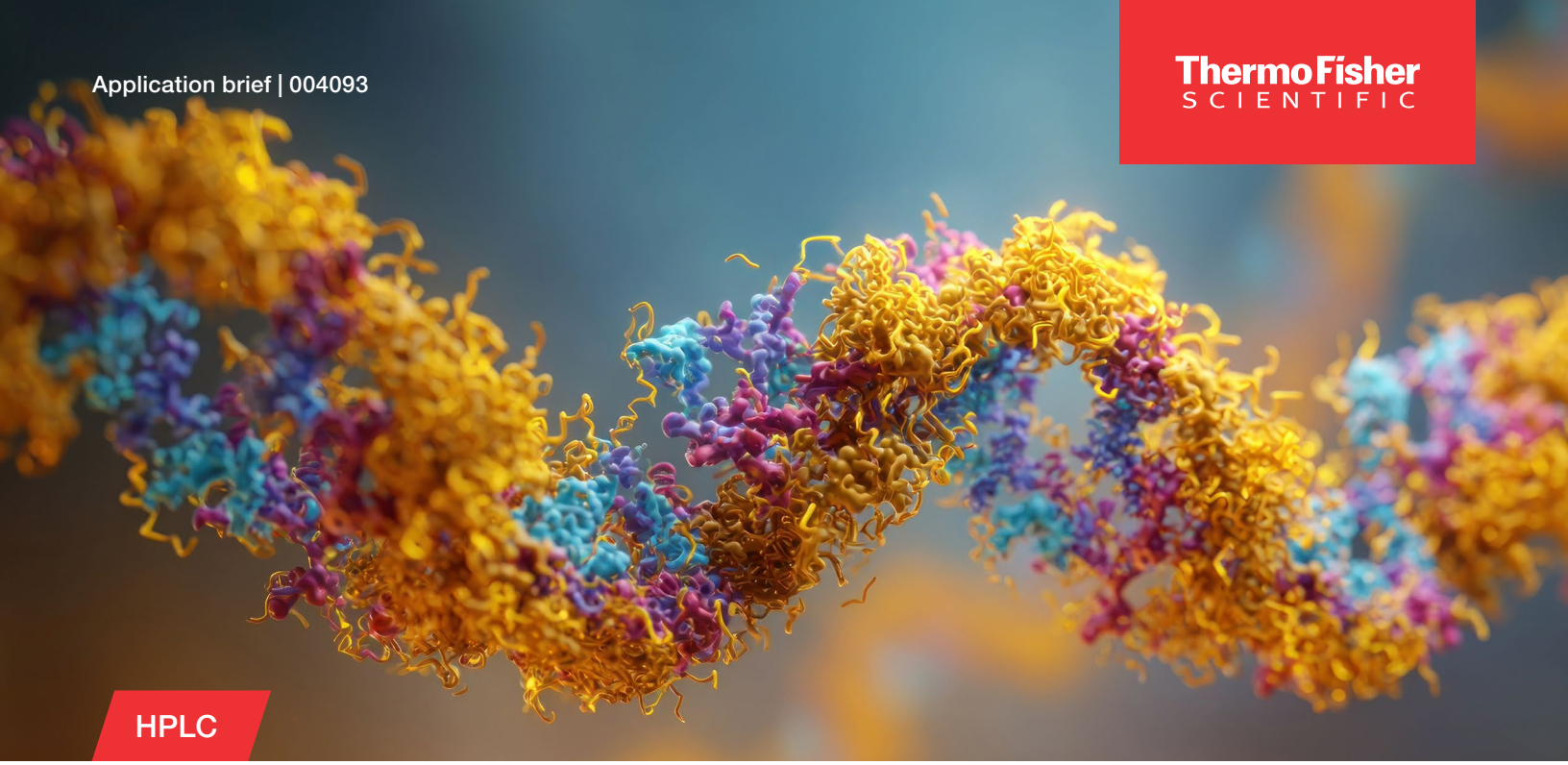
Application brief | 004093



HPLC

Automated in-needle OPA/FMOC derivatization of amino acids analysis with the Thermo Scientific Vanquish Core HPLC system operated under Empower 3.8.0 CDS

Authors

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Keywords

HPLC, Vanquish Core HPLC system, amino acids, derivatization, OPA, FMOC, custom injection program, Accucore column, Empower 3.8.0 release, SII for Empower

