

## INTRODUCTION

Tramadol is an analgesic used to treat moderate to moderately severe pain. A specific analytical method has been established and validated for the determination of tramadol and its major metabolite o-desmethyl tramadol in human plasma. The method was validated over the range 5.00 to 500ng/ml for both analytes.

## METHODOLOGY

### Sample Preparation and Extraction - Plasma Samples

Control human plasma samples were fortified with solutions containing tramadol and o-desmethyl tramadol. These plasma samples were then extracted by liquid/liquid extraction using methyl tert butyl ether (MTBE). The resultant supernatant was evaporated to dryness followed by reconstitution in mobile phase.

### Sample Analysis

The LC-MS/MS system comprised of:

- Applied Biosystems MDS SCIEX API 3000 Tandem Mass Spectrometer with the TurboIonSpray™ Source operating in the Positive Mode.
- Agilent HP1100 Series Quaternary Pump and Degasser.
- CTC HTC PAL Autosampler.

Chromatography was performed on:

- Phenomenex Polar RP column (50 x 4.6 mm, 5 micron).

Mobile Phase: 20mM Ammonium Acetate/Acetonitrile (50/50; v/v)

Injection Volume: 10µl

Mass Transitions:

- Tramadol: 264.1amu - 58.1amu
- O-desmethyl tramadol: 250.1amu - 58.1amu
- Internal Standard (diazepam): 272.0amu - 147.1amu

## VALIDATION RESULTS

Back-calculated concentrations for the plasma calibration samples are shown in Tables 1 and 2. The calibration curves showed good linearity over the range 1.00 - 500 ng/ml. Typical calibration curves are displayed in Figure 1 and 2. Examples of chromatography from a calibration standard containing 1.00 and 500 ng/ml of each analyte are presented in Figures 3 and 4.

Intra-day statistics are presented in Tables 3 and 4.

The inter-day (three occasions) bias for tramadol was 0.8, 0.0, -0.4, -1.0 and -2.2% at 5.00, 12.5, 50.0, 400 and 500ng/ml respectively. The inter-batch CV for tramadol at these concentrations was 7.3, 4.7, 3.2, 2.8 and 4.0% respectively. The inter-batch (three occasions) bias for o-desmethyl tramadol was 3.8, 0.8, 2.2, -3.3 and -6.2% at 5.00, 12.5, 50.0, 400 and 500ng/ml respectively. The inter-batch CV for o-desmethyl tramadol at these concentrations was 6.9, 6.2, 5.5, 5.9 and 5.8% respectively. These results demonstrate good accuracy and precision over the range 1.00 to 500 ng/ml for both analytes.

Both analytes were also shown to be stable in plasma for 24hours at room temperature, over 3 freeze/thaw cycles and plasma extracts containing the analytes demonstrated stability for up to 72 hours when stored at 4°C±3°C.

## CONCLUSION

A sensitive and versatile bioanalytical LC-MS/MS method was developed and validated to determine concentrations of tramadol and o-desmethyl tramadol in human plasma samples.

## TABLES

**Table 1: Back Calculated Concentrations from the Calibration Curve for Tramadol**

	Concentration of Tramadol (ng/ml)							
	5.00	10.0	20.0	50.0	100	200	300	500
	5.20 4.84	9.54 10.4	19.2 19.8	49.0 51.6	102 108	187 203	295 312	459 519
Mean	5.02	9.97	19.5	50.3	105	195	304	489
Bias	0.4	-0.3	-2.5	0.6	5.0	-2.5	1.3	-2.2
SD	0.255	0.608	0.424	1.84	4.24	11.3	12.0	42.4
CV (%)	5.1	6.1	2.2	3.7	4.0	5.8	4.0	8.7

**Table 2: Back Calculated Concentrations from the Calibration Curve for O-Desmethyl Tramadol**

	Concentration of O-Desmethyl Tramadol (ng/ml)							
	5.00	10.0	20.0	50.0	100	200	300	500
	5.17 5.04	8.86 9.91	19.1 21.6	50.2 54.8	96.4 112	190 205	280 301	470 506
Mean	5.11	9.39	20.4	52.5	104	198	291	488
Bias	2.2	-6.1	2.0	5.0	4.0	-1.0	-3.0	-2.4
SD	0.0919	0.742	1.77	3.25	11.0	10.6	14.8	25.5
CV (%)	1.8	7.9	8.7	6.2	10.6	5.4	5.1	5.2

