Application Note: 447

The Importance of Linear Dynamic Range for Small Molecule and Targeted Peptide LC-MS/MS Quantitation

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Key Words

- Bioanalysis
- Linear Dynamic Range
- Pulse Counting
- Targeted Peptide Quantitation
- TSQ Vantage

Introduction

Mass spectrometry (MS) can be divided into ion generation, ion separation, and ion detection. While the techniques of ionization and mass analysis receive much attention, ion detection is less frequently reviewed. The impact of ion detection on quantitative analysis, however, is significant, especially for applications requiring wide dynamic range and high sensitivity such as small molecule quantitation during drug research, and protein quantitation. The demand for simpler methods and quick turn-around of results is high in today's competitive research environments. Typical MS detectors have to perform at very high count rates (> 10^6 counts/s) with minimum refresh times for a wide dynamic range of responses and have low noise characteristics for very sensitive limits of detection.[1]

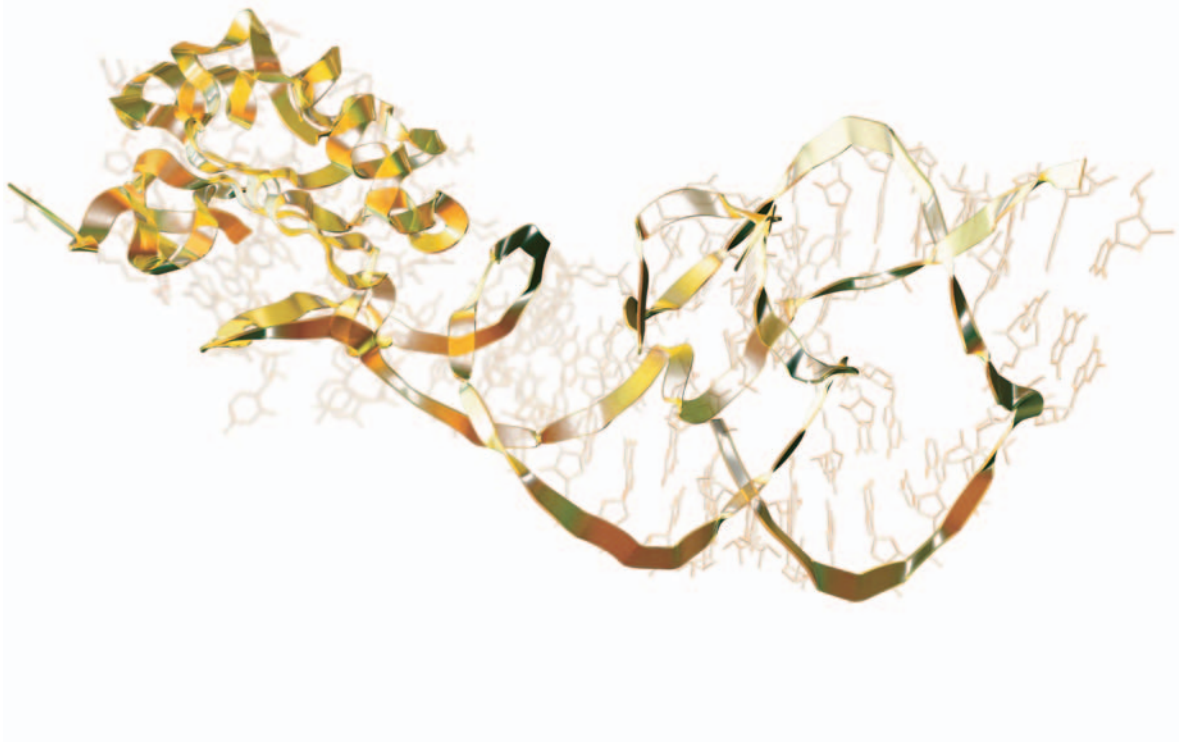
Continuous-dynode electron multipliers (CDEM) are typically used in a triple stage quadrupole because they are compact, rugged, and customizable. The CDEM technique can either be used as "analog" counting mode or "pulse" counting mode. In the pulse counting mode, individual electron pulses from each incident ion are counted as discrete events, in an attempt to buffer against noise created by thermal events or electron impact ionization (EI) of background gases. The limitation of the pulse counting technique is a fall off in linearity due to pulse "pile-up" effects where multiple ion events at high incident rates cannot be distinguished.[1]