Application benefits

- Fully automated workflow for the OPA/FMOC derivatization of amino acids within the Thermo Scientific™ Vanquish™ Split Samplers and subsequent liquid chromatographic (LC) analyses with proven robustness
- Instrument control, data acquisition, and data processing under Waters™ Empower™ 3.8.0 software, fully compatible with Thermo Scientific™ Standard Instrument Integration (SII) for Empower 1.3

Goal

Demonstrate the ease of use of the Thermo Scientific™ Vanquish™ Core HPLC System and Thermo Scientific™ Accucore™ C18 HPLC Column for amino acid analysis through the implementation of automated in-needle derivatization within a Waters Empower environment

Introduction

Amino acids (AAs) are essential building blocks of life and are commonly analyzed in both scientific research and industrial applications. Although over 500 AAs occur naturally, most analyses focus on the 20 proteinogenic α-AAs. These are typically separated using techniques like reversed-phase (RP), hydrophilic interaction (HILIC),[1] or ion-exchange (IEX) chromatography, combined with different detection methods.

Because many AAs do not naturally absorb UV light, a common approach is to enhance their detectability through pre-column derivatization—using ortho-phthaldialdehyde (OPA) for primary AAs and 9-fluorenylmethyloxycarbonyl chloride (FMOC-Cl) for secondary AAs—to produce highly fluorescent compounds. However, manual derivatization can be tedious and prone to inconsistent results. This is where automation offers a clear advantage: it simplifies workflows, reduces hands-on time, improves reproducibility, and enhances lab safety—making it especially valuable in routine analytical environments.

A previous customer application note[2] demonstrated in-depth how OPA/FMOC derivatization of AAs can be fully automated using in-needle sample preparation (Figure 1), controlled by the autosampler of a LC system and managed through the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS). While the flexibility in hardware selection was showcased in a previous document,[2] the workflow implementation with another vendor's CDS is the focus of this application brief. Specifically, it demonstrates how the same automated derivatization process was successfully implemented using Waters Empower 3.8.0 software with the SII for Empower 1.3 plugin on a Vanquish Core HPLC system. Some key steps for instrumental setup and data processing for successful operation within the Empower environment are highlighted.

Experimental

Experimental details on chemicals used, sample, reagent, and standard preparation, as well as detailed method parameters are given in the in-house experiments of reference 2.

Instrumentation

Thermo Scientific Vanquish Core HPLC system consisting of:

- Thermo Scientific™ System Base Vanquish™ Core (Cat. No. VC-S01-A-02)
- Thermo Scientific™ Vanquish™ Quaternary Pump C (Cat. No. VC-P20-A-01)
- Thermo Scientific™ Vanquish™ Split Sampler CT (Cat. No. VC-A12-A-02)
- Thermo Scientific™ Vanquish™ Split Sampler Sample Loop, stainless steel, 100 µL (Cat. No. 6851.1950)
- Thermo Scientific™ Vanquish™ Column Compartment C (Cat. No. VC-C10-A-03) with passive pre-heater, 5 µL, 0.25 × 580 mm, stainless steel (Cat. No. 6732.0180)
- Thermo Scientific™ Vanquish™ Fluorescence Detector F, D-PMT (Cat. No. VF-D51-A)
- Thermo Scientific™ Vanquish™ Fluorescence Standard Flow Cell, 8 µL, biocompatible (Cat. No. 6079.4230)

