CAPSULES notes and applications from BASi
October 2004
377

Ondansetron and Granisetron

LC/MS/MS Assay for Ondansetron and Granisetron in Human and Dog Plasma

Introduction

BASi has developed a LC/MS/MS method for the analysis of ondansetron and granisetron in human or dog plasma. This method utilizes liquid-liquid extraction to produce a fast, simple, sensitive, specific, and robust method with excellent linearity, precision, and accuracy. Although there are several methods for ondansetron in the literature, BASi now has a LC/MS/MS method for ondansetron and granisetron in human or dog plasma. Using only 0.100 mL of plasma per sample, a LLOQ was established at 1 ng/mL. The standard curve showed linearity up to 1000 ng/mL.

Ondansetron and granisetron are oral antiemetic, antinauseant drugs used primarily for patients undergoing radiation, including total body irradiation and fractionated abdominal radiation.

Method Summary

Ondansetron and granisetron concentrations in human or dog plasma are quantified using a 96-well automated liquid-liquid extraction process followed by LC/MS/MS analysis with positive ion electrospray. Plasma samples (0.100 mL) are basified after addition of tropisetron internal standard and extracted with ether. Following evaporation and reconstitution (0.400 mL) in mobile phase samples are injected (5 µL) onto a 2.1 x 50 mm, 5 µM, C18 column and eluted using a methanol/formic acid mobile phase.

Method Performance Data

The method range for ondansetron and granisetron is 1 – 1000 ng/mL. The standard curve for each run is prepared in duplicate at ten concentrations. Precision and accuracy data for the standard calibrators are shown in **T1** and linearity as determined from the coefficient of determination was > 0.99 for all runs.

Validation samples (VS) were prepared at five concentrations spanning the method range. Validation samples (one lower limit of quantification, one low level, two mid-range and one upper limit of quantification) were injected as six replicates in each of three validation runs. The precision and accuracy data are shown in **T2**.

T1. Between-run (n=12 Human, n=4 Dog) accuracy and precision for standard calibrators.

Sample	Human Ondansetron		Human Granisetron	
Name	Mean	CV	Mean	CV
SC-1	1.01	3.2	1.00	4.9
SC-3	2.91	4.2	2.96	3.1
SC-7.5	7.77	4.6	7.59	3.9
SC-10	9.86	3.5	9.84	3.8
SC-30	30.1	2.9	30.3	2.5
SC-75	77.2	4.2	76.5	3.0
SC-100	99.0	3.6	99.6	2.3
SC-300	292	5.2	294	4.8
SC-750	747	6.8	757	6.2
SC-1000	1015	2.6	998	2.2

Sample	Dog Ondansetron		Dog Granisetron	
Name	Mean	CV	Mean	CV
SC-1	1.01	5.6	1.01	5.4
SC-3	2.93	3.0	2.91	4.5
SC-7.5	7.39	5.6	7.51	7.6
SC-10	10.0	2.8	9.90	6.8
SC-30	30.9	3.8	31.0	7.2
SC-75	75.1	2.2	75.1	6.2
SC-100	101	3.5	101	7.8
SC-300	297	4.4	295	8.2
SC-750	727	7.0	726	7.1
SC-1000	1029	3.4	1028	3.2

T2. Between-run (n=18 Human, n=12 Dog) accuracy and precision for validation samples.