In the analog mode, the incident ion creates a cascade of electrons which generates a current that is then converted to a voltage, followed by digitization. Advances in electronics and better vacuum have virtually eliminated common detector noise such as Johnson, Shot, Flicker, & EI of background gases. The key advantage is that a single incident ion can be amplified by 10^6x or more giving high sensitivity and wide dynamic ranges.[1] This is the key reason the CDEM in analog mode with advanced electronics is used in the Thermo Scientific TSQ Vantage mass spectrometer.

The benefit of having a wide linear dynamic range is especially important for small molecule quantitation during drug research, and for targeted peptide quantitation. In small molecule quantitation during drug research, the dosage form can cause an order of magnitude difference in quantitative results. In proteomics, the dynamic range of proteins in real samples can be in excess of 10 orders of magnitude with further complications arising from varying ionization efficiencies for the representative enzymatic peptide fragments. Thus having a wide dynamic range makes experimentation simpler by avoiding re-assays and dilution issues.

Goal

Demonstrate linear dynamic range in excess of four orders of magnitude using a simple fit for small molecule and biomolecule quantitative applications.



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Experimental Conditions

Chromatographic Conditions, Small Molecule

Small Molecule:	Paroxetine
HPLC Pump:	Thermo Scientific Accela pump
Autosampler:	CTC™ PAL (CTC Analytics, Basel, Switzerland)
Column:	Thermo Scientific Hypersil GOLD C18 50 mm x 2.1 mm (3 µm) column

HPLC Method, Small Molecule

A linear gradient of 10% Solvent B (acetonitrile containing 0.1% formic acid) to 95% B over five minutes was used to chromatograph paroxetine. Solvent A was water containing 0.1% formic acid. The flow rate was 1 mL/min. Injection volumes of 10 µL were used.

Chromatographic Conditions, Biomolecule

Biomolecule:	Horse Heart Myoglobin Tryptic digest
HPLC Pump:	Thermo Scientific Surveyor MS pump
Autosampler:	MicroAS autosampler (Spark Holland, Netherlands)
Column:	PicoFrit® C18 column from New Objective (75 µm x 100 mm)

HPLC Method, Biomolecule

A linear gradient from 2% B (0.1% FA/100% ACN) to 50% B in 45 min was used to chromatograph horse heart tryptic digest fragments in *E. coli* protein matrix. Buffer A was 0.1% FA/2% ACN/98% H_2O. Post-split flow rate: 300 nL/min. Sample loading: Directly loaded on column. Injection amount: 1 µL.

Mass Spectrometer Conditions, Small Molecule

Mass Spectrometer:	TSQ Vantage™
Ionization Mode:	HESI-II in positive ion mode
Ion Sweep Gas:	5 au
Ion Transfer Tube Temp:	300 °C
Sheath Gas:	60 au
Aux Gas:	30 au
Resolution:	0.7 Da (FWHM) on Q1 and Q3
Scan Time:	0.2 s
Scan Width:	0.002 Da
Chrom Filter:	5 s
Selected Reaction Monitoring:	Paroxetine 330.20 > 192.1 Da Alprazolam 309.1 > 281.0 Da (ISTD)
Collision Energy:	22 V
Collision Gas Pressure:	1.5 mTorr

Mass Spectrometer Conditions, Biomolecule

Mass Spectrometer:	TSQ Vantage
Ionization Method:	Ion Max source equipped with a column adapter for nanoflow (New Objective, Woburn, MA) in positive ion mode
Ion Sweep Gas:	0
Ion Transfer Tube Temp:	180 °C
Sheath Gas:	0
Aux Gas:	0
Resolution:	0.7 Da (FWHM) on Q1 and Q3
Scan Time:	0.02 s
Scan Width:	0.002 Da
Chrom Filter:	15 s

Multiple Selected Reaction Monitoring

Peptide ALELFR Representing Myoglobin:	374.72 > 435.3 Da 374.72 > 564.3 Da 374.72 > 677.5.3 Da
Collision Energy:	374.72 > 435.3 Da 10 V 374.72 > 564.3 Da 10 V 374.72 > 677.5.3 Da 12 V
Collision Gas Pressure:	1.5 mTorr

