



A Rapid iMethod™ Test for the Analysis Amino Acids

iMethod™ Test for aTRAQ™ Kits 1.0 including Cliquid® AA 3.0 SW License

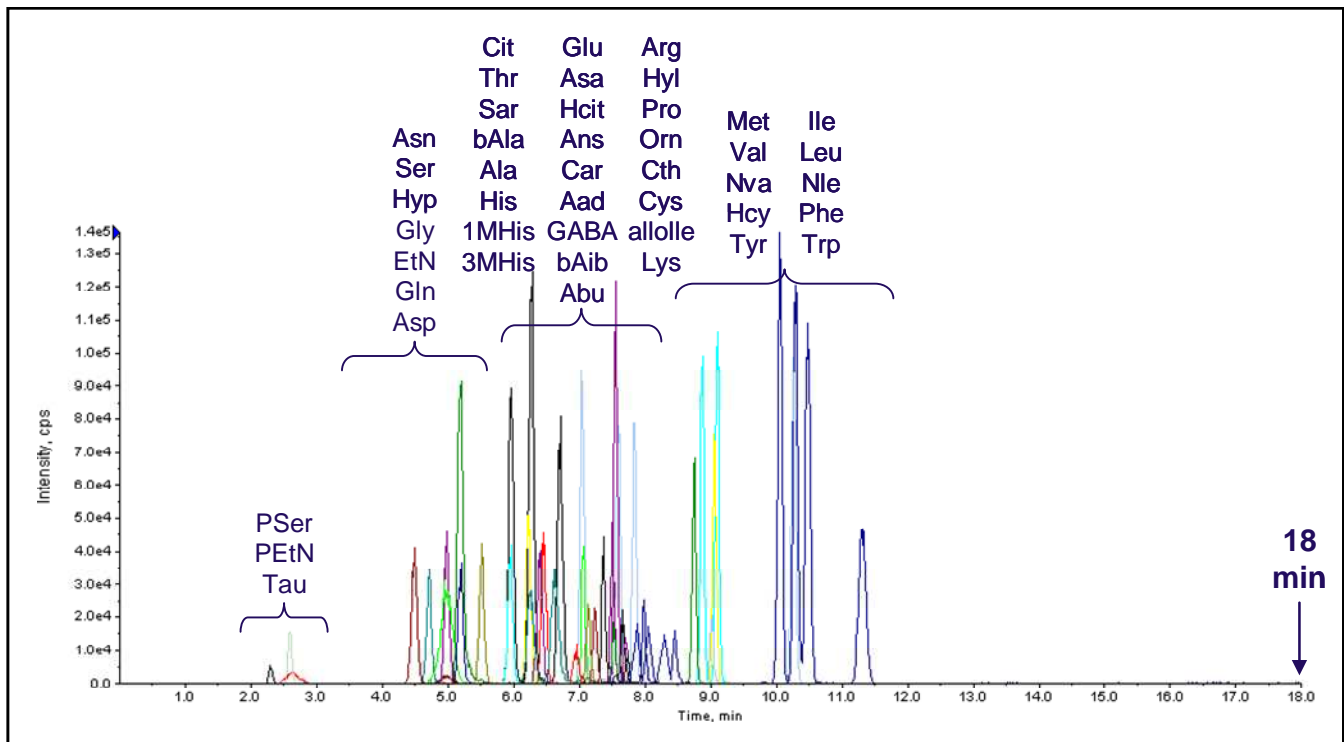
The AB SCIEX iMethod™ Test for aTRAQ™ Kit 1.0 with Cliquid® 3.0 Software for Routine Amino Acid Analysis provides an easy-to-learn, easy-to-use, browser-based application. Through the use of a license, the Cliquid® AA functionality is accessible through the Cliquid® 3.0 software that automates the process of acquiring data and generating reports.

The iMethod™ Test for aTRAQ™ Kit benefits from the sensitivity and specificity of LC/MS/MS analysis using either a triple quadrupole or QTRAP® system operating in MRM mode. HPLC separation is performed using a C18 column. The gradient, wash, and equilibration require a total of 18 minutes, maximizing throughput. The use of the Scheduled MRM™ Algorithm maximizes dwell time while monitoring of large numbers of MRM transitions, resulting in optimum data quality and reproducibility. This iMethod™ Test also employs aTRAQ™ Reagents to increase the precision and accuracy of quantitation by providing an internal standard for each amino acid analyzed. aTRAQ™ Reagents also provide an extra degree of confirmation by using

