LC/MS Application Note

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Determination of the Dynamic Range for the Varian 1200L Electrospray (ESI)-LC/MS/MS Using a "CNS" Drug and its Major Hydroxylated Metabolite as the Test Compounds

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Introduction

The objective of this work is to establish the dynamic range of the Varian 1200L using TX1360 and its major OH-Metabolite as the model compounds. The internal standard selected for this work, Buspirone, is a structurally related central nervous system (CNS) drug.

Background

TX1360 is a pharmaceutical agent currently under development as a CNS drug. A large number of Absorption Distribution, Metabolism, and Excretion (ADME) studies as well as Pharmacokinetic (PK) studies have been completed during the development of TX1360. Subjects in these studies ranged from healthy volunteers (adult and pediatric) to liver-impaired subjects. In addition, *in vitro* and *in vivo* interaction studies have also been carried out.

The concentration range of TX1360 from these subjects ran from a low of 50 pg/mL to a high of 40 ng/mL in plasma. TexMS Analytical Services developed a procedure that meets the Lower Limit of Quantitation required for reliable determination of half-life in PK studies.

Older model mass spectometers used at TexMS exhibited detector saturation at 25 ng/mL using the developed procedure. Many of the study samples exceeded 25 ng/mL which necessitated revalidation. In order to bring the samples into the TX1360 validated curve range, new standard curves and the dilution of a significant number of samples was required. The additional work was costly both to TexMS Analytical Services and the project's sponsor. The 1200L mass spectrometer's linear dynamic range easily provided the solution to the problem of detector saturation at high sample concentration.

Instrumentation, Materials, and Reagents

- Varian 1200L LC/MS equipped with ESI source.
- All other chemicals were reagent grade or HPLC grade.
- Buspirone (Catalog No. B-7148) from Sigma-Aldrich Corp. (St. Louis, Missouri, USA).

Experimental Procedure

The Varian 1200L was used in its Selected Reaction Monitoring (SRM) mode. TX1360, TX1370 and Buspirone calibration solutions covering a range from 50 fg/µL to 80 ng/µL were accurately prepared by serial dilution. A fixed amount of the internal standard at 50 pg/mL was added to all calibration points. An "on-column" injection of 10 µL for each calibration standard was used.

HPLC Conditions

Column	50 mm x 1.0 mm 5 µm C-18			
Solvent A	deionized water			
Solvent B	80:20, (v: v), acetonitrile: water with 15 µL formic acid/100 mL			
LC Program	Time (min:sec)	%A	%B	Flow (mL/min)
	0:00	95	5	0.2
	6:00	55	45	0.2
	6:01	95	5	0.2
Injection Volume	10 µL			

MS Parameters

Collision Gas	2.1 mTorr Argon
API Drying Gas	23 psi at 300 °C
API Nebulizing Gas	60 psi
Scan Time	1 sec
SIM Width	0.7 amu
Needle	5200V
Shield	400V
Capillary	-40V
Detector	2000V

Scan Parameters

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
TX 1360	Propietary	121	-25
TX 1370	Propietary	121	-25
Buspirone	386	121	-25

Results and Discussion

The LC method used a 6-minute run cycle with TX1360 giving a retention time of 2.3 minutes and the OH-metabolite eluting at 2.1 minutes. Lower Limit of Quantitation (LLOQ) and the Upper Limit of Quantitation (ULOQ) are shown in Figures 1 and 2, respectively. The data presented in these figures have not been subjected to any "peak smoothing".

The linearity of the detector response was found to be excellent (Figures 3 and 4) for both the analyte and its OH-metabolite. Both figures are a plot of the peak area ratio (analyte/ISTD) versus analyte concentration. Visual inspection of the figures reveals that excellent linearity was achieved using the Log-Log Linear model. The r^2 for TX1360>121 and TX1370>121 are both 0.999.

