

Fast Quantitative Analysis of Pergolide using APCI on the Finnigan TSQ Quantum Discovery

Chromatography and Mass Spectrometry Application Note

Nicola Hughes¹, Antony Harvey,² Witold Winnik,³ and Gary Paul³

¹Biovail Contract Research, Toronto, Ontario, Canada; ²Thermo Finnigan, San Jose, CA, USA; ³Thermo Electron, Somerset, NJ, USA

Overview

In Application Note 309,^[1] extremely low systemic plasma levels of the ergoline derivative cabergoline were analyzed on the Finnigan TSQ Quantum using the ESI source to demonstrate performance gains over previous generation triple quadrupole mass spectrometers.

In this report, the APCI source is used to demonstrate the Finnigan TSQ Quantum's ability to perform fast quantitative analysis of another ergoline derivative, pergolide. An analytical method is developed which is suitable for quantitative analysis of pergolide in plasma following oral administration at all possible dosage regimens (low to high dose). The results of unit and enhanced mass-resolution analysis on the Finnigan TSQ Quantum are also compared to demonstrate sensitivity improvements that can be made to analytical methods using the enhanced mass-resolution feature of the triple quadrupole mass spectrometer.

Introduction

Pergolide (Figure 1) is a synthetic ergoline derivative that is used for the treatment of Parkinson's disease, providing therapeutic activity at doses as low as 0.75 to 3.0 mg per day.^[2,3] However, due to the severe side effects of pergolide treatment, therapy is often initiated at daily doses of only 0.05 mg.

The development of simple, fast, accurate, and precise methods for the determination of potent drugs, such as pergolide, requires ultimate performance from an analytical technique. Additionally, for a method to be of practical use for analysis following any given dosage, the method must have a broad dynamic range, preferably without detector saturation at higher analyte concentrations.

Although ESI has found widespread application in the development of sensitive detection methods for bioanalytical applications, it is more prone to matrix suppression than APCI and often requires longer chromatographic run times to compensate. In this study, the APCI source was used in combination with a

relatively high chromatographic flow rate (0.8 mL/min) to assess the performance of the Finnigan TSQ Quantum Discovery in the development of a sensitive detection method with an extremely short run time (~1 minute).

Additionally, the performance in the enhanced mass-resolution mode of the Finnigan TSQ Quantum Discovery for the quantitative analysis of pergolide in plasma is tested. Utility of enhanced mass-resolution can achieve mass separation of an analyte of interest from isobaric matrix/chemical interferences in the SRM experiment. This can result in a further improvement in quantitative performance for analytes present in complex biological matrices, and improved LLOQ (lower limit of quantitation) sensitivities relative to those obtained at unit mass-resolution.^[4,5,6] The increase in analyte sensitivity achieved using enhanced mass-resolution analysis is also accompanied by an extended linear dynamic range for these assays.^[4,6]

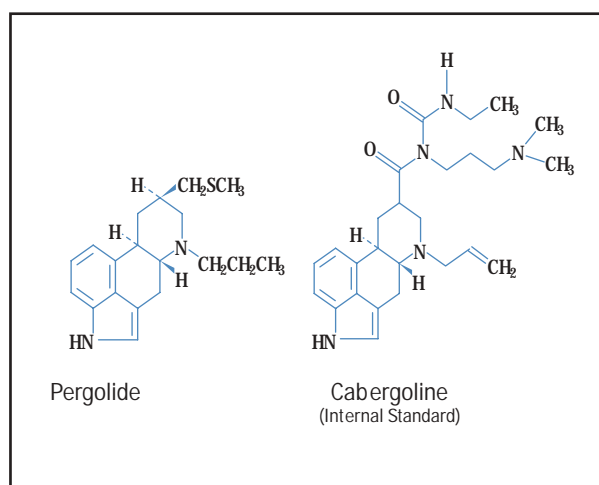


Figure 1. Structures of pergolide and cabergoline.

