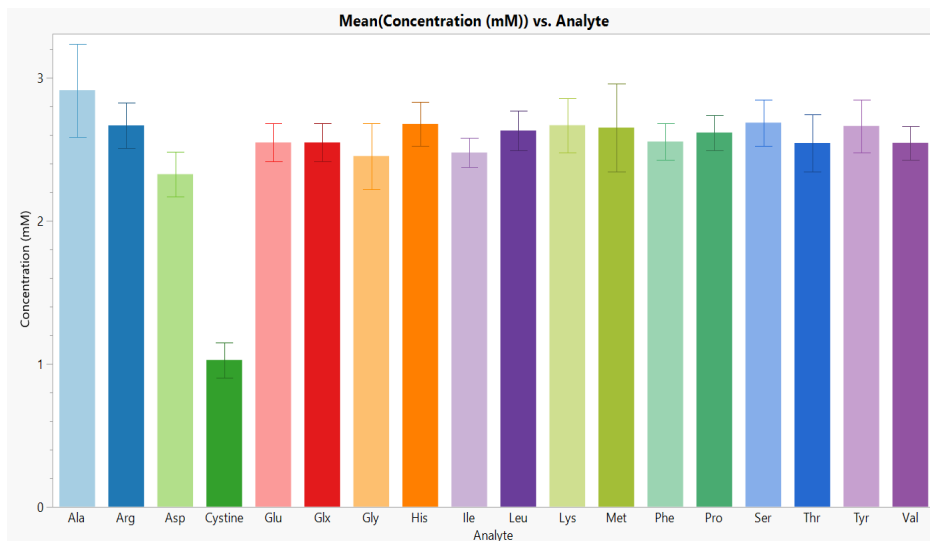


Figure 5. Standard error of all analytes

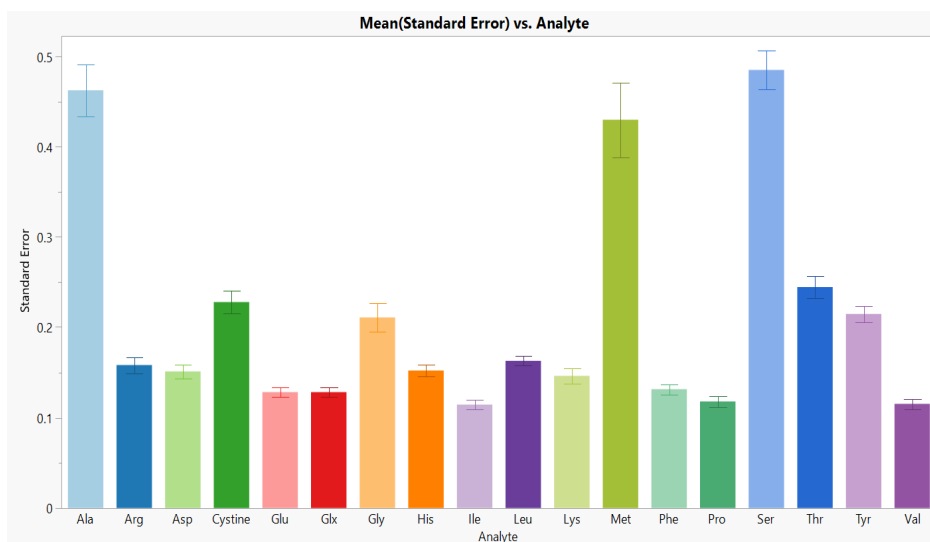


Table 3. Uncertainty contributions for references and REBEL XT System

Contributor	Uncertainty	Details
Reference	Specification tolerance (t)	Given on specification sheet
Reference	Pipette accuracy (a)	Average difference between pipetted weight values and target value
Reference	Pipette precision (p)	Standard deviation of pipetted weight values
Reference	Total reference error	$\sqrt{t^2 + a^2 + 2p^2}$
REBEL XT	REBEL Assay Accuracy	Average difference from expected value across all analytes and runs.
REBEL XT	REBEL Assay Precision	Standard deviation across all analytes and runs.

Table 4. Final Calculations for REBEL XT Validation study

Reference Error Analysis	
Specification tolerance	4.00%
Pipette Accuracy Error	0.62%
Pipette Precision (RSD)	0.20%
Total Reference Error	4.06%
REBEL Error Analysis	
Average Error	2.85%
Average Precision (Single Reps)	7.01%
Average Precision (4-rep average)	5.39%

Table 5. Test statistics for 95% confidence interval

Statistic	Lower	Upper
t value	2.02	
χ^2 values	23.65	58.12

Table 6. Confidence Limits by Analyte

Analyte	%Error		%RSD	
	Low	High	Low	High
Ala	13.00	20.24	9.16	14.36
Arg	4.83	8.67	4.85	7.61
Asp	-9.07	-4.69	5.53	8.67
Cys	-21.64	-13.96	9.70	15.20
Glu	0.31	3.69	4.27	6.70
Gly	-4.83	1.23	7.66	12.01
His	5.36	9.04	4.66	7.31
Ile	-2.20	0.50	3.42	5.37
Leu	3.65	7.03	4.28	6.71
Lys	4.54	9.12	5.78	9.06
Met	2.42	9.92	9.49	14.88
Phe	0.63	3.87	4.09	6.41
Pro	3.22	6.28	3.86	6.06
Ser	5.57	9.45	4.91	7.70
Thr	-0.69	4.37	6.39	10.02
Tyr	4.36	8.84	5.66	8.87
Val	0.37	3.39	3.83	6.00

Conclusion

The REBEL XT System performs well within its advertised accuracy and precision standards, 15% and 20% respectively, utilizing both biological and technical replicates across a statistically significant number (40) of sample analyses. Analytes exhibiting the highest error (Alanine, Cystine, Methionine) have struggled historically using traditional analysis methods. This straightforward yet statistically significant sample study demonstrates how the REBEL XT System allows for excellent reproducibility and confidence in data obtained for amino acid profiling.

Customer Service

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