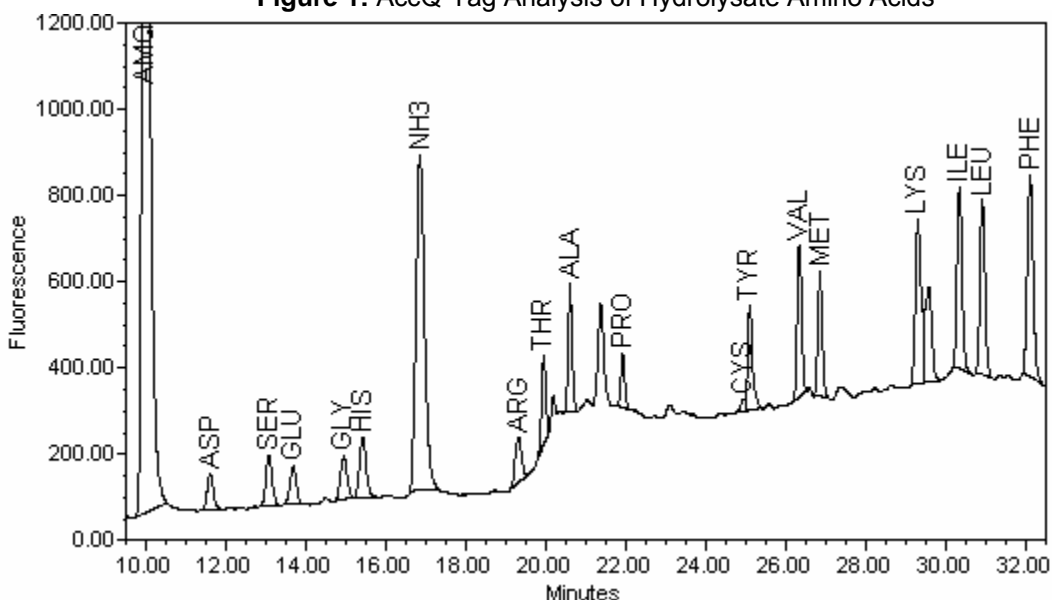


## Waters® 2475 Multi- $\lambda$ Fluorescence Detector: High Sensitivity Amino Acid Analysis

Waters Performance PerSPECTives (720000425EN and 720000448EN) focus on the technology that enables the Waters® 2475 Multi- $\lambda$  Fluorescence Detector to provide superior results for high-sensitivity detection. This Performance PerSPECTive focuses on the enhanced performance of the 2475 detector, compared to previous technologies, using Waters AccQ•Tag™ method for pre-column derivatization and analysis of amino acids. It also demonstrates how detection sensitivity can be increased for sample-limited applications using narrow-bore chromatography (2.1 mm x 150 mm vs. 3.9 x 150 mm columns).

Figure 1: AccQ•Tag Analysis of Hydrolysate Amino Acids



System: Waters Alliance® System (2695 Separations Module)  
 Detector: Waters 2475 Multi- $\lambda$  Fluorescence Detector at 250 nm excitation, 395 nm emission  
 Data: Waters Empower™ Software  
 Column: Waters AccQ•Tag 3.9 X 150 mm at 37° C (Part # WAT052885)  
 Mobil Phase: A=AccQ•Tag Eluent A (Part # WAT052890)  
 B=CH<sub>3</sub>CN  
 C=Water  
 Flow Rate: 1.0 mL/minute  
 Gradient: AccQ•Tag Method  
 Sample: 5  $\mu$ L injection of 0.20 pmol/ $\mu$ L Derivatized Amino Acid Standards (except for cystine, 0.10 pmol/ $\mu$ L)

