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High-sensitivity quantitation of cabergoline and pergolide using a triple-quadrupole mass spectrometer with enhanced mass-resolution capabilities

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Quantitative analysis of pharmaceuticals with low systemic plasma levels requires the utmost in sensitivity and selectivity from the analytical method used. A recently introduced triple-quadrupole mass spectrometer with unique enhanced mass-resolution capability was evaluated in the analysis of two such drugs, cabergoline and pergolide, in plasma. Liquid chromatographic/electrospray ionization selected reaction monitoring determination of cabergoline in plasma at unit mass-resolution demonstrated improved sensitivity (50 fg on-column), coupled with suitable accuracy and precision over a broad linear dynamic range covering five orders of magnitude (50 fg to 5 ng on-column). Liquid chromatographic/atmospheric pressure chemical ionization selective reaction monitoring determination of pergolide in plasma also attained a high level of sensitivity (500 fg on-column) at unit mass-resolution, with accuracy and precision values well within pharmaceutical industry standards. Again, a linear dynamic range covering five orders of magnitude (500 fg to 50 ng on-column) was achieved for the assay. Utility of the enhanced mass-resolution feature of the triple-quadrupole mass spectrometer in the determination of pergolide resulted in an improvement in analyte sensitivity (250 fg on-column) and linear dynamic range (250 fg to 50 ng on-column). **Copyright © 2003 John Wiley & Sons, Ltd.**

KEYWORDS: electrospray; atmospheric pressure chemical ionization; enhanced mass-resolution; quantitative analysis; linear dynamic range

INTRODUCTION

Pharmaceuticals with potent activity achieve their desired pharmacological effect when administered at low doses. Systemic plasma levels are consequently extremely low and, hence, require highly sensitive analytical techniques for their detection. Cabergoline and pergolide, the structures of which are shown in Fig. 1, are synthetic ergoline derivatives with powerful dopaminergic activity which have therapeutic activity at pg ml^{-1} concentrations in plasma.[1–5] Cabergoline has an extremely long duration of biological activity, making it highly advantageous over classical dopamine reference therapy in terms of efficacy, tolerability, administration schedule and patient compliance. Cabergoline is highly effective when dosed at only 0.25 mg twice weekly in the treatment of several hyperprolactinemic conditions,[1,2] although it is administered at higher doses in the treatment of Parkinson's disease.[3] Pergolide is also effective in the treatment of Parkinson's disease, being administered at doses of 0.75–3 mg per day.[4] However, owing to the severe nausea and vomiting that are frequent side-effects of pergolide, therapy is often initiated at a low daily dose of only 0.05 mg.

Systemic plasma levels of these ergoline derivatives can, thus, be extremely low and, as a result, determinations of the plasma pharmacokinetic profiles in human subjects are scarce because classical techniques such as high-performance liquid chromatography (HPLC) have insufficient detection limits.[5,6] Radioimmunoassay (RIA) was the method of choice for many years because of its lower limits of quantitation (LLOQ) of 12 and 20 pg ml^{-1} for cabergoline and pergolide, respectively.[7,8] However, using RIA, maximum cabergoline plasma concentrations (C_{max}) have been estimated at only 67 pg ml^{-1} following 1.5 mg doses.[1] RIA is therefore of insufficient sensitivity for comprehensive cabergoline determination, because an LLOQ of 12 pg ml^{-1} would only allow cabergoline to be detected for just over two biological half-lives after moderately high dosage levels, and would not be nearly sensitive enough to monitor low-dose administration. RIA has also shown limited utility in the determination of pergolide as it is extensively metabolized *in vivo* and RIA does not have adequate specificity to distinguish unequivocally levels of the parent drug from its inactive metabolites.[8] More sensitive and

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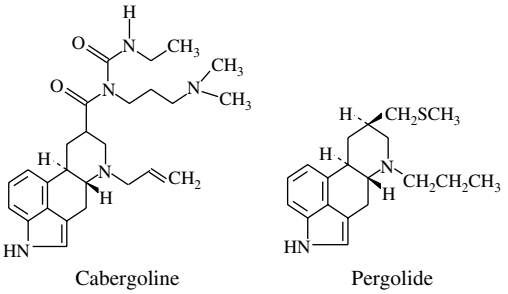

Figure 1. Structures of cabergoline and pergolide.

selective techniques are obviously warranted for the accurate quantitative analysis of these ergoline derivatives.

In more recent years, liquid chromatography coupled with atmospheric precision ionization tandem mass spectrometry (LC/API-MS/MS) has found widespread application in the quantitative analysis of pharmaceuticals in biological matrices. The use of selected reaction monitoring (SRM) on a triple-quadrupole mass spectrometer has shown significant improvement in sensitivity over RIA in the determination of both cabergoline[9,10] and pergolide[11] in plasma, with LLOQs of the order of 2–5 pg ml^{-1}. The achievement of such sensitive methods, however, required plasma volumes as large as 1.5 ml and highly selective solid-phase extraction strategies, and/or liquid–liquid extraction with back-extraction, to maximize analyte enrichment.[9–11] Sample preparation techniques such as these are extremely labor intensive and generally impractical for routine high-throughput analysis for pharmacokinetic applications.