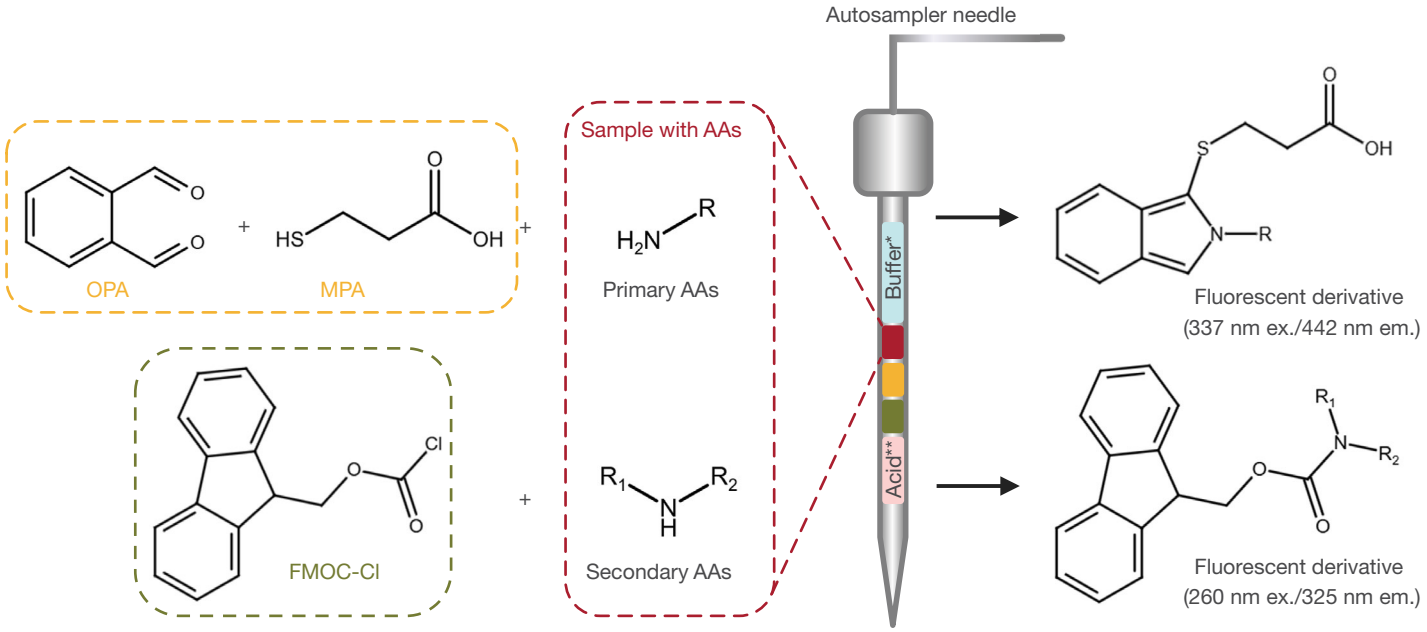


Figure 1. In-needle derivatization of primary and secondary AAs with OPA/MPA and FMOC-Cl reagents for subsequent LC analysis of the fluorescent derivatives.[2] *Borate buffer pH 10, **acetic acid for quenching.

Consumables

- Thermo Scientific™ Accucore™ C18 Column, 3 × 150 mm, 2.6 µm (Cat. No. 17126-153030)
- Thermo Scientific™ Unigard™ Direct-Connection Guard Cartridge Holder (Cat. No. 852-00)
- Thermo Scientific™ Accucore™ C18 Guard Cartridge, 3 × 10 mm, 2.6 µm (Cat. No. 17126-013005)
- Thermo Scientific™ SureSTART™ 2 mL Amber Glass Short Thread Screw Top Vials, 100/pack, Level 2 (Cat. No. 6ASV9-2P)

Data processing and software

For data acquisition and analysis, Empower CDS was used (version 3.8.0) together with the Thermo Scientific SII for Empower plugin (version 1.3).

The plugin enables the operator to configure and control the Vanquish Core HPLC system under Empower software. As shown in Figure 2A, the Empower interface opens with all familiar control elements. A right-click on the module panels opens the instrument view (Figure 2B) and enables the change of parameter settings. A wizard guides you through all steps to set up a new instrument method (Figures 3 and 4), which can be placed in the method set and then be selected on the Empower interface in the drop-down menu. Some more detailed guidelines can be reviewed in reference 3.

The in-needle derivatization of the AAs was realized by a *Custom Injection Program* (CIP) set up within the instrument method editor of Empower CDS. The CIP mode was selected as *Replace normal injection.* The individual commands were then entered into the table as shown in Figure 5.

