

# High Sensitivity ESI, APCI Quantitation of Cabergoline and Pergolide

## in Plasma on the High Resolution TSQ Quantum

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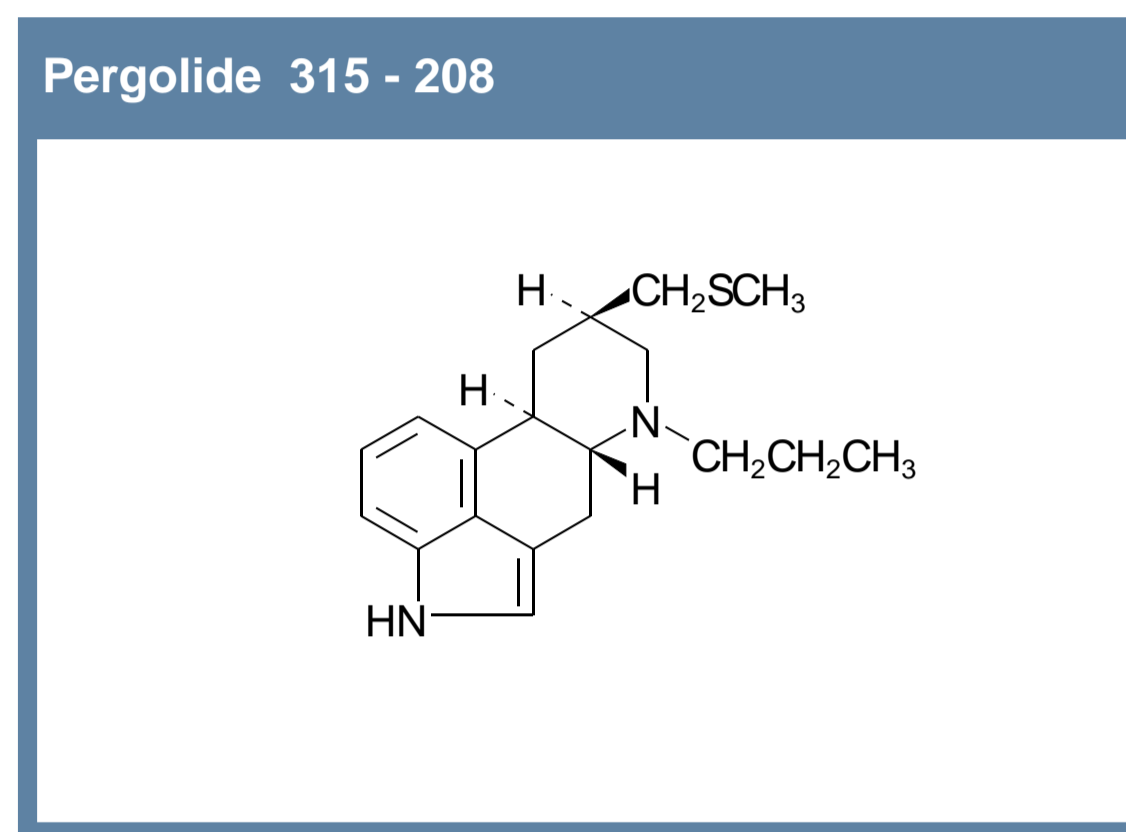
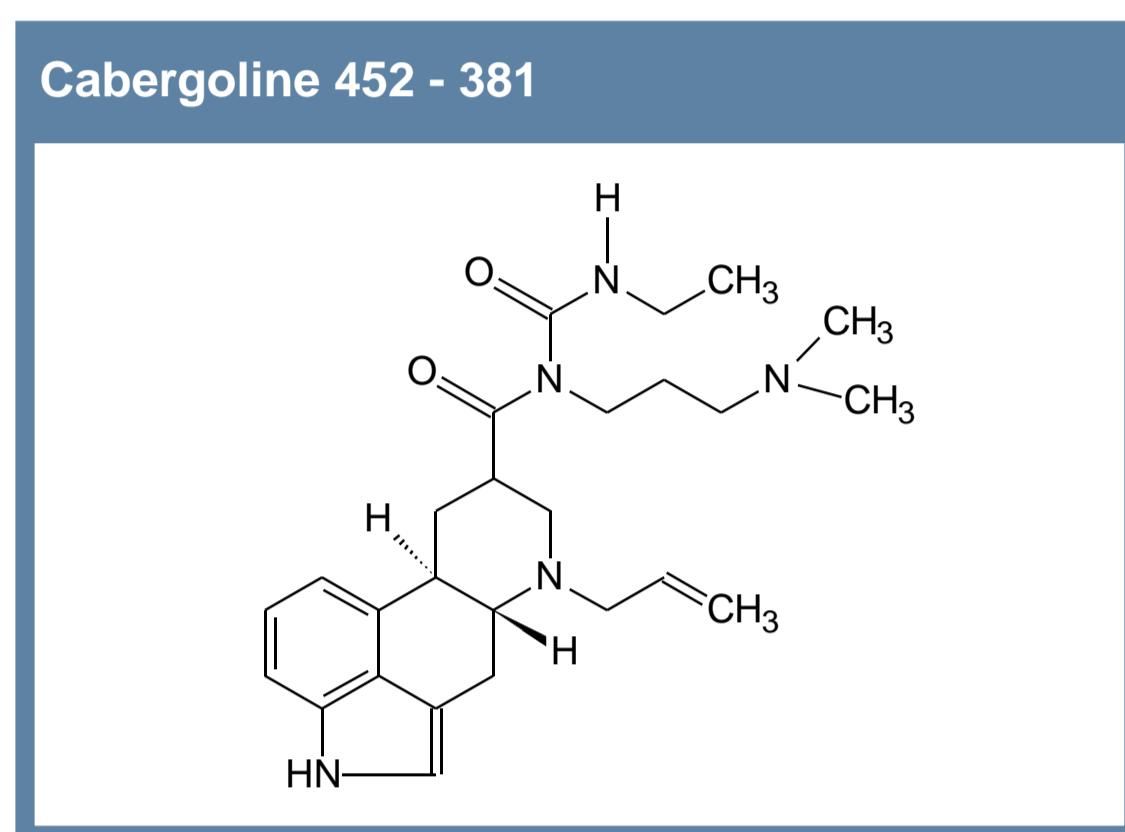
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### Overview