Sample	Human Ondansetron		Human Granisetron	
Name	Mean	CV	Mean	CV
QC-1	1.11	5.6	1.08	6.8
QC-3	3.25	5.1	3.08	5.0
QC-100	109	3.1	105	3.9
QC-750	798	8.8	776	9.4
QC-1000	1046	7.9	1003	7.8

Sample	Dog Ondansetron		Dog Granisetron	
Name	Mean	CV	Mean	CV
QC-1	1.01	6.8	1.04	5.7
QC-3	3.04	4.2	2.93	4.8
QC-100	103	4.2	97.3	6.4
QC-750	819	3.2	783	4.2
QC-1000	1085	3.7	1027	6.5

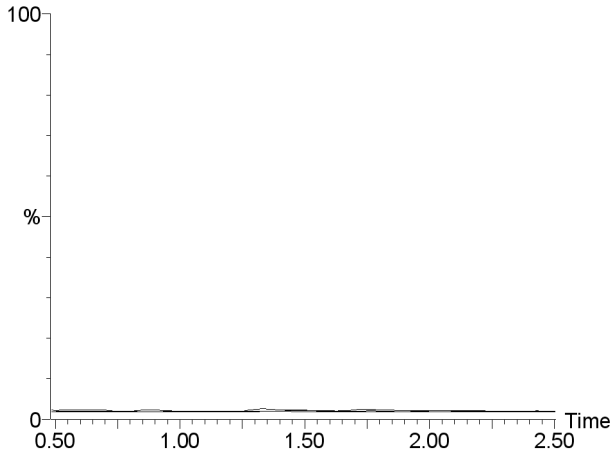
Ruggedness and Selectivity

The ruggedness of the method was tested by exposing validation samples to normal sample preparation practices. Freeze/thaw stability, heat treatment stability (for deactivation of the HIV virus), short-term (room temperature) stability, long-term frozen stability (up to 20 days), and autosampler stability were examined with acceptable accuracy and precision. Over-curve samples were diluted 1000-fold into the range of the method with acceptable accuracy and precision.

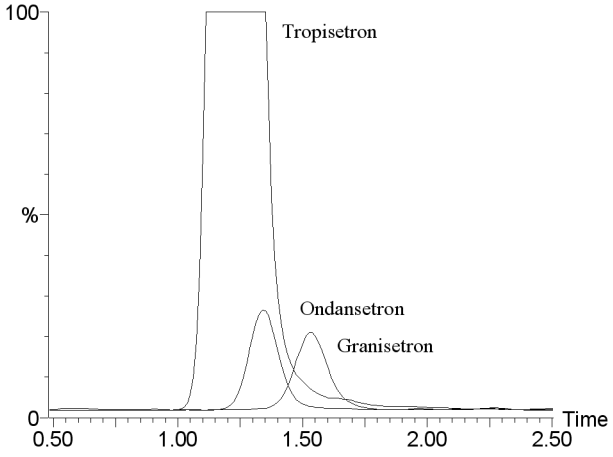
The selectivity of the method was examined by testing six different lots of plasma and a panel of over-the-counter drugs. No matrix interferences were detected between the six different lots of plasma and no interference was detected from a panel of over-the-counter drugs. Representative chromatograms are shown below in **F1**.

F1. Example Chromatograms

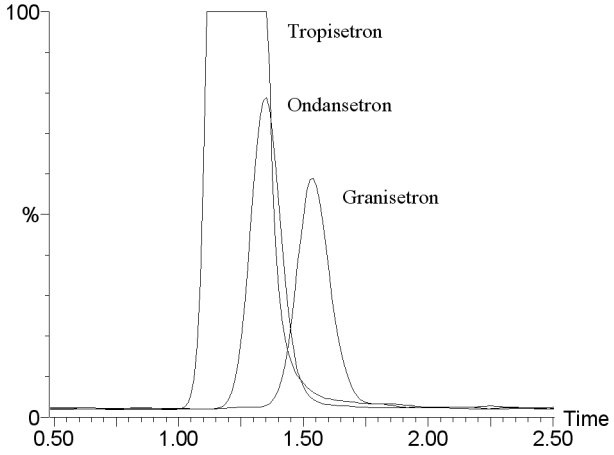
Extracted Blank Plasma (Same scale as the LLOQ)



LLOQ Validation Sample Level (1 ng/mL)



Low Validation Sample Level (3 ng/mL)



Mid Validation Sample Level (100 ng/mL)