It is clear that the development of simple, sensitive, fast, accurate and precise methods for the determination of potent drugs, such as cabergoline and pergolide, with extremely low systemic plasma levels requires the utmost in performance from a mass spectrometer. Additionally, for the method to be of practical utility for analysis following any given dosage requires that the dynamic range be as broad as possible, preferably without detector saturation at higher levels.

Recently, significant advances have been made in triple-quadrupole mass spectrometer design which have led to improvements both in sensitivity and selectivity. One such triple-quadrupole mass spectrometer, the Finnigan TSQ Quantum Discovery, has shown increased analyte sensitivities in LC/electrospray ionization (ESI)-SRM and LC/atmospheric pressure chemical ionization (APCI)-SRM at unit mass-resolution, sometimes down to the mid-to-low femtogram level on-column, relative to those obtained on a previous generation instrument.[12–15] In addition, linear dynamic ranges for these quantitative assays over four orders of magnitude have been reported with suitable precision and accuracy.[12,13,16] The unique ability of this triple-quadrupole mass spectrometer to achieve enhanced-resolution mass separation of an analyte of interest from isobaric matrix interferences in LC/API-SRM can result in a further improvement in quantitative performance through the added specificity. Improved SRM detection limits, relative to those obtained at unit mass-resolution, have been reported with an enhanced mass-resolution setting on the first-quadrupole mass analyzer (Q1), for analytes present in complex biological matrices commonly encountered in pharmacokinetic applications.[13,17,18] The increase in analyte sensitivity through enhanced mass-resolution is also accompanied by an extended linear dynamic range for these assays.[13,18]

In the work described here, the Finnigan TSQ Quantum Discovery was used in the development of highly sensitive detection methods for cabergoline and pergolide suitable for quantitative pharmacokinetic applications. LC/API-SRM detection methods were developed in plasma, applicable to quantitative analysis following oral administration at all possible dosage regimens (low to high dose). For the cabergoline assay in plasma, quantitation through LC/ESI-SRM was performed at unit mass-resolution. For the determination of pergolide in plasma, LC/APCI-SRM was compared at unit and enhanced mass-resolution to see whether the additional specificity afforded to the SRM experiment by enhanced mass-resolution leads to an improved detection method.

EXPERIMENTAL

Chemicals and reagents

Cabergoline (purity >99%) was chemically synthesized. Pergolide mesylate (purity >98%) was supplied by Sigma Chemical (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.

Preparation of standard solutions and samples

Primary stock solutions of cabergoline and pergolide were prepared at a concentration of 1 mg ml^{-1} in methanol and stored at −25 °C. To mimic minimally extracted plasma samples, separate standards of cabergoline (1 μg ml^{-1}) and pergolide (10 μg ml^{-1}) were prepared by spiking the methanolic stock solutions into a bovine plasma solution that had been precipitated with two volumes of acetonitrile. Lower concentration plasma standards were prepared from dilutions of the highest standards with precipitated bovine plasma such that the final concentration in bovine plasma covered a linear dynamic range of five orders of magnitude for cabergoline (10 pg ml^{-1} to 1 μg ml^{-1}) and more than five orders of dynamic range (50 pg ml^{-1} to 10 μg ml^{-1}) for pergolide. Prior to analysis, a fixed concentration of pergolide (200 ng ml^{-1}) was added as an internal standard to each cabergoline standard level and a fixed concentration of cabergoline (100 ng ml^{-1}) was added as an internal standard to each pergolide standard. For each assay, the plasma standards were directly injected in replicates of $n \geq 5$ into the HPLC system without further sample clean-up.

LC/MS/MS equipment

HPLC analysis of cabergoline was carried out on an Thermo Electron Corporation LC Surveyor System (Thermo Electron Corporation, San Jose, CA, USA), which includes a Surveyor MS pump, a built-in degasser and a Surveyor autosampler. LC was performed using isocratic conditions on a 2.1 × 150 mm, 5 μm, Xterra MS18 column (Waters, Milford, MA, USA) with a mobile phase of acetonitrile–25 mM

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ammonium acetate (60 : 40, v/v) and a flow-rate of 0.3 ml min^{-1}. The injection volume was 5 µl. Detection was carried out using a Finnigan™ TSQ® Quantum Discovery triple-quadrupole mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA), equipped with an ESI ion source. The mass spectrometer was operated in the positive ESI multiple reaction monitoring (MRM) mode under unit mass-resolution conditions, with a Q1 resolution of 0.7 Da FWHM and a Q3 resolution of 0.7 Da FWHM. The SRM transitions of m/z 452 → 381 [collision energy 19 eV, argon collision gas pressure 1.5 mTorr (1Torr = 133.3 Pa), scan time 0.5 s] for cabergoline and m/z 315 → 208 (collision energy 27 eV, argon collision gas pressure 1.5 mTorr, scan time 0.5 s) for the pergolide internal standard were monitored. The ESI parameters were optimized to obtain the highest $[M + H]^+$ abundance and were as follows: spray voltage 4.6 kV, nitrogen sheath gas flow 75 units, nitrogen auxiliary gas flow 25 units and ion transfer tube temperature 360 °C.

