

**Quantification of Clostebol in Urine by LC-MS/MS**

**Giacomo Luca Visconti, Tobia Barchi**

**Background**

Clostebol is an anabolic steroid whose detection in urine is critical both for sports anti-doping and occupational health monitoring. Its extremely low urinary concentrations and potential structural interferences require an assay of the highest sensitivity and specificity. The aim of this work is to develop and validate a ready-to-use LC-MS/MS kit capable of rapid and reliable quantification of Clostebol in urine samples.

**Methods**

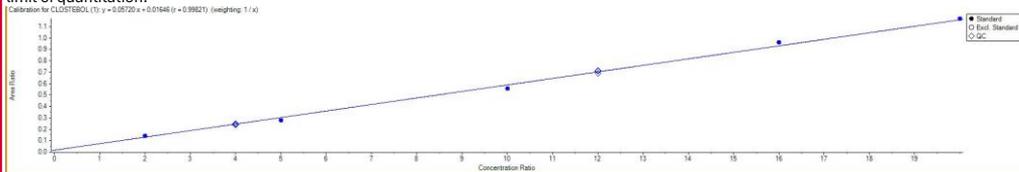
The Eureka Lab Division Clostebol Urine Kit protocol begins by treating a 2 mL urine sample with reagents A (500 µL, buffer solution) and B (20 µL, internal standard solution), followed by extraction with reagent C (3 mL x 2 times, extraction solution) and centrifugation. The residue is evaporated at 45 °C and reconstituted with reagents D (125 µL, reconstituent solution 1) and E (125 µL, reconstituent solution 2). After brief vortexing and a second centrifugation, 200 µL of the processed sample are transferred to a vial, and 10 µL are injected into the chromatographic system. Separation occurs on a C18-silica column at a mobile-phase flow rate of 0,3 mL/min. The total run time is six minutes, including wash and conditioning cycles. Calibration is performed over six points (0–20 ng/mL) with two quality-control levels (4 ng/mL and 12 ng/mL), using a deuterated internal standard (testosterone D3) to ensure accuracy and precision.

**Results**

Intra-series repeatability tests yielded coefficients of variation below 4%, while inter-series repeatability remained under 9% for QC1 (4 ng/mL) and under 2% for QC2 (12 ng/mL). The limit of quantitation is 0,5 ng/mL. Further improvements can be carried out for achieving a LOQ of 0,05 ng/mL. The calibration curve demonstrates excellent linearity ( $r^2 > 0.99$ ). Representative chromatograms display well-resolved peak at 1 ng/mL confirming method performances.

Intra-series repeatability (CV%)	inter-series repeatability (CV%)
< 4 %	< 9 % (QC1) ; < 2 % (QC2)
LLOQ	Linearity
0,5 ng/mL (*)	from 0,5 ng/mL to 20 ng/mL (*)

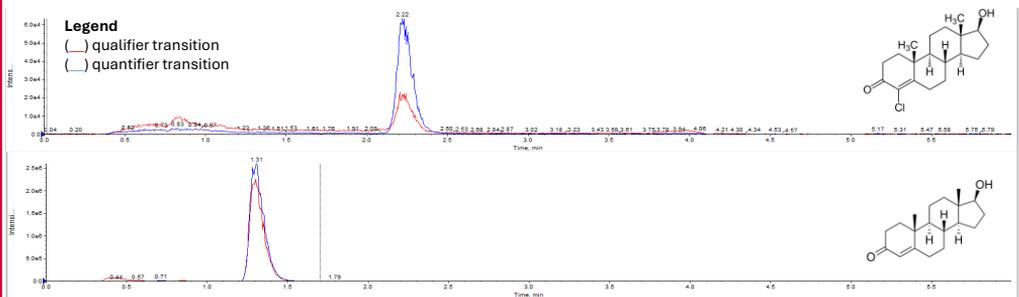
The analysis have been performed on SCIEX4500; (\*) Further improvements can be carried out for achieving a LOQ of 0,05 ng/mL and a higher upper limit of quantitation.



**Chromatograms**

The first chromatogram corresponds to a sample with concentration of 1 ng/mL.

The second one corresponds to internal standard in the same sample.



**Conclusions**

This method delivers high sensitivity with an LOQ down to 0,05 ng/mL, robust precision (intra- and inter-series CVs below 15%), and a rapid analysis time of six minutes. The standardized protocol and ready-to-use kit make it an ideal solution for clinical and occupational laboratories requiring reliable urine Clostebol monitoring.