Key Words

- Finnigan™ TSQ Quantum Discovery™
- Quantitation
- Enhanced Mass Resolution
- Pharmacokinetic Application
- APCI Method
- Linear Dynamic Range
- High-throughput Analysis

Goals

- 1) Develop a fast, sensitive bioanalytical method using APCI.
- 2) Demonstrate a broad linear dynamic range for the assay.
- 3) Use enhanced mass-resolution capability to lower method LLOQ.

Experimental Conditions

Chemicals and Reagents: Pergolide mesylate (purity >98%) was supplied by Sigma Chemical Company (St. Louis, MO, USA). Cabergoline (purity >99%) was chemically synthesized. HPLC-grade acetonitrile and methanol, and reagent grade ammonium acetate were purchased from EM sciences (Gibbstown, NJ, USA). Bovine plasma was acquired from Sigma Chemical Company.

Standard and Sample Preparation: Stock solutions of pergolide and the internal standard cabergoline (Figure 1), were each prepared at concentrations of 1 mg/mL in methanol and stored at -25°C . A plasma solution was prepared by precipitating bovine plasma with a 2 \times volume of acetonitrile. A 10 $\mu\text{g/mL}$ pergolide plasma standard was prepared by spiking the precipitated bovine plasma with the pergolide stock solution. Working plasma standards were then prepared

by sequentially diluting the 10 $\mu\text{g/mL}$ pergolide plasma standard with the precipitated bovine plasma solution to produce a series of standard concentrations of greater than five orders of magnitude (50 pg/mL to 10 $\mu\text{g/mL}$). Prior to analysis, the pergolide standards were spiked with cabergoline such that each standard had a fixed internal standard concentration of 100 ng/mL of cabergoline. The plasma standards were then ready for direct injection into an HPLC—no further sample clean up was necessary.

Sample Analysis: HPLC analysis was performed on a SurveyorTM LC System. The chromatographic separation was performed using isocratic conditions on a BetaBasicTM-18, 5 μm , 2 \times 50 mm column using a mobile phase of methanol/water/formic acid (98:2:0.1). The method used an LC flow rate of 0.8 mL/min, and the injection volume was 5 μL .

Detection was performed on the Finnigan TSQ Quantum Discovery triple quadrupole mass spectrometer equipped with the APCI source. The mass spectrometer was operated under both unit and enhanced mass-resolution conditions, and the APCI settings were optimized to obtain the highest $[\text{M}+\text{H}]^{+}$ abundance.