Quantitative analysis of pharmaceuticals with low systemic plasma levels requires the utmost in sensitivity and selectivity from a triple quadrupole mass spectrometer. The TSQ<sup>®</sup> Quantum was evaluated in the analysis of two such drugs, cabergoline and pergolide. LC/ESI/SRM analysis of cabergoline using unit resolution demonstrated excellent sensitivity (50 fg on column), accuracy, precision and a linear dynamic range of 5 orders of magnitude. LC/APCI/SRM analysis of pergolide also demonstrated excellent sensitivity (500 fg on column), accuracy, precision and 5 orders of linear dynamic range. Utility of high mass resolution on the TSQ Quantum in the APCI/SRM analysis of pergolide led to a two-fold increase in sensitivity and linear dynamic range.

### Introduction

Cabergoline and pergolide have potent dopaminergic activity and are indicated in hyperprolactinaemic disorders and Parkinson's Disease. Due to their efficacy and long-lasting activity, therapeutic doses are typically less than 1 mg. Plasma concentrations are consequently very low and sensitivity has been a major issue governing the development of bioanalytical methods. In the past, RIA has been the method of choice because of the high sensitivity, but recently dramatic advances have been made in triple quadrupole instrument design and notable improvements in sensitivity and selectivity have been reported with the first high resolution triple quadrupole mass spectrometer, the TSQ Quantum. This, coupled to its reputed excellent dynamic range capability, makes it ideally suited for the quantitative LC/API/SRM analysis of cabergoline and pergolide in plasma for pharmacokinetic applications.



### Method

Cabergoline and pergolide were spiked into bovine plasma, which had been precipitated with acetonitrile, so as to mimic complex biological extracts. The spiked extracts, covering  $\geq 5$  orders of linear dynamic range, were analyzed in replicates of 5, using ESI for cabergoline and APCI for pergolide. HPLC was performed on a Thermo Finnigan Surveyor<sup>®</sup> system using isocratic conditions on a C18 LC column (2.1 x 150 mm) with a mobile phase of acetonitrile/ammonium acetate. Detection was carried out using the Thermo Finnigan TSQ Quantum, operated in positive ion SRM mode. Sensitivity, linearity, accuracy and precision were assessed using unit and high mass resolution modes.