HPLC analysis of pergolide was performed on the LC Surveyor System. LC was performed using isocratic conditions on a 2 × 50 mm, 5 µm, BetaBasic-18 column (Thermo Electron Corporation, Bellefonte, PA, USA) with a mobile phase of methanol–water–formic acid (98 : 2 : 0.1) and a flow-rate of 0.8 ml min^{-1}. The injection volume was 5 µl. Detection was carried out on the Finnigan TSQ Quantum Discovery, equipped with an APCI ion source. The mass spectrometer was operated in the positive APCI-MRM mode under unit and enhanced mass-resolution conditions. When operating at unit mass-resolution, the mass spectrometer was set at Q1 resolution 0.7 Da FWHM and Q3 resolution 0.7 Da FWHM. When operating in enhanced mass-resolution mode, the mass spectrometer was set at Q1 resolution 0.2 Da FWHM and Q3 resolution 0.7 Da FWHM. The SRM transitions of m/z 315 → 208 (collision energy 25 eV, argon collision gas pressure 1.3 mTorr, scan time 0.12 s) for pergolide and m/z 452 → 381 (collision energy 19 eV, argon collision gas pressure 1.3 mTorr, scan time 0.12 s) for the cabergoline internal standard were monitored. The APCI parameters were optimized to obtain the highest $[M + H]^+$ abundance and were as follows: APCI vaporizer temperature 420 °C, discharge current 17 µA, nitrogen sheath gas flow 65 units, nitrogen auxiliary gas flow 0 units and ion transfer tube temperature 250 °C.

RESULTS AND DISCUSSION

Investigation of the ESI/MS/MS behavior of cabergoline indicated that the m/z 381 fragment ion was most abundant and, as such, suitable for quantitative analysis by SRM. The ESI/MS/MS spectrum for cabergoline $[M + H]^+$, obtained under similar experimental conditions to those in this study, is shown in a paper by Paul *et al*.[19] In that study, different isobaric ion structures were proposed for the m/z 381 fragment ion, using the predictive Mass Frontier 3.0™ software package (Thermo Electron Corporation, San Jose, CA, USA).[19] The most likely ion structure of m/z 381 was then determined through accurate mass measurement of the fragment ion on the Finnigan TSQ Quantum Ultra AM (Thermo Electron Corporation, San Jose, CA, USA).[19]

The APCI/MS/MS spectrum for $[M + H]^+$ of pergolide is shown in Fig. 2. The m/z 208 ion was clearly the most abundant fragment ion and, hence, used in subsequent APCI-SRM quantitation studies. Elucidation of the APCI/MS/MS fragmentation pathways leading to m/z 208 formation was again achieved using the Mass Frontier 3.0 software. The fragmentation pathways and accompanying fragment ion structures are shown in Fig. 3. MS/MS/MS work on the Finnigan LCQ™ Advantage ion trap mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA) verified the occurrence of the proposed fragmentation pathways. In order to confirm the fragment ion structures, accurate mass determinations of the m/z 267 and 208 fragment ions

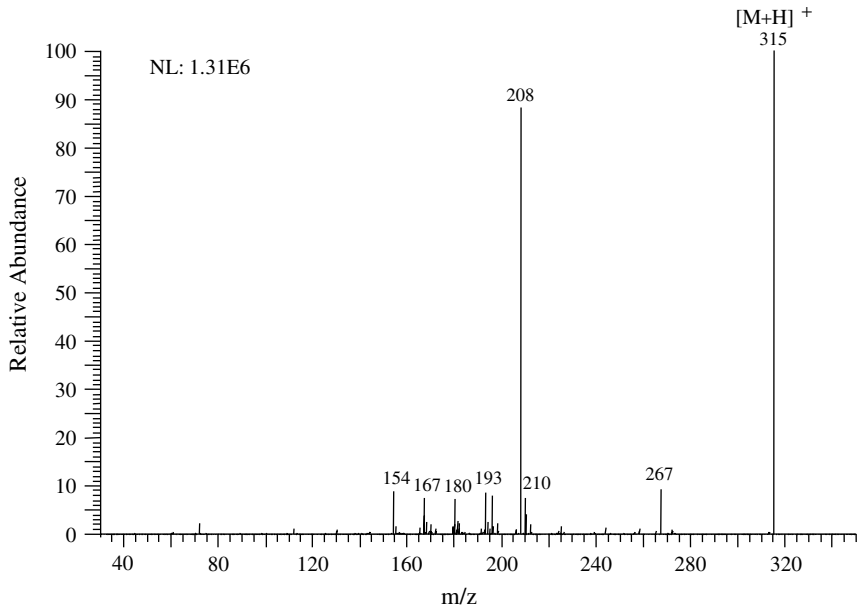

Figure 2. LC/APCI-MS/MS spectrum of pergolide $[M + H]^+$ at *m/z* 315. Collision energy = 25 eV; collision gas pressure = 1.3 mTorr.