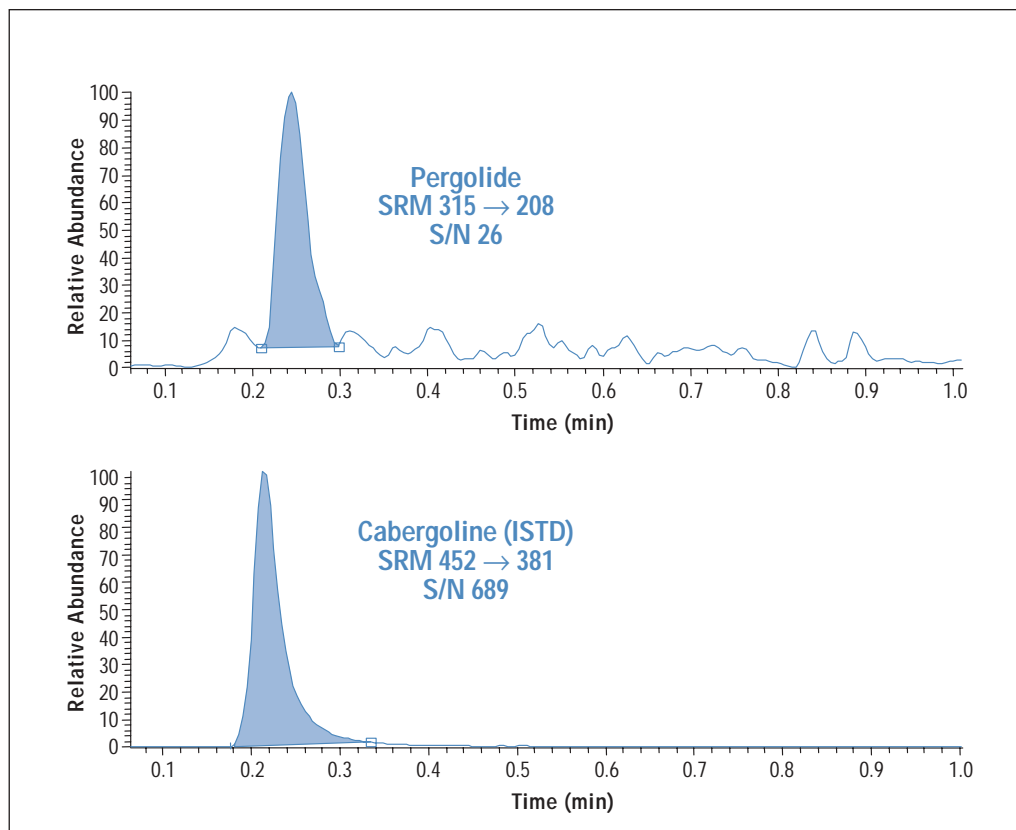


Figure 2. LC/APCI/SRM chromatogram of 500 fg on column of pergolide (m/z 315 \rightarrow 208) and 500 pg on column of cabergoline internal standard (m/z 452 \rightarrow 381) in plasma under unit mass-resolution conditions.

The general MS conditions were as follows:

Source: APCI
Ion Polarity: Positive
Discharge Current: 17 μ A
APCI Vaporizer Temperature: 420 $^{\circ}$ C
Sheath / Auxiliary Gas: Nitrogen
Sheath Gas Pressure: 65 arbitrary units
Auxiliary Gas Pressure: 0 arbitrary units
Capillary Temperature: 250 $^{\circ}$ C
Scan Type: SRM
Collision Gas: Argon
Collision Gas Pressure: 1.3 mTorr

The cabergoline SRM conditions were as follows:

Parent Mass: m/z 452
Product Mass: 381
Scan Width: 0.6 u
Scan Time: 0.12 s
Collision Energy: 19 eV
Q1 Peak Width (enhanced mass-resolution):
0.20 u FWHM
Q1 Peak Width (unit mass-resolution): 0.70 u FWHM
Q3 Peak Width: 0.70 u FWHM

The pergolide SRM conditions were as follows:

Parent Mass: m/z 315
Product Mass: m/z 208
Scan Width: 0.6 u
Scan Time: 0.12 s
Collision Energy: 25 eV
Q1 Peak Width (enhanced mass-resolution):
0.20 u FWHM
Q1 Peak Width (unit mass-resolution):
0.70 u FWHM
Q3 Peak Width: 0.70 u FWHM