### Results

Bioanalytical methods for the pharmacokinetic analysis of cabergoline and pergolide in plasma require detection limits of 1-2 pg/mL (< 1 pg on column) and 5-10 pg/mL (< 5 pg on column), respectively. Using older generation mass spectrometers, the development of methods with this level of sensitivity has typically included exhaustive sample enrichment procedures. In addition, lengthy chromatographic run times, often involving gradient elution, have been necessary to facilitate separation of matrix interferences from the analytes of interest. Here, using the TSQ Quantum, minimal-treated plasma samples were analyzed using isocratic conditions with chromatographic run times of less than 4 minutes. Using ESI with unit resolution, a lower limit of quantitation (LOQ) of 50 fg of cabergoline on column was easily detectable (Figure 1) which is almost 20-fold lower than the LOQ obtained on the older generation TSQ 7000. Linearity over 5 orders of magnitude ( $R > 0.999$ ), with excellent accuracy and precision were also demonstrated (Table 1).

Figure 1. LOQ and Linear Dynamic Range for ESI/SRM Analysis of Cabergoline in Plasma

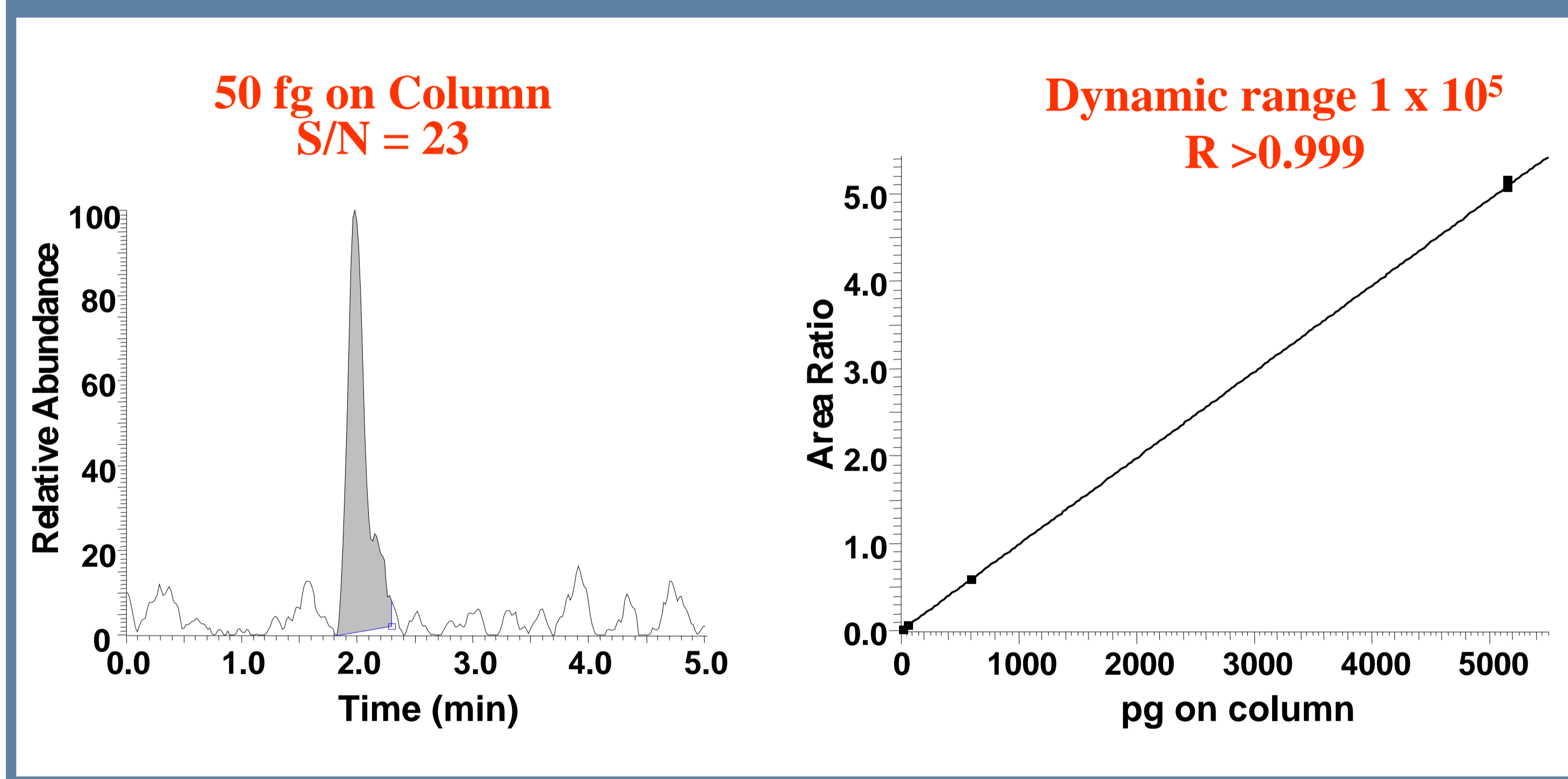


Table 1. Accuracy and Precision for ESI/SRM Analysis of Cabergoline in Plasma - 5 Orders of Linear Dynamic Range

Nominal Amount (pg on column)	Mean Amount (pg on column)	Accuracy (%RE)	Precision (%CV)
0.050	0.057	13.6	9.3
0.500	0.508	1.6	8.4
5.000	4.709	-5.8	2.3
50.000	49.861	-0.3	1.0
500.000	462.354	-7.5	1.3
5000.000	5038.062	0.8	0.5