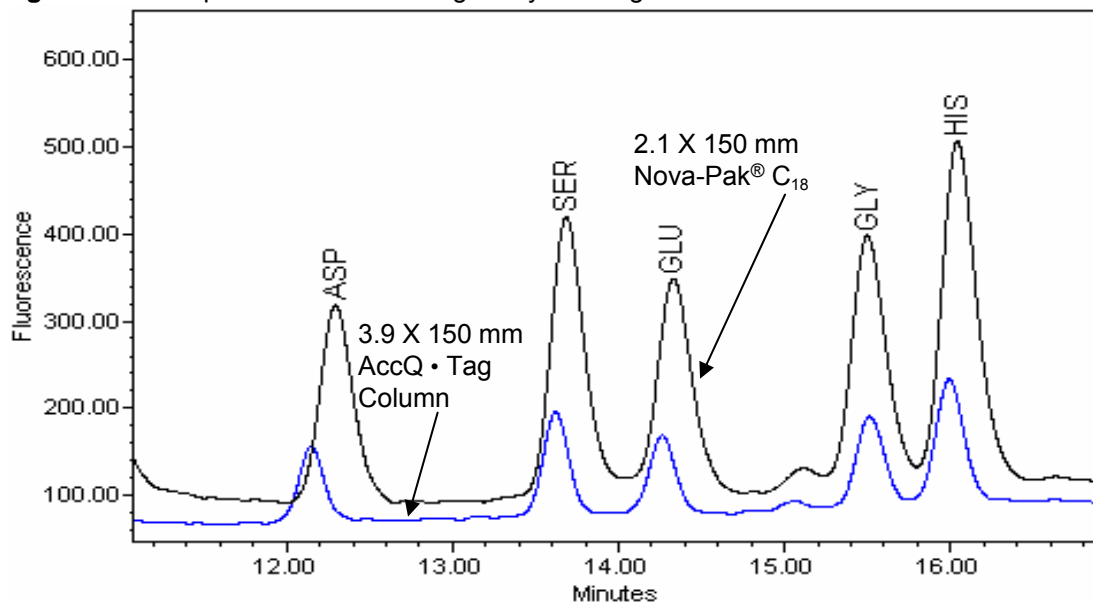
**Higher Sensitivity for AccQ•Tag Analysis.** Figure 1 shows an injection of 1.0 pmol (0.50 pmol for Cys) of derivitized amino acid standards. Signal-to-noise values ranged from 8 for Cys to over 130 for Phe. On average for the 17 amino acids in the standard, the Waters 2475 detector increased sensitivity more than 4 times over results obtained using previous fluorescence detector technologies offered by Waters.

**Table 1:** Signal-to-noise values of derivitized Amino Acid Standards (1 pmol, CYS=0.05 pmol). Note the increases in sensitivity over the entire range of target compounds.

		ASP	SER	GLU	GLY	HIS	ARG	THR	ALA	PRO	CYS	TYR	VAL	MET	LYS	ILE	LEU	PHE
474	3.9X150	5	8	6	7	9	7	13	19	8	2	16	23	19	25	27	24	29
2475	3.9X150	24	32	23	27	39	28	57	81	35	8	71	98	79	104	120	110	131
2475	2.1X150	79	115	89	104	143	122	123	265	146	33	299	376	305	325	431	400	492

**Increased Sensitivity for Sample Limited Applications.** Further increases in sensitivity can be achieved in a number of ways. For example, analysts can inject larger sample volumes onto the column resulting in greater peak response. Unfortunately this approach is not always feasible. Often, only small sample volumes are available for use in different analyses. In these sample limited situations, sensitivity can be increased using a smaller internal diameter (i.d.) column. Injecting the same sample mass onto a column of reduced i.d. produces larger peaks by increasing compound concentration within a given eluted peak volume. Figure 2 and Table 1 clearly show an average peak response increase of ~ 3.5 times for the 17 target compounds, consistent with the theoretical calculations (see Performance PerSPECTive 720000288EN). Implementing this change is straightforward; no physical changes to the Alliance HPLC system are required. Replacing the column, using the same gradient profile, and adding an extra 6 minutes of equilibration time are all that is needed. In addition to added sensitivity, a reduction in solvent usage is also realized.

**Figure 2:** First 5 peaks of an AccQ•Tag Analysis using 3.9 and 2.1 mm i.d. columns.



**Summary:**

- The Waters 2475 Multi-λ Fluorescence Detector offers higher levels of sensitivity for a variety of applications including AccQ•Tag derivitized amino acid analysis.
- Further increases in sensitivity of sample-limited material can be achieved using narrow-bore chromatography.
- Due to the unique flowcell design of the Waters 2475 detector combined with Alliance technology, analysts can use narrow-bore chromatography without compromising chromatographic resolution or peak shape.