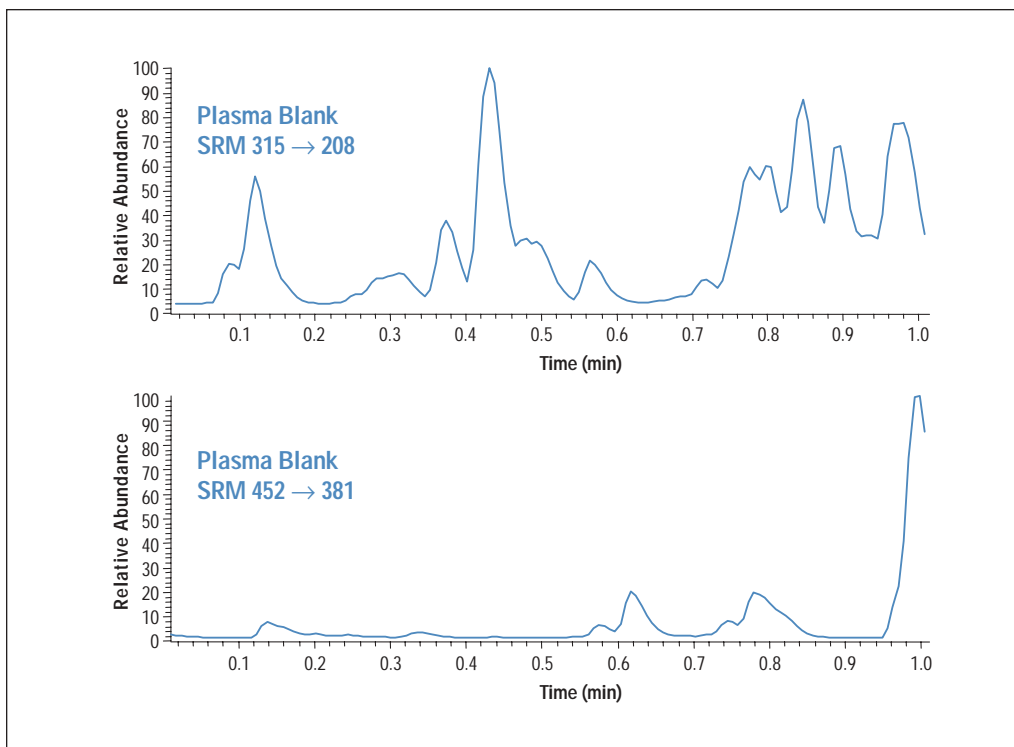


Figure 3. LC/APCI/SRM chromatograms of drug-free plasma under unit mass-resolution conditions.

Results and Discussion

The quantitative results for pergolide in plasma obtained at unit mass-resolution on the Finnigan TSQ Quantum Discovery are shown in Figures 2–4 and in Table 1. Under unit mass-resolution conditions, the LLOQ for pergolide was 500 fg on column (Figure 2), where no interfering peaks were observed in the corresponding extracted ion chromatogram of the blank plasma (Figure 3). In a previously developed LC/ESI/MS method for the quantitation of pergolide in plasma, an LLOQ of 5 pg/mL was reported on an older generation triple quadrupole mass spectrometer, which represented 3 pg of analyte on column.^[7] Hence, the running of this assay by APCI on the Finnigan TSQ Quantum Discovery results in a significant improvement in detection limit. Previous comparative APCI/SRM studies have shown analyte sensitivities with up to an order of magnitude improvement on the Finnigan TSQ Quantum, relative to the older generation TSQ 7000 at unit mass-resolution.^[8,9] Such improvements in detection limits means that the same target LLOQ can be achieved using a much lower per-assay plasma volume and/or a simpler extraction procedure. For example, the previous ESI quantitation method required a very high plasma volume (1.5 mL), a highly selective extraction/enrichment procedure, and a longer run time of 3.5 minutes.^[7] In the current

APCI experiment on the Finnigan TSQ Quantum Discovery, minimally-treated samples were analyzed considerably faster (1 minute run time, Figure 2) using a very small injection volume (5 μ L). A linear dynamic range covering 5 orders of magnitude was achieved with a correlation coefficient of $R=0.998$, using a weighting factor of $1/x^2$ (Figure 4). For many triple quadrupole mass spectrometers, linearity over 3 orders of magnitude poses no problem for analytes in solution (no matrix), but detector saturation is commonly observed with extracted plasma samples, which therefore restricts the practical range of the method. The Finnigan TSQ Quantum Discovery, with the ability of providing greater than 5 orders of linear dynamic range, provides the following significant advantages:

- Bioanalytical methods covering >3 orders of linear dynamic range can be achieved even with the “dirtiest” extracted samples.
- Detector saturation poses no problem in the development of bioanalytical methods for analytes present in vastly different combinations, such as drugs and their metabolites or drug combination products; high sample throughput would not be compromised.
- For a given analyte, a single method can be developed for all pharmacokinetic applications, negating the need for the use of multiple methods covering overlapping ranges.