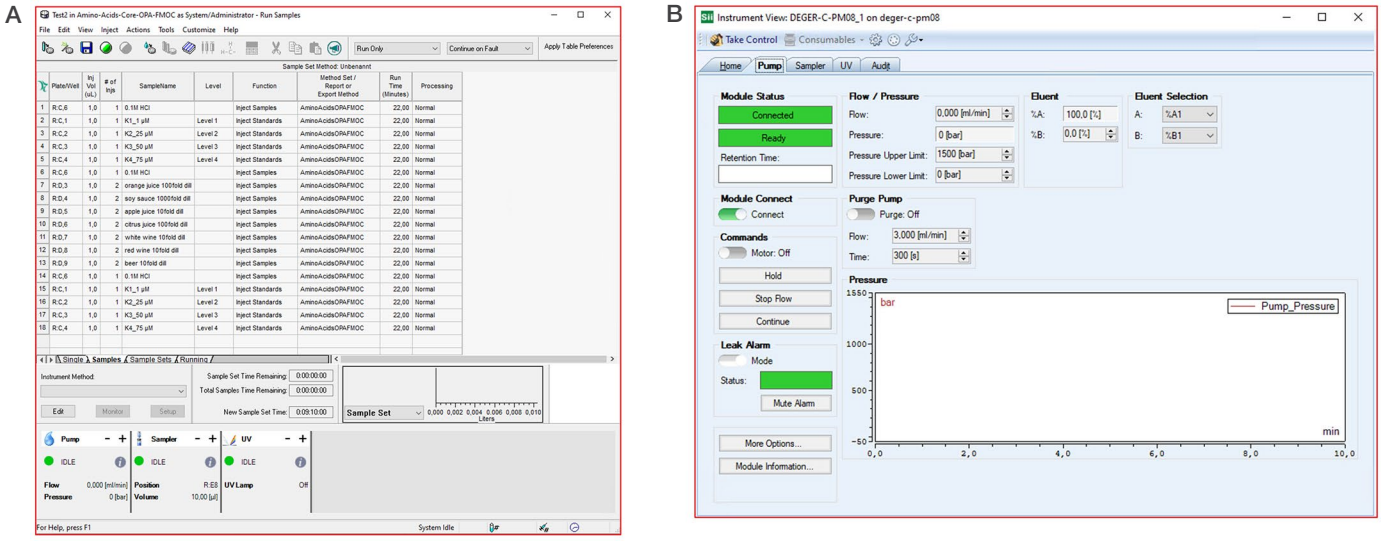


Figure 2. Screenshots of (A) Empower interface with sequence example and (B) SII for Empower instrument view.

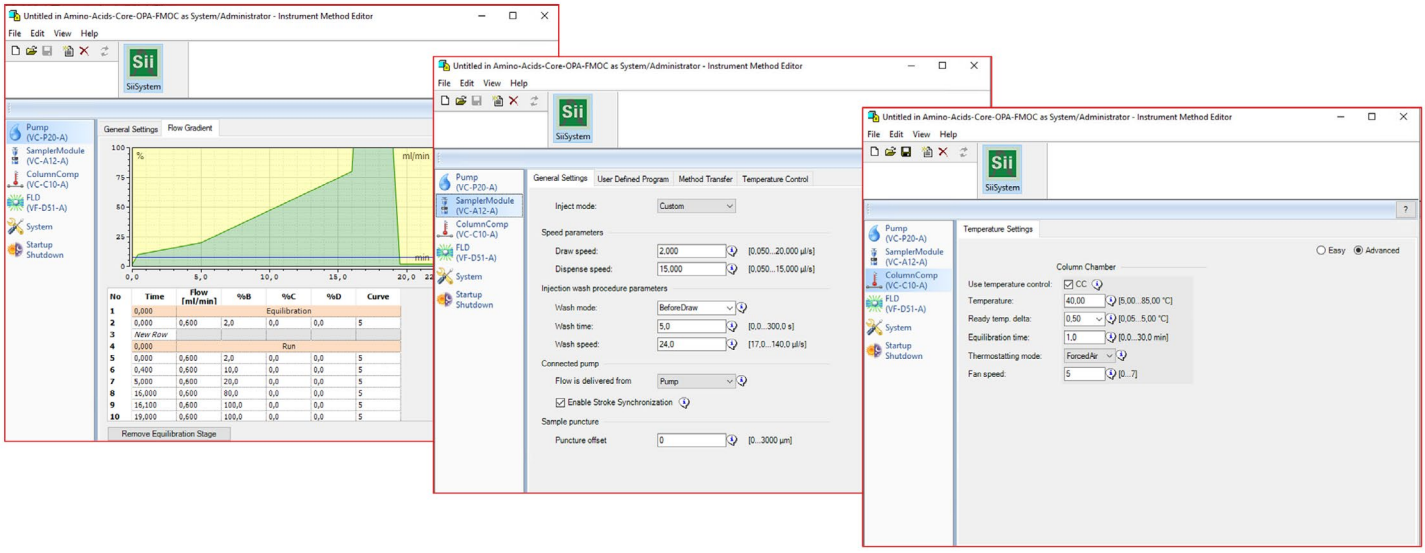


Figure 3. Screenshots of SII for Empower instrument method wizard for pump, sampler, and column compartment.

