

Rapid Method for Identification and Quantification of Dantrolene and its Oxidative Metabolite in Equine Plasma using a Dual Cell Linear Ion Trap Mass Spectrometer

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Overview

Purpose: To evaluate the performance improvement of a dual cell linear ion trap versus a standard LTQ linear ion trap for quantification of Dantrolene in equine plasma

Methods: Equine plasma samples were analyzed by LC-MS/MS using an LTQ XL™ mass spectrometer and a novel dual cell linear ion trap (LTQ Velos™ mass spectrometer). Pharmacokinetic curves were calculated using calibrants and quality control samples

Results: The dual cell linear ion trap afforded >5X improvement in sensitivity and a 1.5X improvement in duty cycle resulting in a 10X reduction in LOQ for 5-Hydroxydantrolene. In addition, complete pharmacokinetic curves were constructed for all time points for both Dantrolene and 5-Hydroxydantrolene.

Introduction

To protect animal health and uphold the integrity of the racing industry, the California Horse Racing Board requires analysis of post-race blood and urine samples from horses in competition. The Kenneth L. Maddy Equine Analytical Chemistry Laboratory is the authorized equine drug testing laboratory for California's six permanent race courses, nine seasonal fair venues, and other performance events and locations. This laboratory is based at the School of Veterinary Medicine in the California Animal Health and Food Safety Laboratory at the University of California, Davis.

Laboratory staff members develop highly specialized methods to document the effects of certain drugs and other substances on equine performance. The equine industry use this faculty research data from the laboratory to make regulatory decisions. Researchers have evaluated the effects of prescribed medications, unauthorized drugs and other substances on the performance of horses. Examples include determining the length of time required for clearance of non-steroidal anti-inflammatory medications from a horse's system, potential performance effects of the diuretic Lasix, and acceptable residue levels of procaine penicillin. Research projects in pharmacology and toxicology focus on controlled studies to establish more effective drug treatments, dosages and clearance times that currently do not exist for many of the hundreds of therapeutic drugs in use.

Using mass spectrometry, laboratory personnel perform testing that can detect and quantify more than 800 drug substances. In September 2005, the laboratory added pre-race blood testing of all thoroughbreds in California for total CO₂ which can indicate "milkshaking," a prohibited procedure believed to increase endurance. In this work we focus on dantrolene, a drug indicated primarily for the prevention and treatment of malignant hyperthermia syndrome, equine post-anesthetic myositis and equine exertional rhabdomyolysis. Dantrolene can be used to enhance the performance of both racing and show horses, therefore, its presence is prohibited in regulatory samples. Dantrolene is rapidly eliminated from the horse (t_{1/2} ~130 min) through metabolism in the liver. Its metabolite (OH-dantrolene) is excreted in the urine.

The present study compares a standard linear ion trap with a modified, faster scanning linear ion trap in a rapid and sensitive method for detection of dantrolene and its oxidative metabolite by liquid chromatography with full scan MS/MS identification and quantification.

Methods

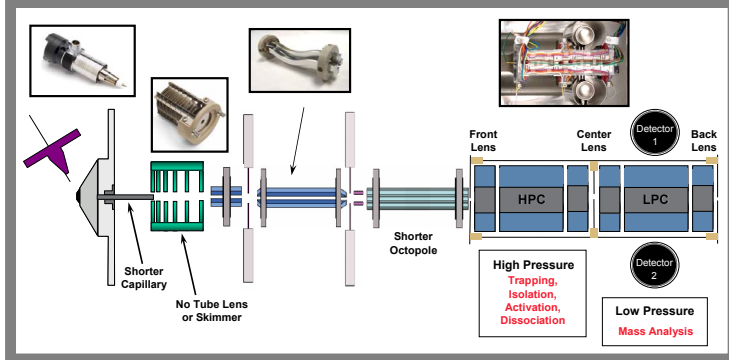
Plasma was obtained at regular time intervals from horses orally administered Dantrium (dantrolene sodium). A 500 µl sample was treated with 600 µl of 9:1 acetonitrile:1 M acetic acid containing 100 ng/ml of nitrofurazone as the internal standard (IS). The sample was refrigerated for 30 minutes and then centrifuged. The supernatant was transferred to autosampler vials and analyzed by LC-MS/MS on a 2.1 mm x 50 mm ACE C₁₈ column. The injection volume was 40 µl for each sample. The analytes were separated with a gradient using 10 to 90% acetonitrile over 5.0 minutes at a flow rate of 400 µl/min.