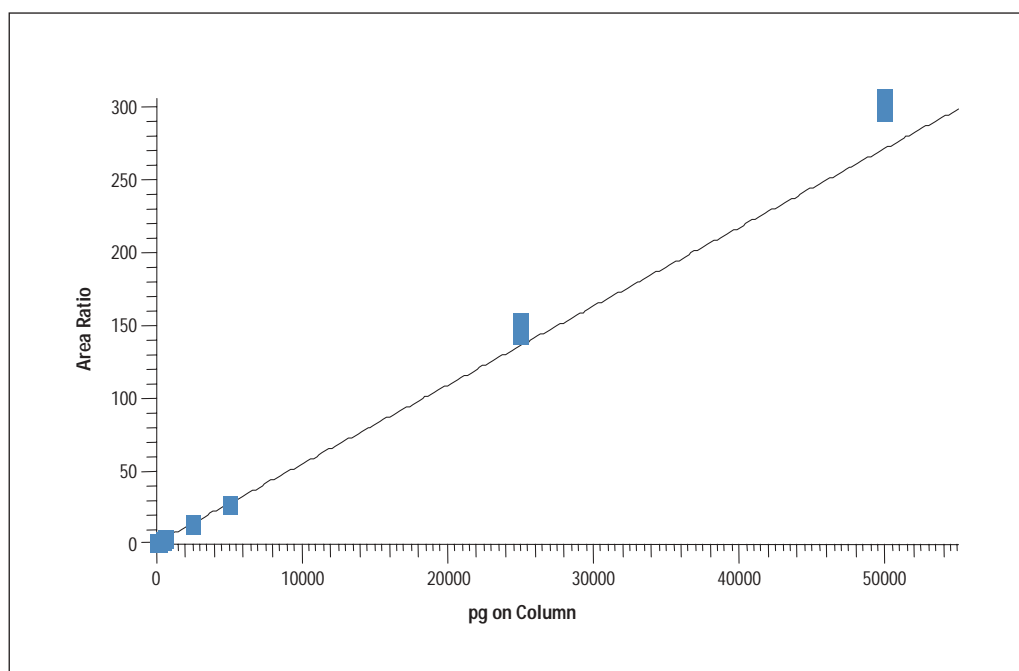


Figure 4. Calibration curve for pergolide in plasma under unit mass-resolution conditions covering 5 orders of linear dynamic range (500 fg to 50 ng on column), $R=0.998$ using $1/x^2$ weighted regression.

Intra-assay accuracy and precision was evaluated for 5 samples at each calibration level. The accuracy and precision over the extended linear dynamic range for pergolide at unit mass-resolution is shown in Table 1. The LLOQ (500 fg on column) gave %RE and %CV of 1.6% and 4.2%, respectively. The %RE and %CV for all other calibration levels (2.5 pg to 50,000 pg on

column) ranged from -5.3% to 11.0% and 0.5% to 5.0%, respectively. Hence, the accuracy and precision values for the APCI/SRM analysis of pergolide in plasma on the Finnigan TSQ Quantum Discovery, are well within pharmaceutical industry specifications over the dynamic range of five orders of magnitude (Table1).