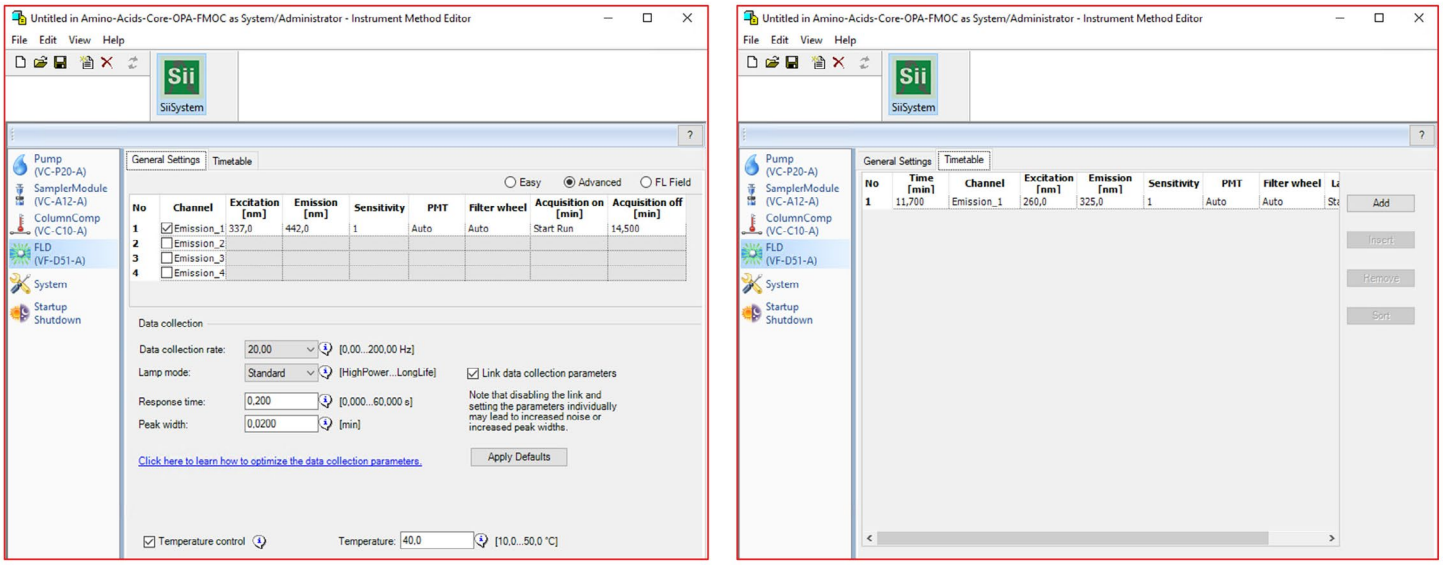


Figure 4. Screenshots of SII for Empower instrument method wizard for the fluorescence detector.

