

Quantitation of Cabergoline at Extremely Low Plasma Concentrations with a Triple Quadrupole Mass Spectrometer

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Overview

Quantitation of low plasma concentrations of the pharmaceutical cabergoline is performed to demonstrate the sensitivity and selectivity of the Thermo Scientific TSQ™ Quantum mass spectrometer. Samples with analyte concentrations ranging five orders of magnitude are analyzed to demonstrate precision and accuracy over a linear dynamic range suitable for pharmacokinetic applications. Analysis of 50 fg of cabergoline on column in minimally treated plasma samples is performed to demonstrate the sensitivity, ruggedness, and practicality of the bioanalytical method in a complex matrix.

Introduction

Pharmaceuticals with potent activity achieve their desired therapeutic effects when administered at low doses. Consequently, their systemic plasma levels are extremely low and require highly sensitive techniques for detection. Cabergoline (Figure 1), a synthetic ergoline derivative with a powerful dopaminergic activity, is usually administered in 0.25 mg, biweekly doses, yielding plasma concentrations in the pg/mL level.^(1,2)

A variety of analytical methods have been employed to quantify low levels of cabergoline in plasma; these include high performance liquid chromatography (HPLC), radioimmunoassay (RIA), and liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS). Techniques utilizing HPLC alone proved to have insufficient detection limits.^(3,4) RIA also does not have an adequate lower limit of quantitation (LLOQ) suitable for monitoring cabergoline when administered at low doses.^(1,5) And while recent LC/MS/MS methods have shown improvement in sensitivity through the use of selected reaction monitoring (SRM), they still require large sample volumes and time-consuming sample preparation.^(6,7) Consequently, these methods either lack the sensitivity, dynamic range, or practicality required for routine, high-throughput pharmacokinetic applications.

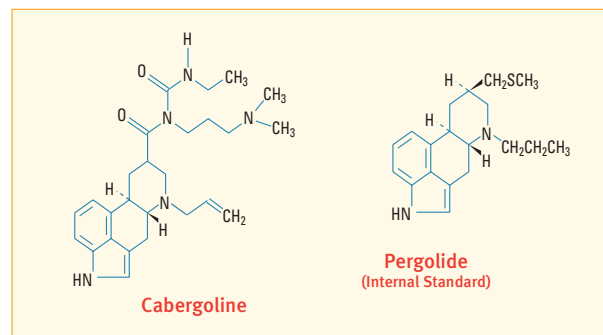


Figure 1: Structures of cabergoline and pergolide.

To assess the feasibility of using the TSQ Quantum to address these application requirements, cabergoline was analyzed in the LC/ESI/MS, unit-resolution mode. Significant improvement in sensitivity was demonstrated on the TSQ Quantum compared to the previous generation triple quadrupole mass spectrometer from Thermo Fisher Scientific, the TSQ 7000.⁽⁸⁻¹¹⁾ Using the TSQ Quantum, low femtogram-levels of cabergoline were resolved from the complex plasma matrix. Excellent precision and accuracy were maintained over five orders of magnitude, demonstrating a linear dynamic range suitable for real-world, pharmacokinetic applications.^(8,9,12)

Goals

- 1) Analyze low-level plasma concentrations of cabergoline in a complex matrix.
- 2) Demonstrate a dynamic linear response of method more suitable for pharmacokinetic applications.
- 3) Compare the method performance of the TSQ Quantum to that of an earlier-generation mass spectrometer.

Experimental Conditions

Chemicals and Reagents: Cabergoline (purity >99%) was chemically synthesized. Pergolide mesylate (purity >98%) was supplied by Sigma Chemical Company (St. Louis, MO, USA). 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: Primary stock solutions of cabergoline and the internal standard pergolide (Figure 1) were each prepared at a concentration of –1 mg/mL in methanol and stored at –25 °C. A plasma solution was prepared by precipitating bovine plasma with a 2 × volume of acetonitrile. A 1 µg/mL cabergoline plasma standard was prepared by spiking the precipitated bovine plasma with the cabergoline stock solution. Working plasma standards were prepared by sequentially diluting the 1 µg/mL cabergoline plasma standard with the precipitated bovine plasma solution to yield final concentrations ranging from 10 pg/mL to 1 µg/mL. Prior to analysis, each standard level was spiked with the pergolide stock solution to produce the fixed internal standard concentration of 200 ng/mL of pergolide. The plasma standards were then ready for direct injection into the HPLC—no further sample clean up was necessary.