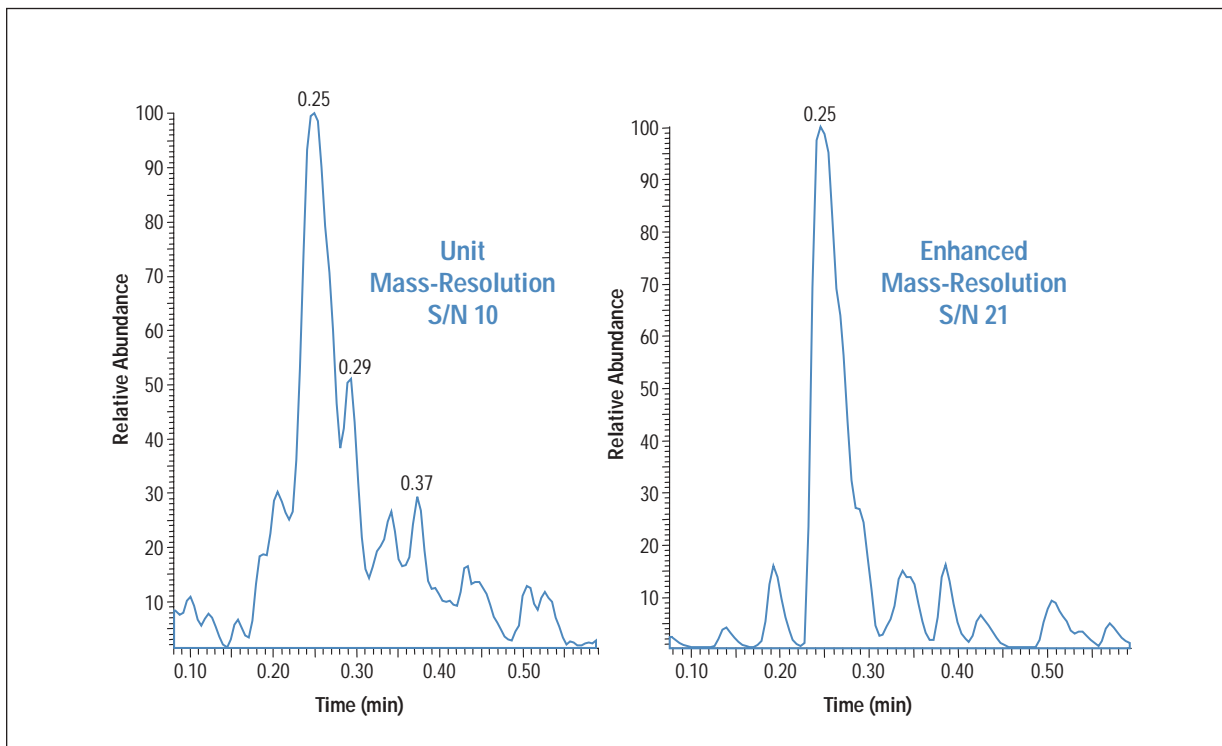


Figure 5. LC/APCI/SRM chromatogram of 250 fg on column of pergolide (m/z 315 \rightarrow 208) in plasma under unit mass-resolution and enhanced mass-resolution conditions.

NOMINAL AMOUNT (PG ON COLUMN)	MEAN AMOUNT (PG ON COLUMN)	ACCURACY (%RE)	PRECISION (%CV)
0.500	0.508	1.6	4.2
2.500	2.375	-5.0	5.0
5.000	4.734	-5.3	4.4
25.000	24.903	-0.4	1.3
50.000	48.715	-2.6	2.7
250.000	242.442	-3.0	1.6
500.000	487.399	-2.5	2.8
2500.000	2461.572	-1.5	1.2
5000.000	4977.895	-0.4	0.5
25000.000	27040.506	8.2	2.5
50000.000	55522.884	11.0	1.1

Table 1. Precision and accuracy of LC/APCI/SRM analysis of pergolide in plasma (mean of $n \geq 5$ injections) under unit mass-resolution conditions (Q1 and Q3 resolution=0.7 u FWHM).

Attempts to lower the LLOQ at unit mass-resolution to 250 fg of pergolide on column proved to be difficult because of isobaric chemical/matrix interferences to the analyte SRM transition, as shown in Figure 5. Typical strategies to improve analyte sensitivity include further time-consuming work on sample preparation techniques to remove interferences, followed by analyte enrichment to increase sensitivity. An alternative approach to lowering the LLOQ involves the manipulation of the chromatographic method to further separate interferences from the analyte, but this lengthens analysis time and, thus, lowers the sample throughput capability.

In this work, the complex plasma samples were assayed under the same fast chromatographic conditions (1 minute run time) using the enhanced

mass-resolution capability of the Finnigan TSQ Quantum Discovery to determine whether this feature could improve the S/N response in APCI/SRM at the lower 250 fg level. As shown in Figure 5, the enhanced mass-resolution on the Q1 mass analyzer, used for parent molecular ion selection (Q1 0.2 μ FWHM), resulted in a dramatic decrease in chemical noise, and a corresponding improvement in S/N ratio. As would be expected, the accuracy and precision at the 250 fg level were also significantly improved in enhanced mass-resolution mode. Hence, at higher mass-resolution, an improved LLOQ of 250 fg on column was achieved for the pergolide assay. Previous LC/ESI/SRM studies have also shown improvements in LLOQ for analytes in complex plasma and brain matrices using the enhanced