Detection was carried out using a standard LTQ XL mass spectrometer and a new LTQ Velos dual cell linear ion trap operated in negative ion mode using 3 full scan MS/MS events for identification and quantification. For quantification the following product ions were selected: m/z 313.2: 270.0; m/z 329.2: 257.0, 285.0, 286.0 for dantrolene and 5-hydroxydantrolene, respectively. Integrated extracted ion chromatographic peak area ratios were calculated relative to the internal standard, nitrofurazone. Peak area ratios were used for quantification.

Mass spectral data were acquired on a new dual cell linear ion trap depicted in the block diagram in Figure 1. Traditional ion traps are operated at a single pressure which affords sub-optimal conditions for trapping, isolation, activation, dissociation and mass analysis performance. Here, we introduce a dual pressure configuration which separates ion manipulation processes such that each can be carried out at its optimum pressure. Trapping, isolation, activation and dissociation occur in the high pressure cell while mass analysis occurs in the low pressure cell resulting in a faster scanning instrument. An S-lens is implemented in the first stage of pumping which affords the LTQ Velos greater sensitivity.

FIGURE 1. Schematic Diagram of Dual Cell Linear Ion Trap with S-Lens (a Stacked Ring Ion Guide)



Results

Area ratios versus internal standard for dantrolene and 5-hydroxydantrolene standards between 10 fg/µL and 100 pg/µL (four orders of magnitude) were used to construct the calibration curve. Two levels of quality control were injected between samples. Plasma was obtained at 24 time points (including baseline) over seven days for four horses dosed with dantrolene. Three full scan MS/MS events were used to monitor the drug, its hydroxylated metabolite and the internal standard on the LTQ XL and on the dual cell linear ion trap.

Extracted ion chromatograms for the three full scan MS/MS events for the 1 pg/µL standard are shown in Figure 2 for the LTQ XL (left hand side) and for the dual cell linear ion trap (right hand side). Retention times for the LTQ XL are slightly longer due to the inclusion of a guard column. All intensities on the dual pressure linear ion trap are at least five times greater than those on the LTQ XL.

FIGURE 2. Extracted Ion Chromatograms for 1 pg/µL Dantrolene (m/z 313), 5-Hydroxydantrolene (m/z 329) and 1 ng/µL Nitrofurazone (IS, m/z 197) on LTQ XL and on the dual cell linear ion trap

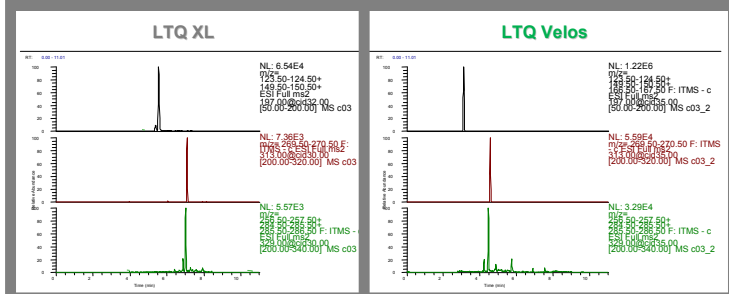
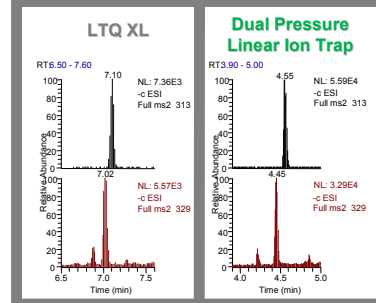


Figure 3 shows the number of scans for each full scan MS/MS event across each chromatographic peak for 1 pg/µL dantrolene and 5-Hydroxydantrolene on the LTQ XL (left) and on the Dual Pressure Linear Ion Trap (right). In addition to an increase in sensitivity (see Figure 4) we also observe approximately a 1.5-fold improvement in duty cycle.