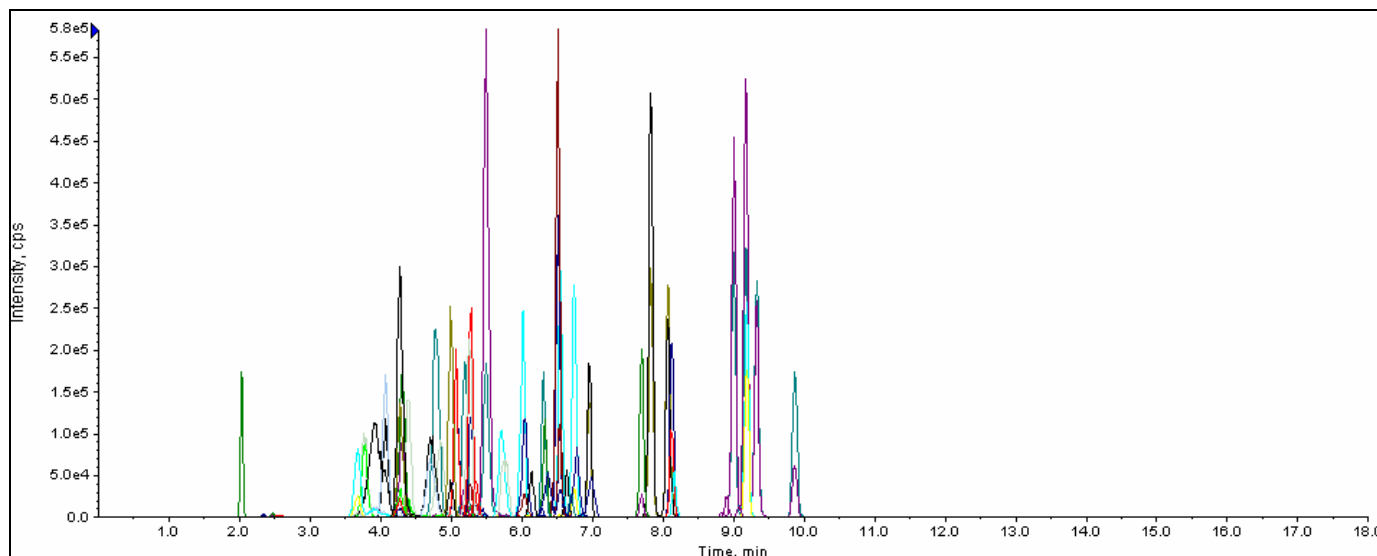
the retention time and the molecular weight of the standard and sample for confirmation of each amino acid.

The protocol is applicable to analyze amino acid in hydrolysates as well as free amino acids in physiological fluids.

- Ability to analyze 45 amino acids in a single run
- High throughput with an analysis time of 18 minutes per sample
- Single universal protocol for a range of physiological fluids and matrices such as plasma, urine, tissue extracts and culture media
- Small sample requirement, 40 µL
- Internal standard for each analyte, leading to accurate and precise analyte identification and quantification
- Wide dynamic range with an LLOQ and ULOQ of <1 µM to >10,000 µM, respectively



Chromatogram of the separation of a mixture of amino acid standards. The amount of each amino acid injected on the column was 10 pmole (except 5 pmole for cystine). Detection was performed using the 3200 QTRAP® system.



Chromatogram of the separation of a mixture of amino acids from a physiological plasma sample. Detection was performed using the 3200 QTRAP® system.

Please note that the results presented above were obtained using a single instrument and single set of standards and samples. Prior to production use, the method should be fully validated with real samples, and the results here may not be typical for all instruments. Variations in LC column properties, chemicals, environment, instrument performance and sample preparation procedures will impact performance, thus these results should be considered as informative rather than representative.

HPLC Apparatus and Conditions

This method has been tested on 3 different HPLC Systems:

- Agilent 1100 LC system
 - Agilent 1200 LC system
 - Shimadzu Prominence LC system
- **Column:** AAA C18 reversed-phase column, 5 μ M, 150 x 4.6 mm (AB SCIEX)
 - **Mobile Phase A:** Water + 0.1% formic acid + 0.01% heptafluorobutyric acid (plus MP modifier)
 - **Mobile Phase B:** Methanol + 0.1% formic acid + 0.01% heptafluorobutyric acid (plus MP modifier)
 - **Flow rate:** 0.8 mL/min.
 - **Column oven temperature:** 50°C
 - **Injection volume:** 2 μ L

MS/MS Detection

- Cliquid® Amino Acid Software Version 3.0 supports methods run on API 3200™, API 4000™, 3200 QTRAP®, and 4000 QTRAP® LC/MS/MS systems
- TurbolonSpray® ion source
- Positive polarity
- Analyst® Software 1.5 or 1.5.1 and Cliquid® Software 3.0 are required to run this iMethod™ Test

Ordering information

Product Name	Part N°
<i>iMethod™ Test for aTRAQ™ Kits 1.0 including Cliquid® AA 3.0 SW License</i>	1039658

This kit includes an iMethod™ Test (methods and report templates) DVD for relative and absolute amino acid quantitation from protein hydrolysate and physiological samples. Installation, set-up, demonstration of the System Suitability test and basic training are included when this product is ordered as part of a system purchase. aTRAQ™ Reagent kits and column need to be ordered separately. Go to www.absciex.com.

Important Note

The purchase and use of certain chemicals listed above may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements.

The iMethod™ Test described above has been developed by AB SCIEX to provide all the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. The performance of this method will need to be verified in a given lab due to potential variations in instrument performance, maintenance, chemicals and procedures used, technical experience, sample matrices and environmental conditions. It is the responsibility of the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

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