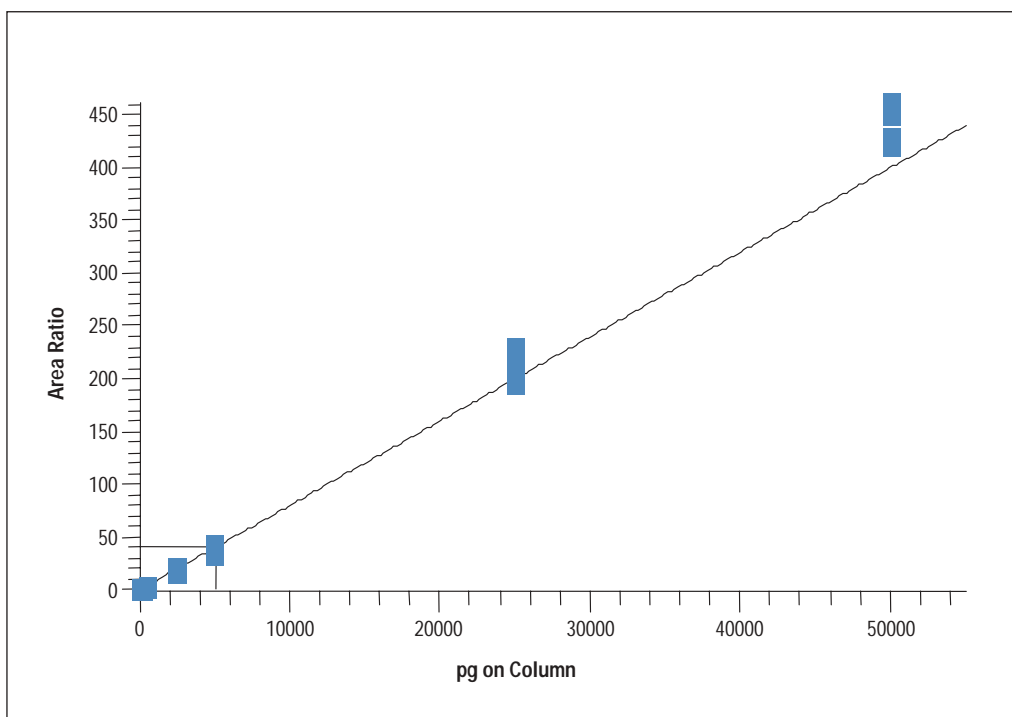


Figure 6. Calibration curve for pergolide in plasma under enhanced mass-resolution conditions covering >5 orders of linear dynamic range (250 fg to 50 ng on column); $R=0.997$ using $1/x^2$ weighted regression.

mass-resolution feature of the Finnigan TSQ Quantum.^[4,6] In all, an improvement in LLOQ of greater than an order in magnitude is achieved for pergolide in APCI on the Finnigan TSQ Quantum Discovery at enhanced mass-resolution, relative to a previous ESI quantitation study.^[7]

The quantitative results for pergolide in plasma at enhanced mass-resolution on the Finnigan TSQ Quantum Discovery are shown in Figure 6 and in Table 2. Intra-assay accuracy and precision were evaluated for $n \geq 5$ samples at each calibration level. An extended linear dynamic range of 2×10^5 was achieved with a correlation coefficient of $R=0.997$, using a weighting factor of $1/x^2$ (Figure 6). The accuracy and precision for pergolide at higher mass-resolution is shown in Table 2. The LLOQ (250 fg on column) gave %RE and %CV of -2.5% and 4.9%, respectively. The %RE and %CV for all other calibration levels (0.5 pg to 50,000 pg on column) ranged from -5.8% to 4.9% and 3.6% to 7.6%, respectively. The accuracy and precision values achieved at enhanced mass-resolution for pergolide in plasma on the Finnigan TSQ Quantum Discovery are again well within pharmaceutical industry specifications.

Conclusions

Highly satisfactory quantitative performance for pergolide in minimally-treated plasma is achieved by APCI/SRM on the Finnigan TSQ Quantum Discovery using an extremely short run time. The criteria required for high throughput analysis of pergolide are, thus, accomplished by this method. In addition, the added sensitivity of the Finnigan TSQ Quantum Discovery coupled with the extended linear dynamic range allows for the monitoring of low and high dose administration of this potent drug within the same assay. The enhanced mass-resolution feature of the Finnigan TSQ Quantum Discovery provides the user a simple, yet rapid, means to further improve method sensitivity and selectivity, without the need for additional sample manipulation.