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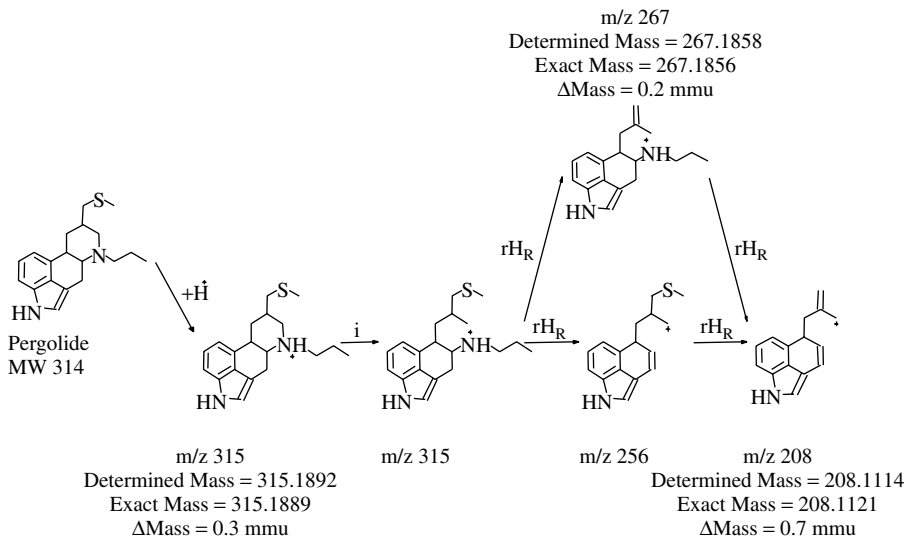

Figure 3. Proposed APCI-MS/MS fragmentation pathways for pergolide *m/z* 208 fragment ion formation. Fragmentation pathways predicted by the Mass Frontier 3.0 software.

were performed on the Finnigan TSQ Quantum Ultra AM. The procedure for accurate mass measurement of fragment ions using the Finnigan TSQ Quantum Ultra AM has been described in detail earlier.[19] The abundance of the *m/z* 256 fragment ion shown in the fragmentation pathway (Fig. 3) was too low in MS/MS for accurate mass measurement (Fig. 2). The experimentally determined accurate masses are shown in Fig. 3, along with the exact masses corresponding to the predicted fragment ion structures. In both cases, excellent agreement within 1 mmu was observed between the experimental and theoretical masses for the proposed fragment ions (Fig. 3). Accurate mass measurement of the pergolide $[M + H]^+$ on the Finnigan TSQ Quantum Ultra AM provided validation of the mass measurement technique, as the experimental $[M + H]^+$ determination differed by only 0.3 mmu from the known exact mass (Fig. 3).

The quantitative LC/ESI-SRM results for cabergoline obtained at unit mass-resolution are summarized in Figs 4 and 5 and Table 1. The LLOQ observed for cabergoline was 50 fg on-column (5 µl injection of 10 pg ml^{-1}), which gave a signal-to-noise (S/N) ratio of 23 (Fig. 4). No interfering peaks were observed in the corresponding extracted ion chromatogram of the blank plasma (Fig. 5). As discussed previously, bioanalytical methods for the pharmacokinetic analysis of cabergoline ideally require detection limits of 1–2 pg ml^{-1} in plasma, or less than 1–2 pg on-analyte on column. This was easily accomplished here, as the LLOQ of 50 fg on-column represents only 5% of a 1 ml plasma sample containing 1 pg of cabergoline. By extrapolation, it is also clear that with such sensitivity, an assay requiring less than a 1 ml sample volume is possible. A significant advantage of a bioanalytical method 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 easily to attain low detection limits in complex plasma samples also negates the need to develop highly selective sample enrichment procedures to minimize matrix interferences and maximize analyte

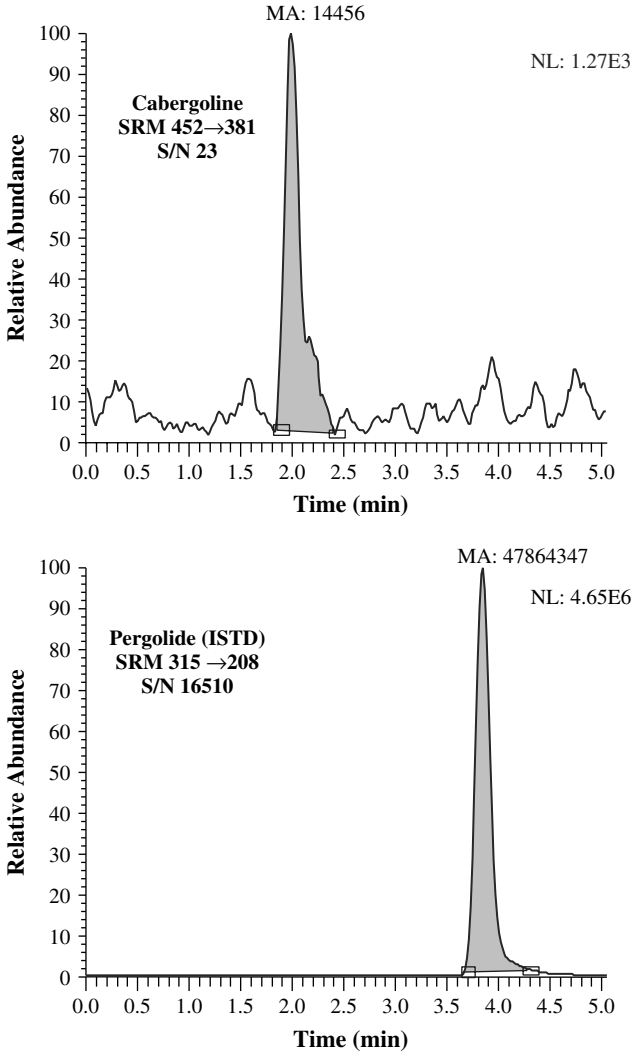

Figure 4. LC/ESI-SRM chromatogram of 50 fg on-column of cabergoline (*m/z* 452 → 381) and 1 ng on-column of pergolide internal standard (*m/z* 315 → 208) in plasma under unit mass-resolution conditions.