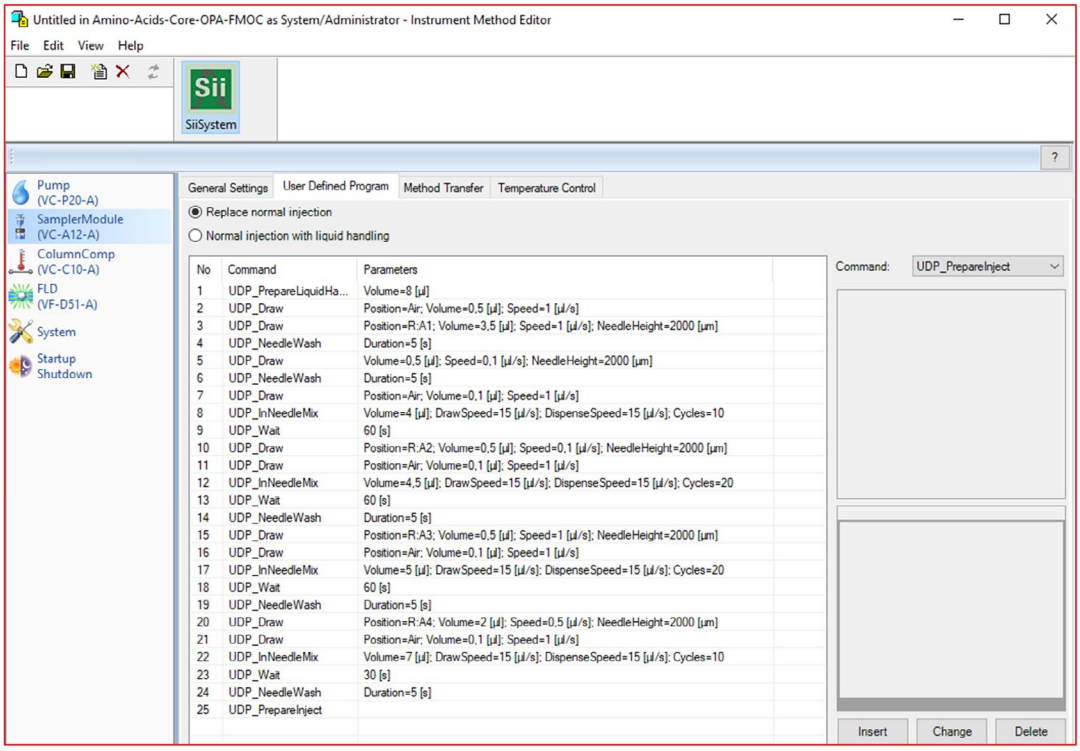


Figure 5. SII for Empower CIP for in-needle derivatization of AAs.

Results and discussion

The in-needle derivatization workflow for AAs with the Vanquish Core HPLC system was easily implemented on an Empower-controlled workstation. Figure 6 shows the AA separation achieved, including retention times, as visualized in the Empower environment. It displays a calibration standard injection—with all tested AAs present at equal concentrations—and a representative test sample injection of soy sauce.

The data processing is approached as usual under the Empower CDS. For example, calibration curves using a quadratic fit were created to estimate the sample concentrations of each individual AA. The fit type, along with the list of AA components and their corresponding amounts, can be adjusted in the processing method settings. Figure 7 gives an exemplary calibration curve for tyrosine (Tyr), as displayed in the calibration curve window, next to some sample results observed for a soy sauce sample in the data review window.