For pergolide, using APCI under unit resolution conditions, the LOQ was 500 fg on column, the dynamic range 5 orders of magnitude with excellent linearity, accuracy and precision (Table 2, Figure 2). Further improvement of the LOQ to 250 fg using unit resolution, was difficult due to poor peak integration from chemical/matrix interferences (Figure 3). Accuracy and precision at the 250 fg level were significantly improved by increasing the mass resolution for precursor ion selection (Q1 0.2 Da FWHM). Using high resolution, there was a dramatic decrease in chemical noise and a corresponding 2X enhancement in signal to noise (Figure 3). Linearity over 5 orders of magnitude ( $R = 0.997$ ) (Figure 4) with accuracy and precision well within pharmaceutical industry specifications were also demonstrated (Table 2). The high resolution feature of this instrument thus gives the user a simple yet rapid means to improve method sensitivity/selectivity without the need for further sample enrichment.

Table 2. Accuracy and Precision for APCI/SRM Analysis of Pergolide in Plasma in Unit and High Resolution

Nominal Amount (pg on column)	UNIT RESOLUTION			HIGH RESOLUTION		
	Mean Amount (pg on column)	Accuracy (% RE)	Precision (% CV)	Mean Amount (pg on column)	Accuracy (% RE)	Precision (% CV)
0.250				0.246	-2.5	4.9
0.500	0.508	1.6	4.2	0.515	4.3	5.7
2.500	2.375	-5.0	5.0	2.399	-4.1	7.6
5.000	4.734	-5.3	4.4	5.150	4.9	7.6
25.000	24.903	-0.4	1.3	25.069	1.4	6.9
50.000	48.715	-2.6	2.7	49.589	-1.4	7.0
250.000	242.442	-3.0	1.6	248.873	-1.3	5.0
500.000	487.399	-2.5	2.8	472.818	-5.8	4.5
2500.000	2461.572	-1.5	1.2	2421.090	-2.4	6.7
5000.000	4977.895	-0.4	0.5	4836.477	-2.6	6.5
25000.000	27040.506	8.2	2.5	26024.648	5.2	5.8
50000.000	55522.884	11.0	1.1	55073.948	4.7	3.6

Figure 2. Linear Dynamic Range for APCI/SRM Analysis of Pergolide in Plasma at Unit Resolution

