



## Gradient Elution LC-ESI-MS Determination of Tramadol in Rat Plasma

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**SUMMARY.** A sensitive and simple liquid chromatography/electrospray mass spectrometry (LC-ESI-MS) method for determination of tramadol in rat plasma using one-step protein precipitation was developed. After addition of ketamine as internal standard (IS), protein precipitation by acetonitrile was used as sample preparation. Chromatographic separation was achieved on an SB-C18 (2.1 mm × 50 mm, 3.5 μm) column with methanol-0.1 % formic acid as mobile phase with gradient elution. Electrospray ionization (ESI) source was applied and operated in positive ion mode; selected ion monitoring (SIM) mode was used to quantification using target fragment ions  $m/z$  264.0 for tramadol and  $m/z$  237.8 for the IS. Calibration plots were linear over the range of 5-500 ng/mL for tramadol in rat plasma. Lower limit of quantification (LLOQ) for tramadol was 5 ng/mL. Mean recovery of tramadol from plasma was in the range 92.8 %-97.4 %. RSD of intra-day and inter-day precision were both less than 10 %. This method is simple and sensitive enough to be used in pharmacokinetic research for determination of tramadol in rat plasma.

**KEY WORDS:** Gradient elution, LC-ESI-MS, Rat plasma, Tramadol.

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