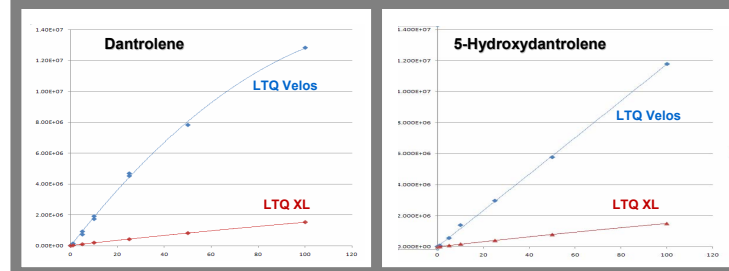
FIGURE 3. Extracted Ion Chromatograms for Dantrolene, 5-Hydroxydantrolene for LTQ XL and the Dual Pressure Linear Ion Trap Showing the Number of Scans Across Each Peak



Simultaneous improvement in sensitivity and duty cycle result in improved performance in quantification experiments. Calibration curves for dantrolene and 5-Hydroxydantrolene are shown in Figure 4 for LTQ XL (left) and for the Dual Pressure Linear Ion Trap (right). The LOQ for 5-Hydroxydantrolene for the dual cell linear ion trap is an order of magnitude lower than that for the LTQ XL based on peak detection. Relative standard deviations were not used for determination of LOQs as only a single injection was made for each standard on the LTQ XL.

Detection of lower levels of metabolite and/or drug in standards indicates the capability for detection of the compound at lower levels in real samples allowing improved assessment of the pharmacokinetic properties

FIGURE 4. Calibration curves for dantrolene (Left) and 5-Hydroxydantrolene (Right) for LTQ XL (Red) and for the LTQ Velos dual cell linear ion trap (Blue)



Pharmacokinetic curves for Dantrolene and 5-Hydroxydantrolene for LTQ XL and for the dual cell linear ion trap for four horses (named Summer, Levi, Eve and Monsieur) dosed with the drug as either paste or capsules are shown in Figure 5. Equine plasma samples were split and analyzed on the two platforms, results show comparable amounts in each sample. However, 5-hydroxydantrolene was detected at all time points on the dual cell linear ion trap but was below the limit of detection on the LTQ XL beyond the 18 hour time point for Monsieur and beyond the 24 hour time point for Levi.

FIGURE 5a. Pharmacokinetic Profiles for Dantrolene and its Metabolite 5-Hydroxydantrolene over 48-72 hours for two horses dosed with Dantrium (Sodium Dantrolene) as Paste

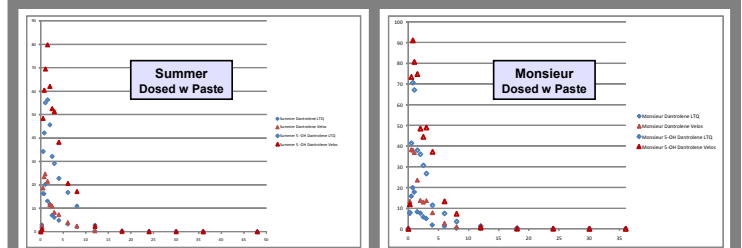
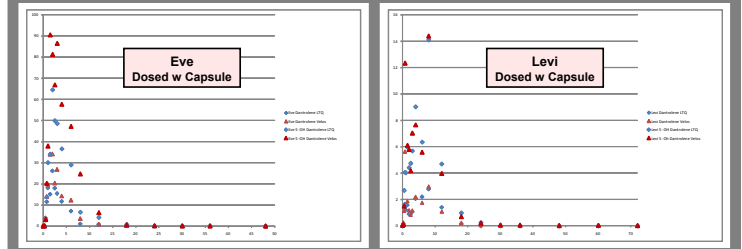


FIGURE 5b. Pharmacokinetic Profiles for Dantrolene and its Metabolite 5-Hydroxydantrolene over 36-48 hours for two Horses dosed with Dantrium (Sodium Dantrolene) as Capsules



Conclusions

We have compared a standard linear ion trap with a dual cell linear ion trap in a rapid and sensitive method for detection of dantrolene and its oxidative metabolite by liquid chromatography with full scan MS/MS identification and quantification and find the following:

1. The faster scan rate afforded by the LTQ Velos results in a 1.5-fold increase in the number of scans across chromatographic peaks.
2. The increase in sensitivity of the LTQ Velos results in a 10-fold increase in signal intensity observed for both Dantrolene and its metabolite 5-Hydroxydantrolene.
3. Equine plasma concentrations of both Dantrolene and 5-Hydroxydantrolene could be measured for all time points using the LTQ Velos whereas lower levels (time points later than 24 hours) could not be detected on the LTQ XL.

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