Sample Analysis: HPLC analysis was performed on the Thermo Scientific Surveyor™ LC System. The chromatographic separation was performed using isocratic conditions on a C18, 5 µm, 2.1 × 150 mm column with a mobile phase of acetonitrile/ammonium acetate (60:40, v/v). The LC flow rate was 0.3 mL/min and the injection volume was 5 µL.

Detection was performed on the TSQ Quantum mass spectrometer equipped with the ESI source.

The general MS conditions were as follows:

Source: ESI
Ion Polarity: Positive
Spray Voltage: 4600 V
Sheath / Auxiliary Gas: Nitrogen
Sheath Gas Pressure: 75 arb units
Auxiliary Gas Pressure: 25 arb units
Ion Transfer Capillary Temperature: 360°C
Scan Type: SRM
Collision Gas: Argon
Collision Gas Pressure: 1.5 mTorr

The cabergoline SRM conditions were as follows:

Parent Mass: 452 *m/z*
Product Mass: 381 *m/z*
Scan Width: 0.7 u
Scan Time: 0.50 s
Collision Energy: 19 eV
Q1 Peak Width: 0.70 u FWHM
Q3 Peak Width: 0.70 u FWHM

The pergolide SRM conditions were as follows:

Parent Mass: 315 *m/z*
Product Mass: 208 *m/z*
Scan Width: 0.7 u
Scan Time: 0.50 s
Collision Energy: 27 eV
Q1 Peak Width: 0.70 u FWHM
Q3 Peak Width: 0.70 u FWHM

Discussion

The quantitative LC/ESI/SRM results for cabergoline on the TSQ Quantum at unit resolution are summarized in Figures 2–4 and in Table 1. The LLOQ observed for cabergoline was 50 fg on column (5 µL injection of 10 pg/mL) which gave a S/N ratio of 23 (Figure 2). Bioanalytical methods for the pharmacokinetic analysis of cabergoline ideally require detection limits of 1–2 pg/mL in plasma. This is easily accomplished on the TSQ Quantum since an LLOQ of 50 fg on column represents only 5% (50 µL) of a 1 mL plasma sample containing 1 pg of cabergoline. By extrapolation, it is clear that extremely sensitive limits of detection (down to the low pg/mL level for cabergoline) can be achieved with small plasma sample volumes.

A significant advantage of using smaller plasma volumes is that it lends itself to improved pharmacokinetic analyses, as replicate and other large samples-per-subject study designs would not be limited by the plasma volume requirements of the assay. The ability to easily attain such low detection limits on the TSQ Quantum with complex plasma samples also negates the need to develop highly selective sample enrichment procedures to minimize matrix interferences and to maximize the amount of analyte injected on the column.^{6–7} The method used here is in marked contrast to a previous method for cabergoline developed on the older generation TSQ 7000 triple quadrupole mass spectrometer, which reported an LLOQ value of 1.86 pg/mL (S/N ratio of 18±13.1).

However, this LLOQ actually represents ~900 fg of cabergoline on column⁶ rather than the 50 fg on column reported here. Furthermore, in order to acquire an accurate, reproducible LLOQ for cabergoline on the TSQ 7000, the assay required use of a 1-mL plasma sample, a complex extraction procedure including a five-fold enrichment of the analyte concentration, and a large injection volume (150 µL) representing 75% of the extracted sample.⁶ In contrast, using the TSQ Quantum, superior cabergoline sensitivity was achieved with minimally-treated samples and a much smaller injection volume (5 µL). Thus, the detection limit for cabergoline on the TSQ Quantum is almost 20 times lower than that attained on the TSQ 7000.^{6,8,11}