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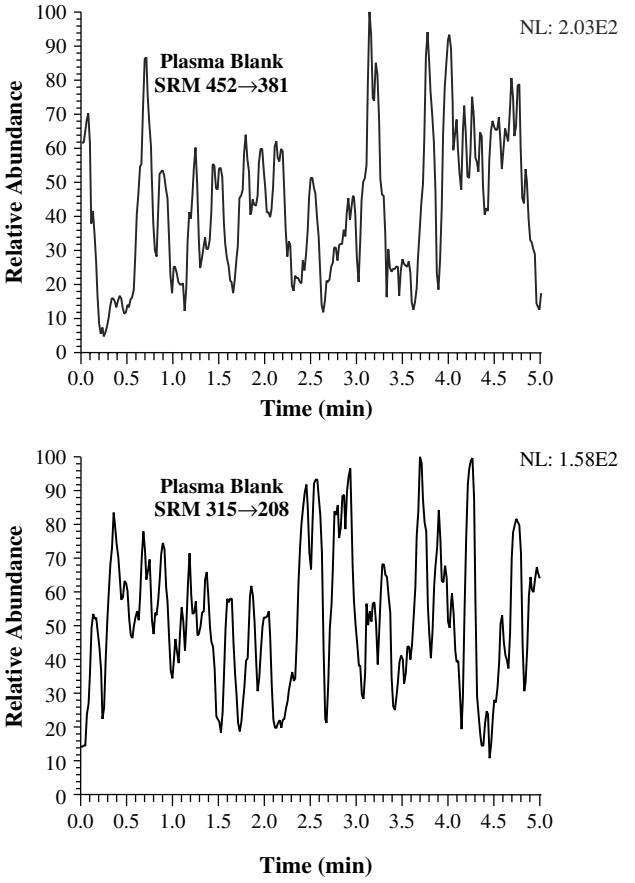

Figure 5. LC/ESI-SRM chromatograms of drug-free plasma under unit mass-resolution conditions.

Table 1. Precision and accuracy in the LC/ESI-SRM determination of cabergoline in plasma (mean of $n \geqslant 5$ injections) under unit mass-resolution conditions (Q1, Q3 resolution 0.7 Da FWHM)

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

enrichment (amount injected on the column), which has been a requirement for previous methods.[9–11] For example, a previous method for cabergoline developed on the older generation TSQ 7000 triple-quadrupole mass spectrometer reported an LLOQ value of 1.86 pg ml^{-1} (S/N ratio of 18 ± 13.1). The attainment of this LLOQ was accomplished using a minimum 1 ml plasma volume, a complex extraction procedure requiring a five-fold analyte enrichment and an injection volume (150 μl) representing 75% of the extracted sample or 900 fg on-column.[10] Thus, the 50 fg detection limit for cabergoline reported using the Finnigan TSQ Quantum Discovery is almost 20 times lower than that obtained on the older generation instrument, which was equipped with the original API source.[9] Our findings are consistent with other LC/ESI-SRM quantitative studies in which LLOQs were found to be one to two orders of magnitude lower on the Finnigan TSQ Quantum Discovery relative to the TSQ 7000.[12–15] Similar cabergoline sensitivities to those obtained on the TSQ 7000 have also been reported using the API 3000.[10]

The large increase in sensitivity achieved on the Finnigan TSQ Quantum Discovery is a direct consequence of the improved design of the API interface, ion optics, collision cell and quadrupoles.[20] A schematic of the Finnigan TSQ Quantum Discovery analyzer is shown in Fig. 6. The new off-axis ESI probe allows for noise reduction and better desolvation, while providing improved ruggedness for high-throughput bioanalytical assays. The increased internal diameter of the ion transfer tube in the Finnigan TSQ Quantum Discovery compared with the heated capillary of the TSQ 7000 (400 to 500 μm) and the larger entrance skimmer diameter (1.45 to 1.78 mm) improves the ion sampling capacity. The use of two square quadrupoles as r.f.-only ion guides in the entrance optics of the new instrument (QOO and QO, Fig. 6) improves the ion focusing and ion transmission efficiency compared with the regular hexapole or octapole designs used in past triple-quadrupole mass spectrometers. A square quadrupole ion guide combines the effects of both quadrupole and higher order multipole fields, providing better focusing of the ion beam with a lower kinetic energy spread (typically 1–3 eV) and better phase space characteristics. While the strong focusing effect of the quadrupolar field dominates at the center of the device, resulting in efficient radial damping, higher order multipole fields created by the square shape of the rods