NOMINAL AMOUNT (PG ON COLUMN)	MEAN AMOUNT (PG ON COLUMN)	ACCURACY (%RE)	PRECISION (%CV)
0.250	0.244	-2.5	4.9
0.500	0.522	4.3	5.7
2.500	2.398	-4.1	7.6
5.000	5.244	4.9	7.6
25.000	25.343	1.4	6.9
50.000	49.285	-1.4	7.0
250.000	246.854	-1.3	5.0
500.000	471.035	-5.8	4.5
2500.000	2439.180	-2.4	6.7
5000.000	4871.028	-2.6	6.5
25000.000	26293.492	5.2	5.8
50000.000	52373.320	4.7	3.6

Table 2. Precision and accuracy of LC/APCI/SRM analysis of pergolide in plasma (mean of $n \geq 5$ injections) under enhanced mass-resolution conditions (Q1 resolution=0.2 u FWHM, Q3 resolution=0.7 u FWHM).

References

1. Hughes, N.; Harvey, A.; Winnik, W.; Dunyach, J.J.; Amad, M.; Splendore, M.; Paul, G. *Quantitation of Cabergoline at Extremely Low Plasma Concentrations with a Triple Quadrupole Mass Spectrometer*; Application Note No. 309; Thermo Electron: San Jose, CA, Nov 2002.
2. Rubin, A.; Lemberger, L.; Dhahir, P. *Clin. Phramacol. Ther.* 1981; **30**: 258.
3. Lemberger, L.; Crabtree, R.; Callaghan, J.T. *Clin Pharmacol Ther.* 1980; **27**: 642.
4. Xu, X.; Tucker, G.; Zhou, Q.; Veals, J.; Korfmacher, W. 50th ASMS Conf Mass Spectrometry and Allied Topics, Orlando, FL, 2002.
5. Schweingruber, H.; Dunyach, J.J.; Olney, T.N.; Taylor, D.; Churchill, M.; Amad, M.; Winnik, W.; Paul, G.; Schoen, A.E.; Campbell, C. 49th ASMS Conf Mass Spectrometry and Allied Topics, Chicago, IL, 2001.
6. Paul, G.; Winnik, W.; Schmidt, C.; Amad, M.; Splendore, M.; Lytle, C.; Hughes, J.E.; Desai, B.; MacKenzie, K.I. 50th ASMS Conf Mass Spectrometry and Allied Topics, Orlando, FL, 2002.
7. Letarte, L.; Tessier, E.; Choinière, M.; Guilbaud, R. 49th ASMS Conf Mass Spectrometry and Allied Topics, Chicago, IL, 2001.
8. Xu, X.; Wainhaus, S.; Tucker, G.; Veals, J.; Korfmacher, W. 49th ASMS Conf Mass Spectrometry and Allied Topics, Chicago, IL, 2001.
9. Folk, B.; Burton, R.P.; Price, P.; Newton, J. 49th ASMS Conf Mass Spectrometry and Allied Topics, Chicago, IL, 2001.

In addition to these offices, Thermo Electron Corporation maintains a network of representative organizations throughout the world.

Australia

+61 2 9898 1244

Austria

+43 1 333 50340

Belgium

+32 2 482 30 30

Canada

+1 800 532 4752

China

+86 10 5850 3588

France

+33 1 60 92 48 00

Germany

+49 6103 4080

Italy

+39 02 950 591

Japan

+81 45 453 9100

Latin America

+1 512 251 1503

Netherlands

+31 76 587 98 88

Nordic

+46 8 556 468 00

South Africa

+27 11 570 1840

Spain

+34 91 657 4930

Switzerland

+41 61 48784 00

UK

+44 1442 233555

USA

+1 800 532 4752

www.thermo.com



Thermo Finnigan LLC,
San Jose, CA USA
is ISO Certified.

AN61536_E 3/04 S