**Table 3: Intra-Occasion Statistics for Tramadol**

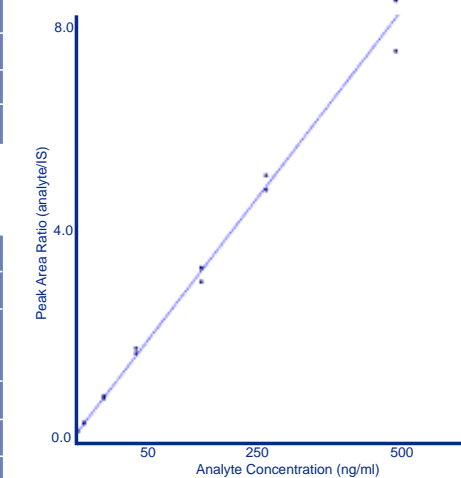
	Concentration of Tramadol (ng/ml)				
	5.00	12.5	50.0	400	500
	4.76 4.95 5.04 5.12 4.57 4.75	11.9 12.8 12.3 12.8 12.1 12.3	47.6 50.4 50.1 49.5 47.8 48.8	370 387 389 398 386 394	487 452 493 475 477 452
Mean	4.87	12.4	49.0	387	473
Bias	-2.6	-0.8	-2.0	-3.3	-5.4
SD	0.207	0.367	1.17	9.63	17.3
CV (%)	4.3	3.0	2.4	2.5	3.7

**Table 4: Intra-Occasion Statistics for O-Desmethyl Tramadol**

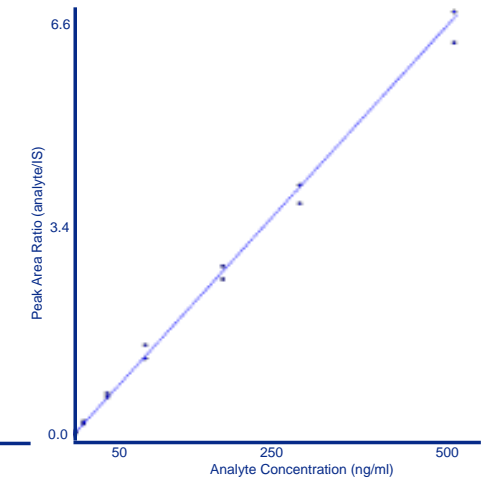
	Concentration of O-Desmethyl Tramadol (ng/ml)				
	5.00	12.5	50.0	400	500
	4.76 4.63 4.97 4.90 4.98 5.19	12.9 12.8 13.3 13.1 13.4 12.3	50.7 51.2 52.9 54.2 51.7 51.5	372 384 395 366 364 371	466 461 474 456 457 434
Mean	4.91	13.0	52.0	375	458
Bias	-1.8	4.0	4.0	-6.3	-8.4
SD	0.194	0.398	1.29	11.9	13.5
CV (%)	4.0	3.1	2.5	3.2	2.9

## FIGURES

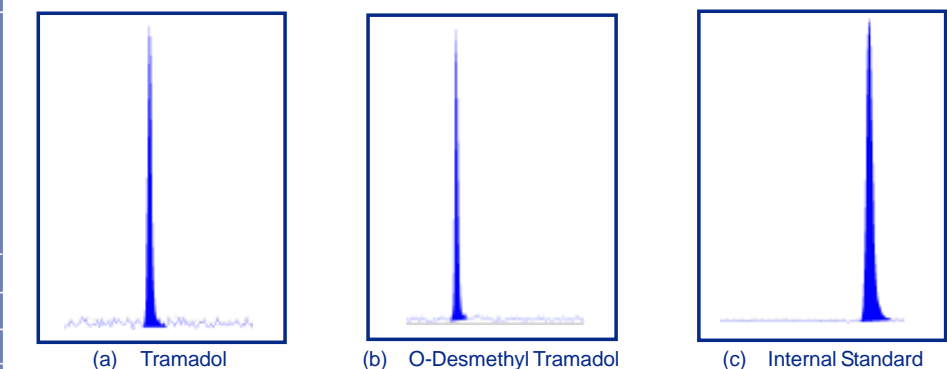
**Figure 1: Representative Calibration Curve for Tramadol**



**Figure 2: Representative Calibration Curve for O-Desmethyl Tramadol**



**Figure 3: Representative Chromatogram from a Calibration Standard Sample containing 5.00ng/ml of Tramadol and O-Desmethyl Tramadol**



**Figure 4: Representative Chromatogram from a Calibration Standard Sample containing 500ng/ml of Tramadol and O-Desmethyl Tramadol**

