Analysis of Phosphopeptides by Automated IMAC in Gallium Form
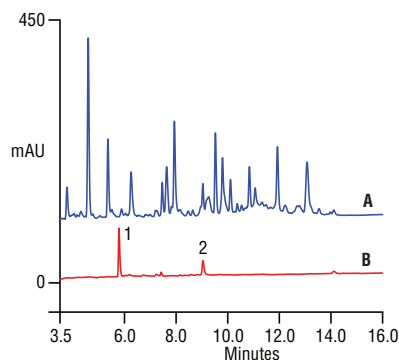

Column:	Thermo Scientific™ Acclaim™ 300 C18 3 µm, 4.6 × 150 mm
Pump:	Thermo Scientific™ Dionex™ Summit P680A DGP6
Mobile Phases:	(A) 70:30 acetonitrile:water (v/v) (B) water (C) 0.40% TFA (v/v) + 1.0 mM $Na_4P_2O_7$

Gradient:	Times	0	15	25
	%A	14	75	75
	%B	61	0	0
	%C	25	25	25

Flow:	1.0 mL/min
Temperature:	TCC-100 thermostat, 30 °C
Injection:	ASI-100 autosampler; 30 µL
Detector:	UVD-340U; UV at 214nm
Note:	See Thermo Scientific Dionex Technical Note 705 for a complete description of the automation and IMAC conditions.
Samples:	Fractionated on Thermo Scientific™ ProPac™ IMAC-10 2 × 50 mm column in gallium form: (A) Unretained fraction (B) Fraction retained by IMAC
Sample Prep:	β-Casein, 2 mg/mL, reduced and carboxymethylated, digestion with immobilized trypsin, diluted 1:1 in 200 mM formic acid.

Peaks: 1. FQSpEEQQQTEDELQDK
2. RELEELNVPGEIVESpLSpSpSpEESITR

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Immobilized metal affinity chromatography (IMAC) is a powerful tool for enriching phosphopeptide isolates from protein digests. Gallium and iron are typically used as the immobilizing metals in IMAC. An automation package consisting of the Thermo Scientific Dionex dual-gradient LC system, ProPac IMAC-10 (for enrichment) and Acclaim 300 analytical column is the perfect solution for this technique.