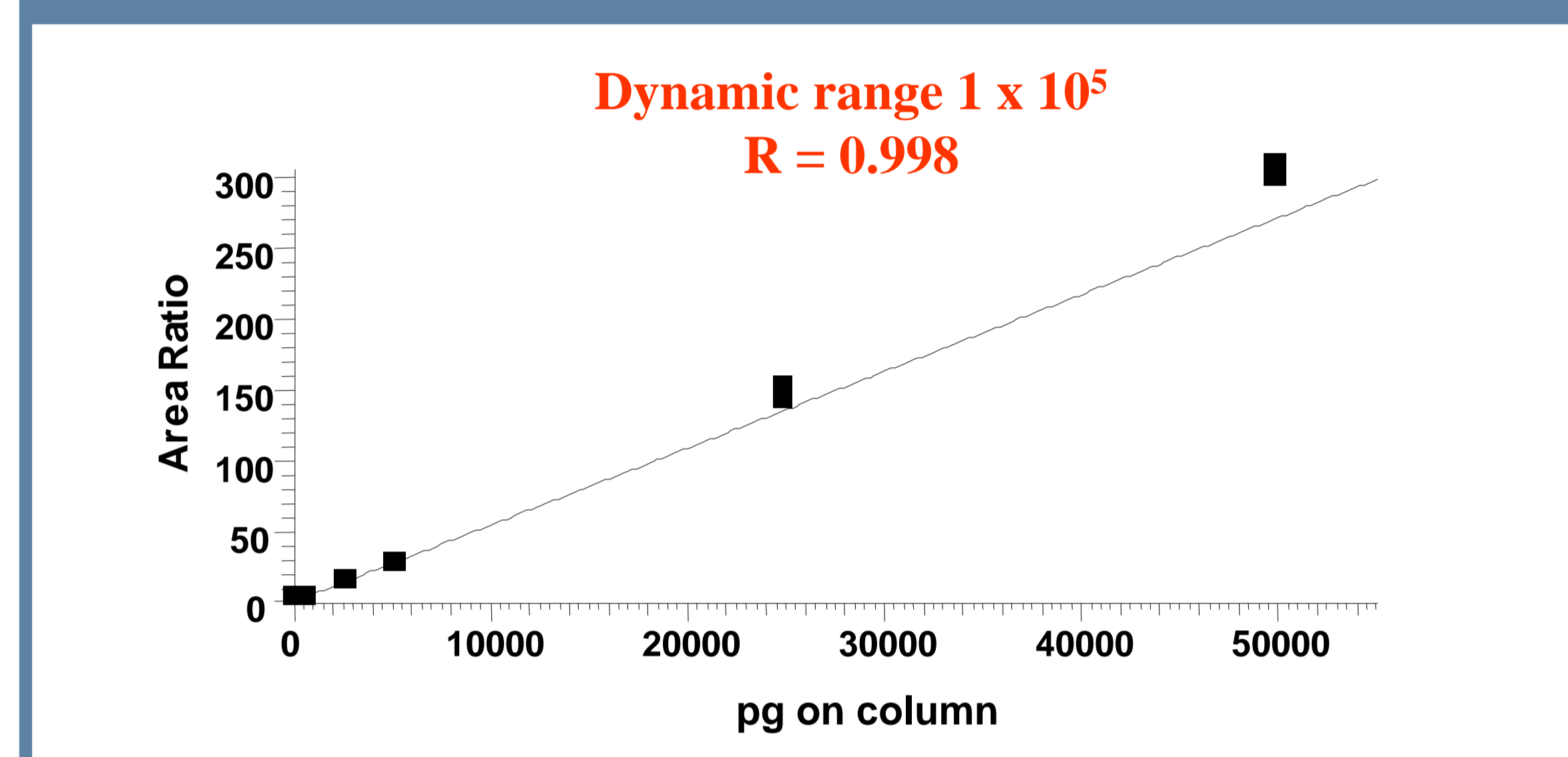


Figure 3. 250 fg of Pergolide in Plasma on Column under Unit and High Resolution Conditions