The calibration curve for cabergoline obtained on the TSQ Quantum shows a linear dynamic range covering five orders of magnitude (1×10^5) with a correlation coefficient of $R > 0.999$ using a weighting factor of $1/x$ (Figure 4). Intra-assay accuracy and precision was evaluated for $n \geq 5$ samples at each calibration level. The accuracy and precision numbers obtained over this extended linear dynamic range are shown in Table 1. The LLOQ (50 fg on column) had an accuracy (%RE) and precision (%CV) of 13.6% and 9.3% respectively. The %RE and %CV for all the other calibration levels (0.5 pg to 5000 pg on column) ranged from –7.5% to 1.6% and 0.5 to 8.4%, respectively. The linear dynamic range for the TSQ Quantum is considerably broader than that achievable on the TSQ 7000 (which was less than two orders of magnitude for this assay).^{6,7} The significance of this extended linear dynamic range is that a single method can now be developed for cabergoline for application in both low and high dose pharmacokinetic analyses.

It is typical for the response of older generation mass spectrometers to be nonlinear and/or for detector saturation to occur for linear dynamic ranges as low as three orders of magnitude. This is particularly problematic for assays where drugs and their metabolites are present in vastly different concentrations and for the analysis of drug combination products. Typically, method development is biased towards optimizing the detection limit for

the lower concentration analyte, sacrificing detector saturation of the higher concentration analytes. Since linear range of the high concentration analyte is compromised, sample dilution and subsequent analysis becomes unavoidable; this contributes considerably to overall sample analysis time and, thus, does not lend itself to high-throughput applications. Hence, the extended linear dynamic range of the TSQ Quantum is extremely beneficial in these situations.

Conclusions

The TSQ Quantum operated at unit resolution shows tremendous utility in the development of highly sensitive detection methods for ergoline derivatives such as cabergoline. Sensitivity on the TSQ Quantum is ~20 times better than that reported on the TSQ 7000 while only requiring a fraction of the sample previously used.

Furthermore, analysis of low plasma concentrations of cabergoline was performed in a complex, minimally treated plasma matrix. The broad dynamic range of the TSQ Quantum, coupled with the excellent precision and accuracy in unit resolution, is of particular importance for applications where detector saturation is typically encountered. The broad dynamic range, therefore, allows for easy development of methods suitable for the analysis of drugs administered at any given dose (low and high), for drugs and their metabolites, and for drug combination products. For these reasons, the TSQ Quantum should find widespread use in the development of sensitive, simple, fast, accurate, and rugged methods for many pharmacokinetic applications

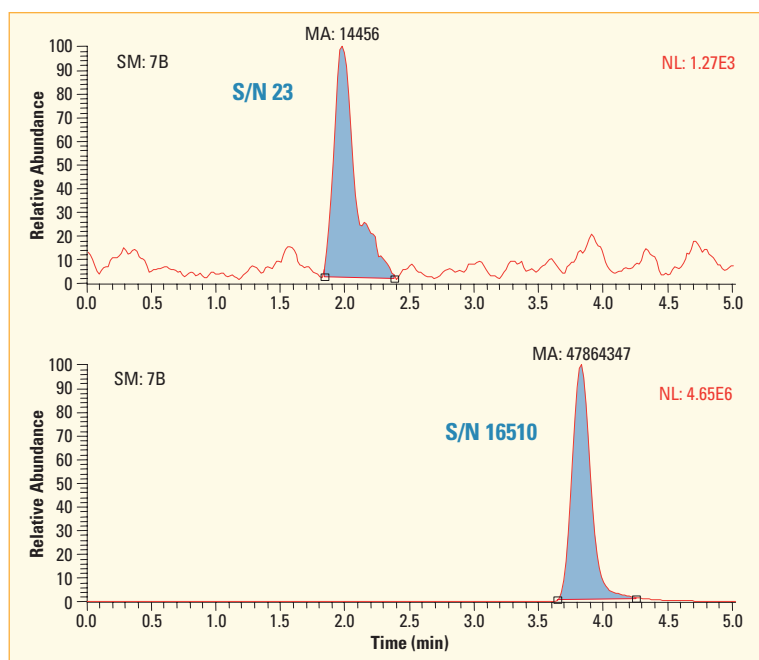


Figure 2: LC/ESI/SRM chromatogram of 50 fg on column of cabergoline (m/z 452→381) and 1 ng on column of pergolide internal standard (m/z 315→208) in plasma under unit resolution conditions.