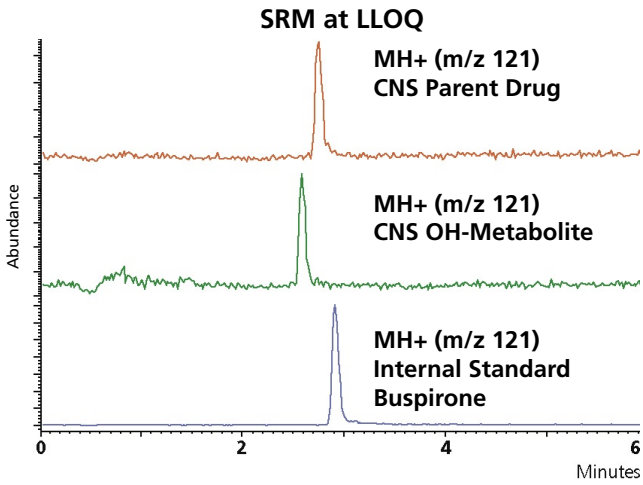

Figure 1. Excellent detector response and peak shape for TX1360 an TX1370 at LLOQ.

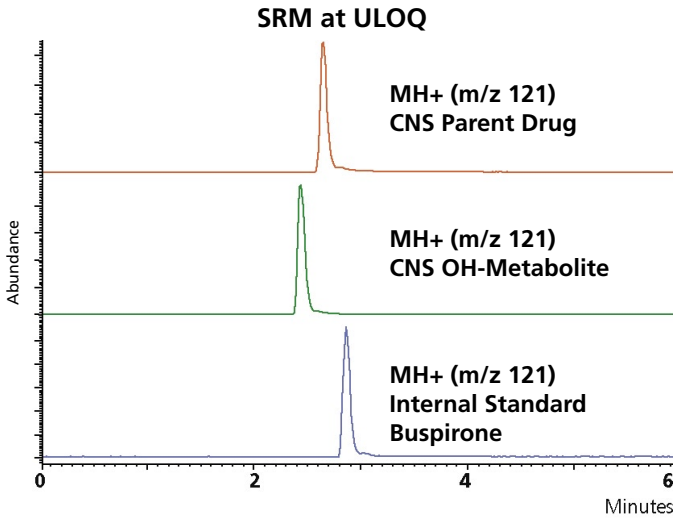

Figure 2. Excellent peak shape for TX1360 and TX1370 at ULOQ.

Calibration Curve TX1360

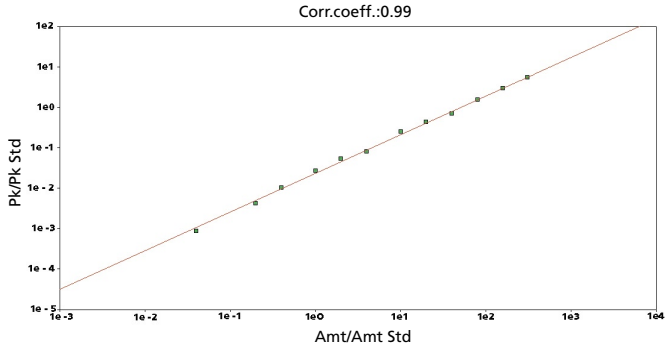

Figure 3. Excellent linear response over several orders of magnitude for TX1360.

Calibration Curve TX1370

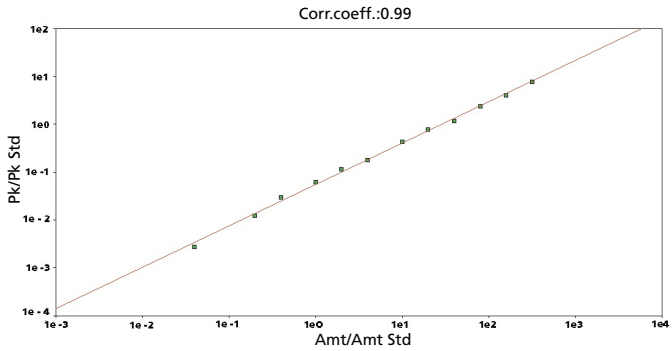

Figure 4. Excellent linear response over several orders of magnitude for TX1370.

Conclusion

The dynamic range for the CNS drug calculated as the ratio of the ULOQ/LLOQ is equal to 5.0E04. The large linear dynamic range of the Varian 1200L allows for the concentration range of these CNS drugs and their analogs to be extended significantly.

Using the 1200L instead of older model triple quad mass spectrometers for this application, TexMS estimated a savings of at least 30% in assay cost.

These data represent typical results.
For further information, contact your local Varian Sales Office.