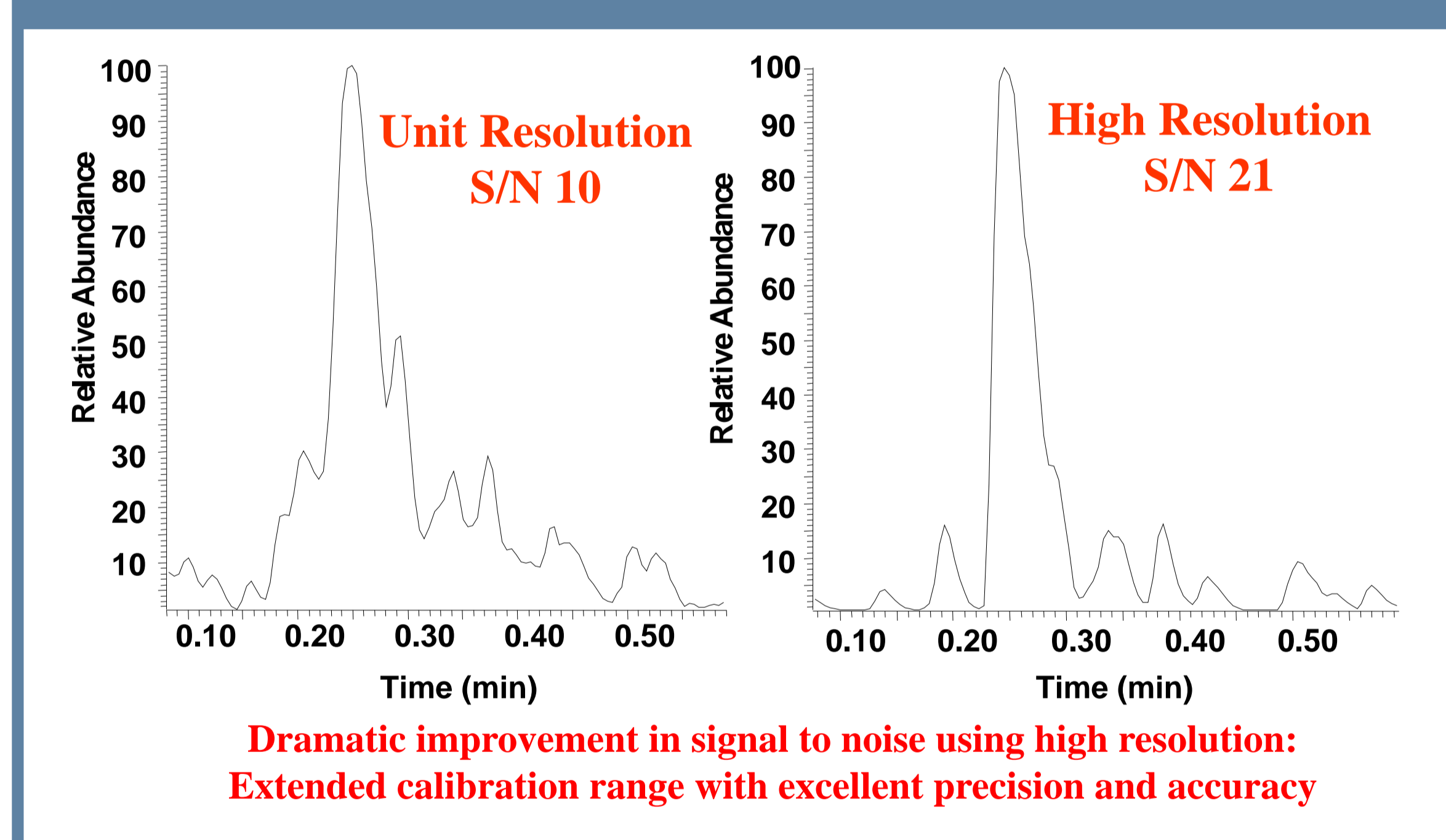
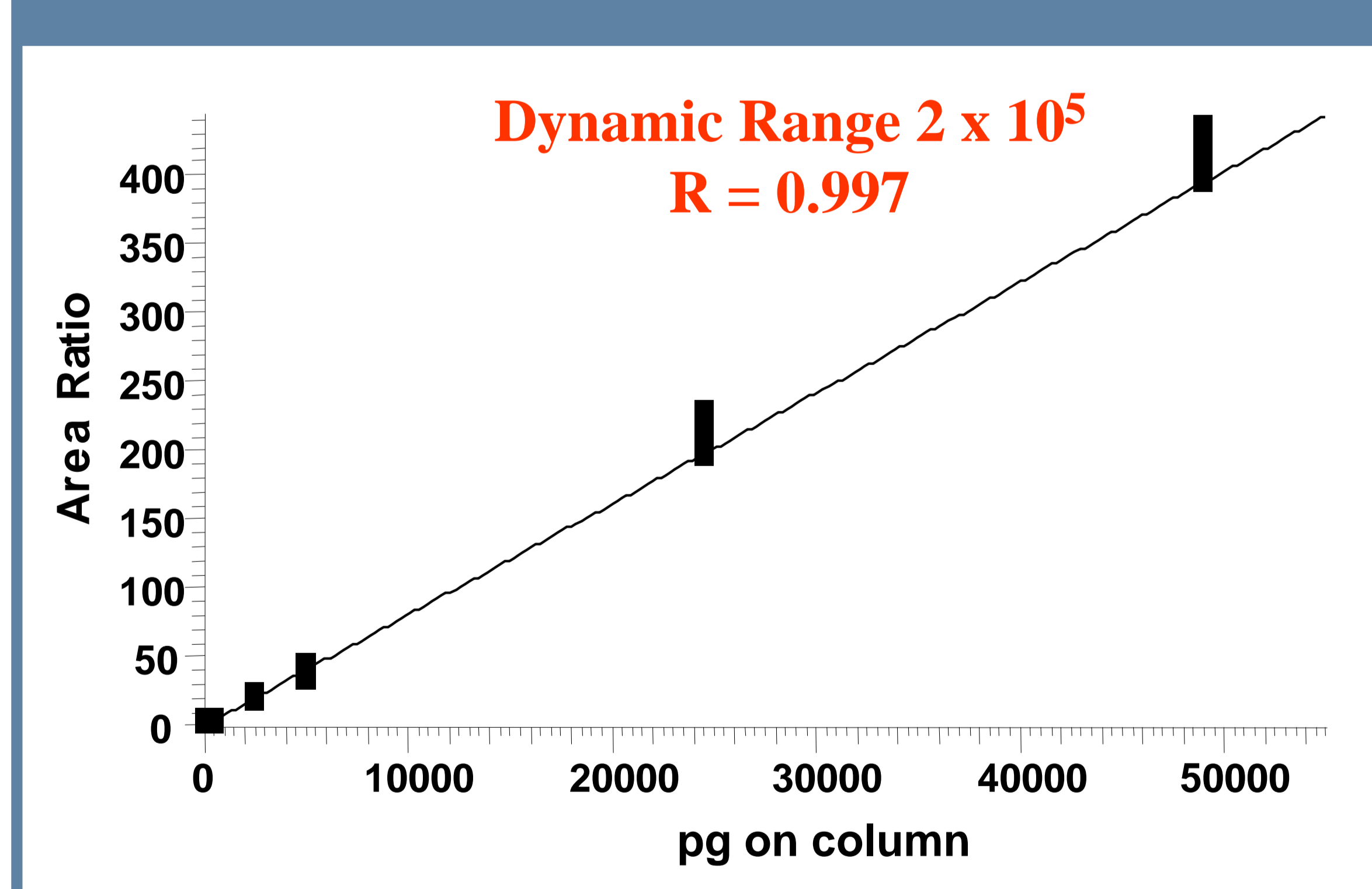


Figure 4. Linear Dynamic Range for APCI/SRM Analysis of Pergolide in Plasma at High Resolution



### Conclusion

The TSQ Quantum has shown great utility in the development of highly sensitive detection methods using ESI and APCI. The additional flexibility of being able to use the high resolution feature to improve the LOQ and extend the dynamic range, by distinguishing analytes of interest from interferences of the same nominal mass, should find great utility in the development of highly sensitive and selective methods for analytes with low systemic plasma levels. The outstanding dynamic range coupled to the excellent precision and accuracy, under unit and high resolution conditions, is of practical importance for most quantitative applications, but is particularly advantageous for those where detector saturation is typically problematical, e.g., multi-dose studies, methods for drug combination products. Overall, the TSQ Quantum should find widespread use in the development of simple, fast, accurate and rugged methods for many pharmacokinetic applications.

Copies of this and other applications as well as technical information can be obtained from:

[www.thermofinnigan.com](http://www.thermofinnigan.com)