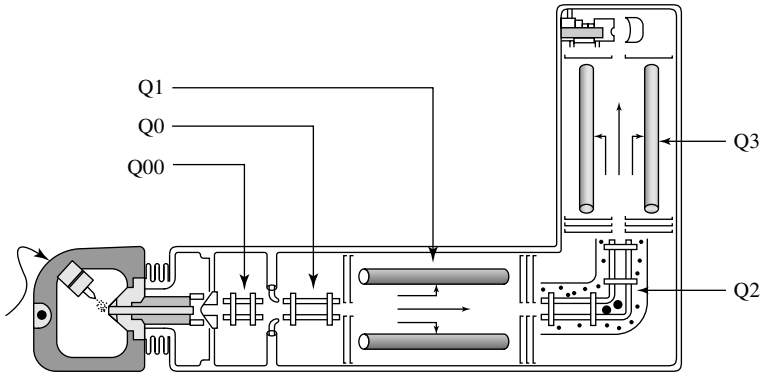

Figure 6. The Finnigan TSQ Quantum Discovery analyzer.

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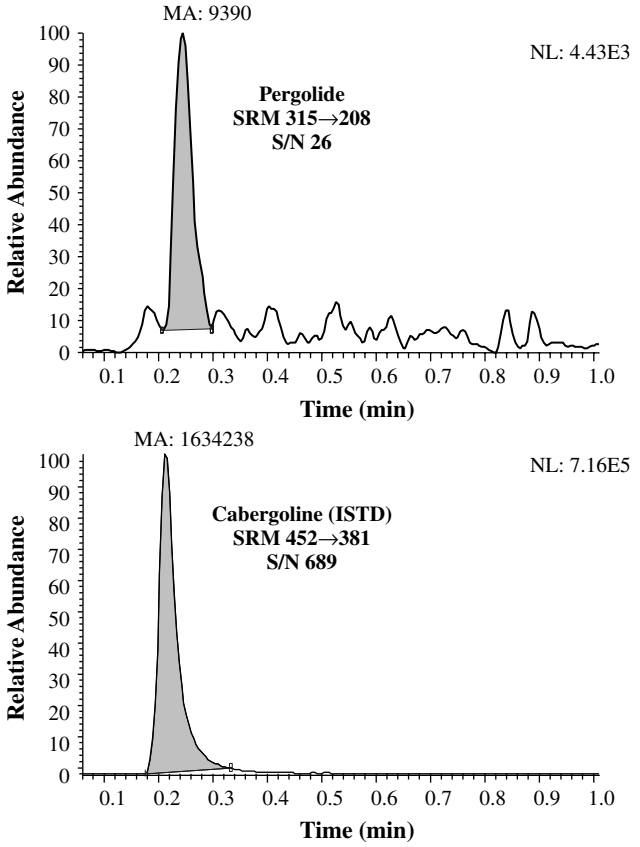

Figure 7. LC/APCI-SRM chromatogram of 500 fg on-column of pergolide (*m/z* 315 → 208) and 500 pg on-column of cabergoline internal standard (*m/z* 452 → 381) in plasma under unit mass-resolution conditions.

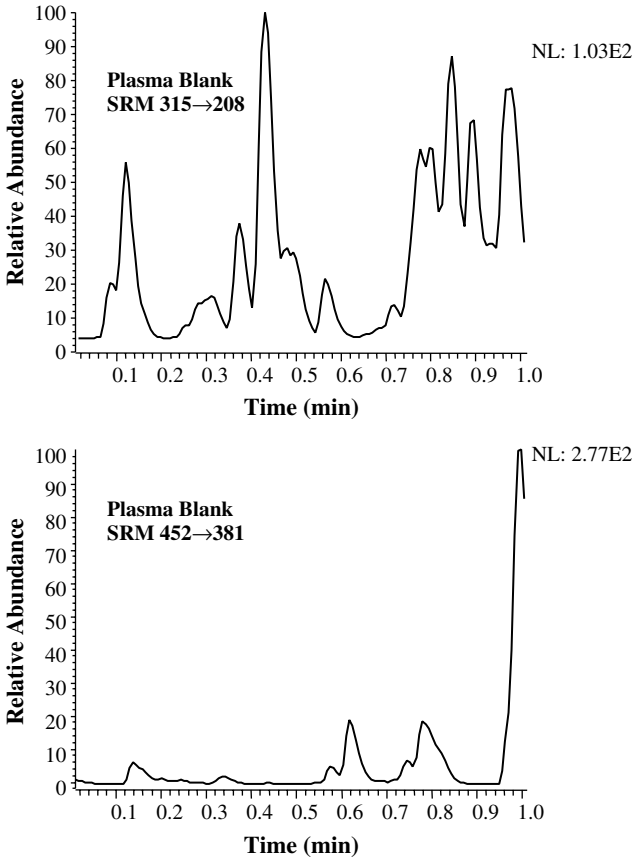

Figure 8. LC/APCI-SRM chromatograms of drug-free plasma under unit mass-resolution conditions.

