

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

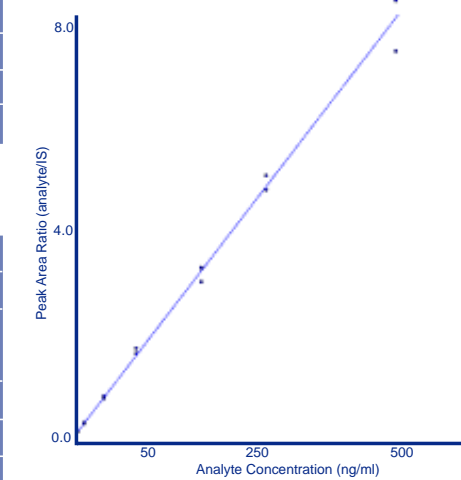


Figure 2: Representative Calibration Curve for O-Desmethyl Tramadol

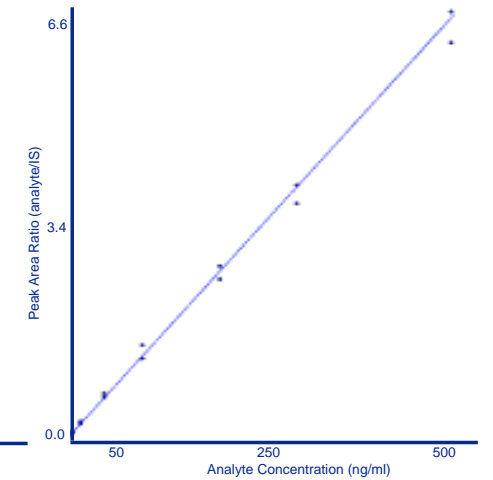


Figure 3: Representative Chromatogram from a Calibration Standard Sample containing 5.00ng/ml of Tramadol and O-Desmethyl Tramadol

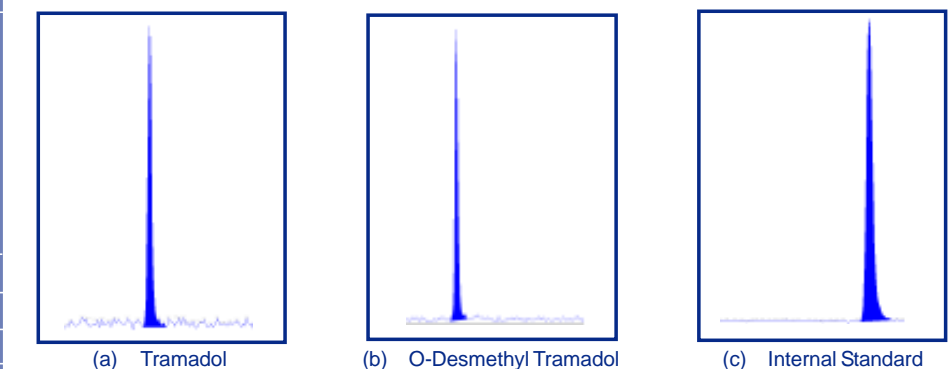


Figure 4: Representative Chromatogram from a Calibration Standard Sample containing 500ng/ml of Tramadol and O-Desmethyl Tramadol

