# An Alternative Approach for Characterization of Impurities and Site-specific Modifications in Protein Drugs

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## INTRODUCTION

Sequence variants and posttranslational modifications (PTMs) such as glycosylation, deamidation, and oxidation are common in recombinant protein pharmaceuticals. They potentially affect the safety, activity and stability of protein drugs. Effective monitoring of such impurities and PTMs requires sensitive and reproducible methods.

In this study, we have applied an Ultra Performance Liquid Chromatography-Data Independent Acquisition Mass Spectrometry (UPLC/MS<sup>E</sup>) approach for characterization and quantification of impurity proteins and site-specific PTMs in protein drugs. The method was evaluated using tryptic digests of yeast enolase, alcohol dehydrogenase (ADH), and a humanized monoclonal antibody (IgG1). Synthetic peptides were used to 1) further confirm the deamidations in the IgG1 "PENNY" distinguish aspartyl and isoaspartyl peptide, and isoforms; 2) exclude potential Met oxidation artifacts produced in the MS ion source; and 3) test ionization efficiency of modified peptides.

# **ADVANTAGES OF LC/MS<sup>E</sup>**

- Unbiased data acquisition
- Unbiased sampling of low-abundance peptides
- Improved detection sensitivity
- Suited for guantification via MS signal intensities

oncentration of impurity proteins identified

UPLC/MS<sup>E</sup> analysis and Hi3 guantitation of Enolase and ADH

- Excellent reproducibility
- Improved analytical speed and efficiency

### Peptides identified by UPLC/MS<sup>E</sup> covered 97% of Enolase protein sequence. MS<sup>E</sup> data also identified peaks corresponding to protein impurities , partial/Non-tryptic peptides, and modified peptides.

Glucose-6-phosphate isomerase	e (Pgi1p – <mark>Px</mark> ); Tri	osephosphate isomerase (Tp
Waters Acquity™ UPLC®	MS System:	Waters SYNAPT™ MS
Acquity <sup>™</sup> PST C18, BEH300, 2.1	x 100 mm, 1.7 µn	ı
0.1% FA in water,	В:	0.1% FA in acetonitrile
0-50% B in 60 min,	Flow Rate:	0.2 ml/min
40 °C,	Detection:	MS <sup>E</sup>
	Glucose-6-phosphate isomerase Waters Acquity™ UPLC® Acquity™ PST C18, BEH300, 2.1 0.1% FA in water, 0-50% B in 60 min, 40 °C,	Glucose-6-phosphate isomerase (Pgi1p - Px); TriWaters Acquity™ UPLC®MS System:Acquity™ PST C18, BEH300, 2.1 × 100 mm, 1.7 µm0.1% FA in water,B:0-50% B in 60 min,Flow Rate:40 °C,Detection:





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	-MS (TI	C)		-14	22.00	23.00	24.00	25.00 26	00 27.00	28.0	0 29.00	30.00 31.00 32.00	33.00 34	00 35.00	36.00 37.0	38.00
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# CONCLUSIONS

- 1. UPLC/MS<sup>E</sup> analysis of protein tryptic digests yielded qualitative and quantitative information about protein impurities and the presence of protein modifications.
- 2. Unbiased data acquisition with UPLC/MS<sup>E</sup> provides high sequence coverage, especially for peptides with low-stoichiometry modifications (<0.5%).
- 3. Reproducible high resolution UPLC peptide separations ensured confident identification of isobaric modifications with sub Da mass differences (e.g., single and double Asn-deamidated peptides)
- 4. Synthetic peptides were helpful for distinguishing modified peptides isoforms, and evaluating the ionization efficiency of peptides and their modified forms.