improves the overall transmission efficiency by reducing the ion noding that occurs within pure quadrupolar fields. These effects result in a tighter, more controlled ion beam passing through the square quadrupole ion guides, with improved ion transmission into the first quadrupole mass analyzer region (Q1, Fig. 6). The same square quadrupole technology is used in the new 90° collision cell (Q2, Fig. 6) where the increased length of the device, coupled with the better ion focusing characteristics, improves the CID efficiency by a factor of two relative to the octapole collision cell employed in the TSQ 7000. In addition, the 90° bend totally eliminates neutral noise, which leads to an improvement in S/N ratio. Finally, the use of true hyperbolic quadrupoles as mass analyzers with an increased field radius (r_0) relative to the TSQ 7000 provides better compensation for ion scattering effects. Coupled with a high-quality r.f. system, the new quadrupole design has improved ion acceptance and transmission efficiencies, in addition to enhanced mass-resolution capabilities which can lead to a further enhancement in quantitative performance on the Finnigan TSQ Quantum Discovery, as will be demonstrated later.[13,18]

The calibration range for cabergoline (Table 1) was linear, covering five orders of magnitude (50 fg to 5 ng on-column), with a correlation coefficient $R > 0.999$ using a weighting factor of $1/x$. Intra-assay accuracy and precision was evaluated for $n \geq 5$ samples at each calibration level. The accuracy and precision values obtained over this extended linear dynamic range are shown in Table 1. The LLOQ (50 fg on-column) gave 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 5 ng on-column) ranged from 7.5% to 1.6% and from 0.5 to 8.4%, respectively. The linear dynamic range reported here is considerably broader than those reported previously for this assay on the API 3000 and TSQ 7000 instruments, which were both less than two orders of magnitude.[9,10] The significance of this extended linear dynamic range is that a single detection method has now been developed for cabergoline suitable for application in both low- and high-dose pharmacokinetic analyses.

The extended linear dynamic range capability of the Finnigan TSQ Quantum Discovery is mainly due to the advanced analog technology present in the detection system. The use of a wide dynamic range electron multiplier coupled with a high-bandwidth electrometer and an analog-to-digital converter allows for single ion event detection, while maintaining excellent linearity at high levels of signal. The improved limit of detection on the Finnigan TSQ Quantum Discovery also increases the overall linear dynamic range of the instrument. This extended linearity compares favorably with typical pulse-counting technologies used on other mass spectrometers, where the dead-time effect in the time-to-digital converters causes systematic errors in peak intensity and mass position at higher ion flux, thus limiting the linear dynamic range.[21] In fact, it is common-place for many of the older generation mass spectrometers to see non-linear responses and/or detector saturation at linear

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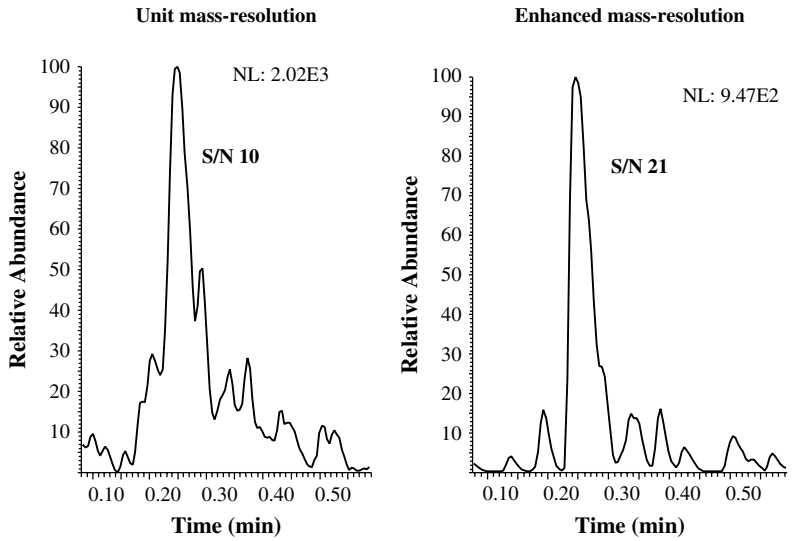

Figure 9. LC/APCI-SRM chromatogram of 250 fg on-column of pergolide (*m/z* 315 → 208) in plasma under unit mass-resolution conditions and enhanced mass-resolution conditions (see Experimental).

dynamic ranges of only three orders of magnitude. This is particularly problematic for methods where drugs and their metabolites are present in vastly different concentrations and also for methods involving drug combination products. In the latter example, in particular, it is common to have plasma concentrations in the low pg ml^{-1} range for one analyte and in the ng ml^{-1} range for the other(s). Typically, method development would be biased towards optimizing the detection limit for the lower concentration analyte and detector saturation of the higher concentration analyte is an unavoidable consequence. In this instance, the linear range of the high-concentration analyte is compromised so sample dilution and separate analysis are necessary. This adds considerably to overall sample analysis time and hence does not lend itself to high-throughput applications. Therefore, extended linear dynamic range is extremely beneficial in these situations.

Next, LC/APCI-SRM quantitation of pergolide at unit and enhanced mass-resolution will be discussed. Similar to analyses performed by LC/ESI-SRM, analyte sensitivities attained by LC/APCI-SRM have been found to be an order of magnitude greater on the Finnigan TSQ Quantum Discovery relative to the previous generation triple-quadrupole mass spectrometer.[12,14] 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, with chromatographic run times that are often longer in order to compensate. In this study, APCI was selected in combination with a relatively high chromatographic flow-rate (0.8 ml min^{-1}) in the development of a sensitive detection method with an extremely short chromatographic run time (<1 min).