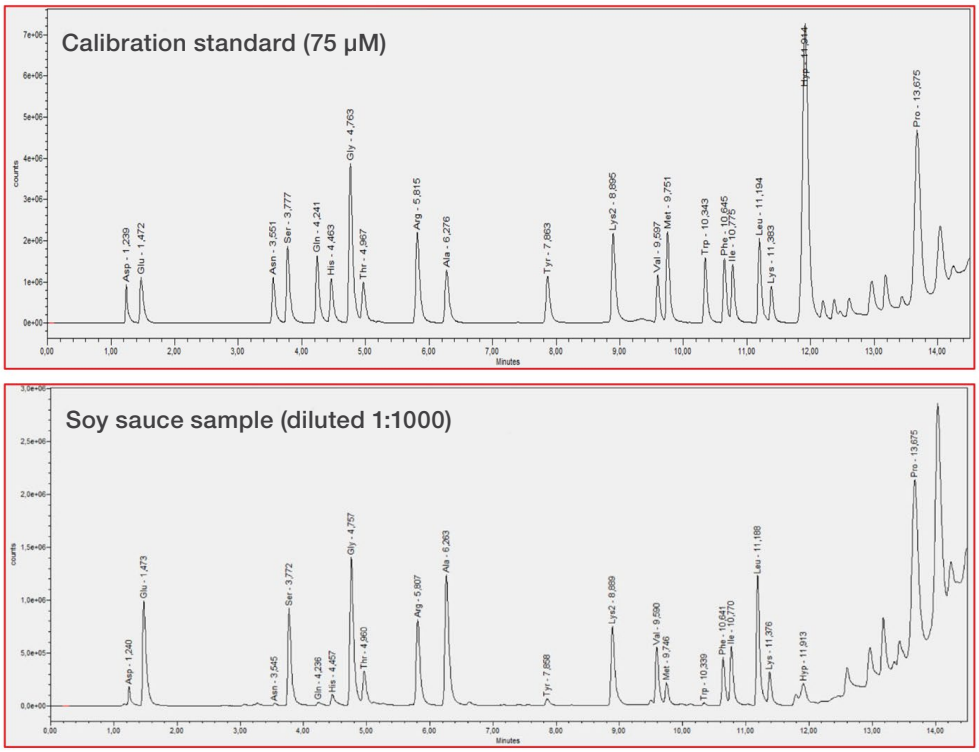
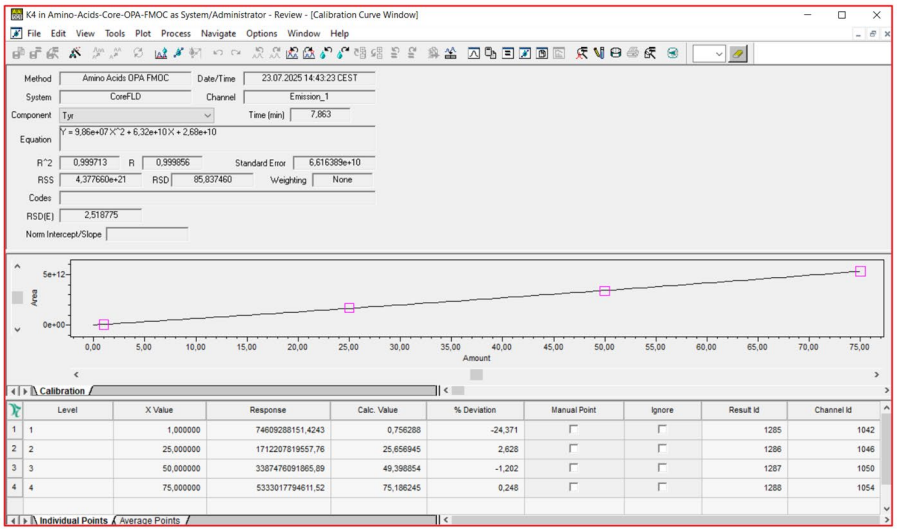


Figure 6. Chromatograms of a 75 µM AAs calibration standard and a soy sauce test sample.

A



B

	Name	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)	Int Type	Amount	Units	Peak Type
1	Asp	1,240	548762267376	0,71	182192445388	BV	17,626	µM	Found
2	Glu	1,473	3941269501373	5,11	991952210898	VB	66,816	µM	Found
3		3,065	73329879402	0,10	14035593717	BV			Unknown
4		3,267	156638195334	0,20	26827378301	VV			Unknown
5	Asn	3,545	87978931442	0,11	22983869109	VV	0,800	µM	Found
6	Ser	3,772	3614526070910	4,69	917533397545	VV	39,436	µM	Found
7	Gln	4,236	142742774332	0,19	29121929121	VV	1,101	µM	Found
8	His	4,457	484652669512	0,63	102739010639	VV	8,496	µM	Found
9	Gly	4,757	5793836387109	7,52	1401608630741	VV	29,721	µM	Found
10	Thr	4,960	1528364515679	1,98	324266408334	VV	27,347	µM	Found
11		5,173	43505407474	0,06	18609693903	VV			Unknown
12		5,260	97422457233	0,13	19115079162	VB			Unknown
13	Arg	5,807	3682961034663	4,78	812896788793	BV	30,144	µM	Found
14	Ala	6,263	5791322621596	7,51	1233794193097	VV	71,181	µM	Found
15		6,625	172672636931	0,22	34346349485	VB			Unknown
16		7,405	113234931171	0,15	9056530981	BB			Unknown
17	Tyr	7,858	300779420155	0,39	64424246021	BB	4,309	µM	Found

2D Channels Peaks Fractions

Figure 7. Data processing under Empower CDS. (A) Tyrosine calibration curve results observed in the calibration curve window and (B) soy sauce sample results observed in the data review window.

Conclusion

- The automated OPA/FMOC derivatization workflow is both effective and highly adaptable across various laboratory hardware and software setups. The cross-platform capabilities streamline its implementation and efficiently support standardized AA analysis in routine labs.
- Vanquish HPLC systems are fully compatible with Empower 3 software using SII for Empower, including support of key features like CIPs, making it an ideal choice for multi-vendor laboratory environments.

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