Results and Discussion

Figures 1 and 2 show over four orders of linear dynamic range for the quantitative analysis of Paroxetine and Myoglobin tryptic fragment (ALELFR) on the TSQ Vantage. Another notable factor is that Paroxetine was quantified using an internal standard (alprazolam) while the Myoglobin tryptic fragment (ALELFR) was quantified using absolute intensity, or, without an internal standard. Both assays demonstrated linearity as indicated by correlation coefficients of 0.9998 (Paroxetine) and 0.9999 (Myoglobin tryptic fragment ALELFR). Having a triple stage quadrupole with a wide linear dynamic range offers substantial advantages: time saved due to lack of re-assays and avoiding complications that are usually associated with dilution (partition, settling, solubility).

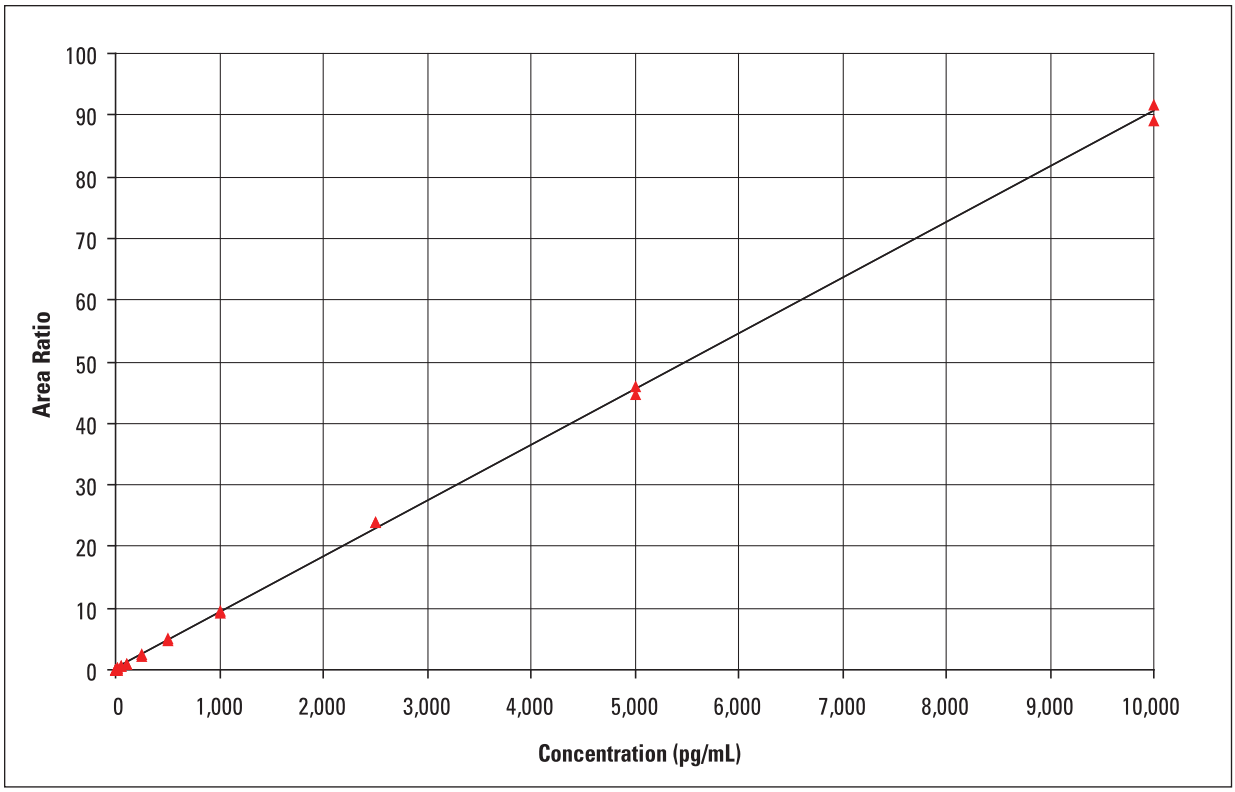

Figure 1: Heated ESI-II based LC-MS/MS analysis of Paroxetine showing four orders of linear dynamic range (1-10,000 pg/mL). A linear gradient of 10% Solvent B (acetonitrile containing 0.1% formic acid) to 95% B over five minutes was used. Solvent A was water containing 0.1% formic acid. The column used was Hypersil GOLD™ C18 50 mm x 2.1 mm (3 µm) and the flow rate was 1 mL/min. Injection volumes of 10 µL were used.