The quantitative results for pergolide in plasma at unit and enhanced mass-resolution are shown in Fig 7–9 and Tables 2 and 3. Under unit mass-resolution conditions, the LLOQ for pergolide was 500 fg on-column (Fig. 7), where no interfering peaks were observed in the corresponding extracted ion chromatogram of the blank plasma (Fig. 8). In a previously developed LC/ESI-SRM method for the quantitation of pergolide in plasma, an LLOQ of 5 pg ml^{-1} was reported on an older generation triple-quadrupole mass spectrometer,[11] which represented 3 pg of analyte on-column (personal communication). Hence, running this assay by APCI on the Finnigan TSQ Quantum Discovery at unit mass-resolution results in a significant improvement in sensitivity. Once again, the previous LC/ESI-SRM method required a very high plasma volume (1.5 ml), a highly selective extraction/enrichment procedure and a run time of 3.5 min, whereas in the present experiment the minimally treated samples were analyzed considerably faster (1 min run time). A linear dynamic range covering five orders of magnitude (500 fg to 50 ng on-column) was achieved, with a correlation coefficient $R = 0.998$ using a weighting factor of $1/x^2$. This is a significantly broader linear dynamic range than that obtained by LC/ESI-SRM, which only covered two orders of magnitude.[11] Intra-assay accuracy and precision were evaluated for $n \geq 5$ samples at each calibration level. Accuracy and precision over the complete calibration range for pergolide were well within pharmaceutical industry standards at unit mass-resolution (Table 2). 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 (2.5–50 000 pg on-column) ranged from −5.3 to 11.0% and from 0.5 to 5.0%, respectively.

Attempts to lower the LLOQ of pergolide to 250 fg on-column at unit mass-resolution were difficult because of isobaric matrix interferences to the analyte signal, as shown in Fig. 9. Strategies that would typically be required to improve analyte sensitivity involve further work on sample preparation techniques to remove/reduce these interferences, followed by enrichment to increase the sensitivity of the analyte. An alternative approach would involve the manipulation of the chromatographic method to separate out the interferences from the analyte, but this would undoubtedly lengthen the analysis time and sample throughput capability, and may also reduce sensitivity. Here, the complex plasma samples were assayed under

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

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 3. Precision and accuracy of LC/APCI-SRM determination of pergolide in plasma (mean of $n \geq 5$ injections) under enhanced mass-resolution conditions (Q1 resolution 0.2 Da FWHM and Q3 resolution 0.7 Da FWHM)

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

the same chromatographic conditions (<1 min run time) using the enhanced mass-resolution capability of the triple-quadrupole mass spectrometer to determine whether this unique instrument feature could improve the LLOQ. As shown in Fig. 9, enhanced mass-resolution on the Q1 mass analyzer used for parent molecular ion selection (Q1 0.2 Da FWHM) resulted in a dramatic decrease in isobaric chemical noise and a corresponding two-fold enhancement in S/N ratio. With this improvement in analyte signal, an LLOQ of 250 fg on-column was achieved for the pergolide assay in enhanced mass-resolution mode, as shown in Table 3. Previous SRM studies have also shown significant improvements in LLOQ for analytes present in plasma and brain matrices using enhanced mass-resolution rather than unit mass-resolution.[13,18] In addition to the increase in the detection limit of pergolide, an increase in the linear dynamic range (250 fg to 50 ng on-column) was also achieved using enhanced mass-resolution, with a correlation coefficient $R = 0.997$ using a weighting of $1/x^2$. Accuracy and precision values for all calibration levels under enhanced mass-resolution conditions were well within pharmaceutical industry specifications (Table 3).

The LLOQ of 250 fg on-column for pergolide in LC/APCI-SRM at enhanced mass-resolution now represents over an order of magnitude improvement in sensitivity to that previously achieved.[11] This sensitivity, coupled with the broad linear dynamic range, makes the Finnigan TSQ Quantum Discovery applicable for the determination of pergolide at any given dosage regime using low plasma volumes and the requirement for minimal sample extraction. Thus, the enhanced mass-resolution feature of the instrument gives the user a simple, yet rapid, means to improve method sensitivity and selectivity without the need for further sample manipulation. This is a feature that should lend itself well to the development of high-throughput pharmacokinetic applications.

CONCLUSIONS

A new generation triple-quadrupole mass spectrometer operated at unit mass-resolution has shown tremendous utility in the development of highly sensitive detection methods for the ergoline derivatives cabergoline and pergolide. The API-SRM sensitivities achieved were 10–20-fold greater than those reported in previous studies. The additional flexibility of being able to use the enhanced mass-resolution feature to improve the LLOQ, through distinguishing analytes of interest from interferences of the same nominal mass, offers the opportunity for improved quantitative performance for other analytes with low systemic plasma levels. The broad dynamic range capability in either unit or enhanced mass-resolution operation is of practical importance for most quantitative applications but is particularly advantageous in situations where detector saturation is an issue. Examples include multi-dose studies (low and high dose), methods for drugs and their metabolites and quantitation of drug combination products. Overall, the advent of an improved-sensitivity triple-quadrupole mass spectrometer with enhanced mass-resolution capabilities should find widespread use in the development of simple, sensitive, fast, accurate and rugged methods for many pharmacokinetic applications.

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