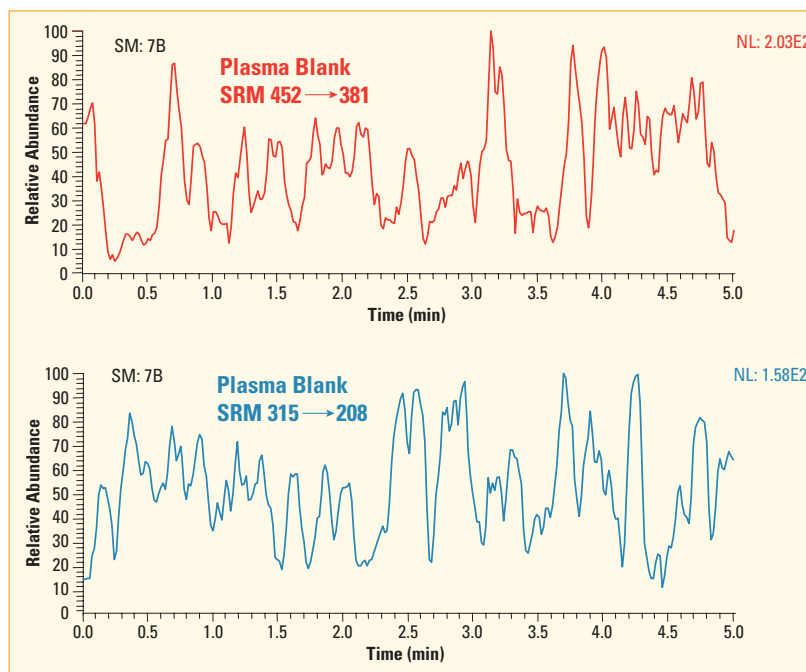


Figure 3: LC/ESI/SRM chromatograms of drug-free plasma under unit resolution conditions.

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

Table 1: Precision and accuracy in the LC/ESI/SRM analysis of cabergoline in plasma under unit resolution conditions. $n \geq 5$ samples at each calibration level.

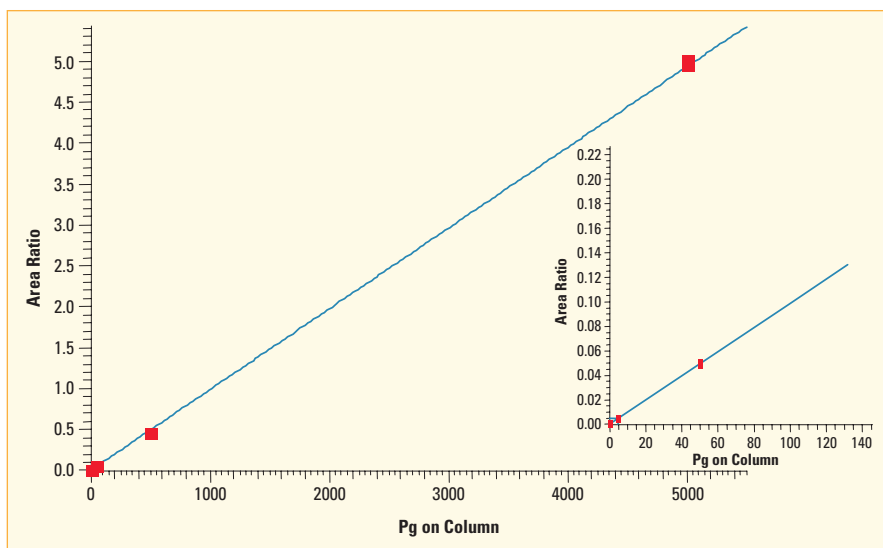


Figure 4: Calibration curve for cabergoline in plasma under unit resolution conditions covering 1×10^5 orders of linear dynamic range (50 fg to 5 ng on column), $R > 0.999$ using $1/x$ weighted regression.

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