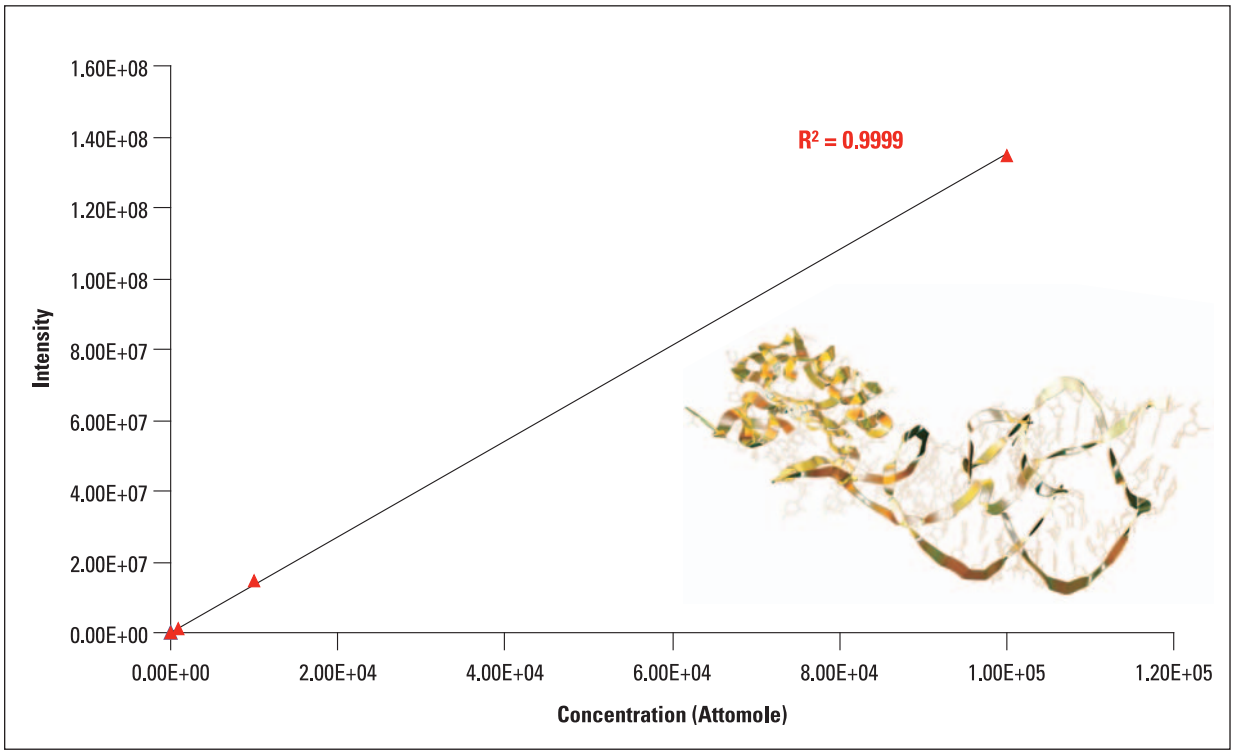

Figure 2: Linear dynamic range of four orders of magnitude for the peptide quantitation experiment. Horse heart myoglobin (0.01 fmol - 100 fmol) was spiked into *E. coli* tryptic digest. Excellent linear calibration curve was generated from 0.01 fmol to 100 fmol for peptide ALELFR without using internal standard.

Conclusions

A linear dynamic range in excess of four orders of magnitude using a simple fit for a small molecule and a biomolecule can be achieved on the TSQ Vantage triple stage quadrupole instrument. This allows for faster results and simpler LC-MS/MS methods for small molecule quantitation during drug research, and for targeted peptide quantitative analysis.

References

1. Koppenaal, D.W., et al. Anal. Chem. p419a. 2005.

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