INTRODUCTION
Curcumin (CU), a derivative of the plant Curcuma longa, is used extensively in the food industry. It is a major component of curry powder, and research has shown that curcumin may prevent cancer and other chronic diseases (1).

A robust, sensitive and accurate analytical method for the LC-MS-MS determination of Curcumin (CU) in equine plasma was developed in our laboratories based on information taken from the literature (2, 3, 4).

This communication describes the experimental conditions used and the results obtained during the validation of the LC-MS-MS method. The Guidance for Industry ‘Bioanalytical Method Validation’ issued by FDA on May 2001 and the GLP recommendations were taken into account as a base for the validation plan.

MATERIALS AND METHODS

TEST ARTICLE
Curcuma (CU), Batch 043030191. Indena, Relatting Date 02:2008, HPLC purity 99.86%.

MATERIALS
Methanol, HPLC grade
Acetonitrile, HPLC-grade gradient
Formic acid 98-100% analytical grade
Purified water – Acetonitrile - Formic Acid (10:90:0.1 v/v).

Blank equine plasma for calibration standards, control samples and QCs
SPE Strip X-35 µm Polymeric Sorbent: 30 mg mL⁻¹ (Phenomenex).

Purified water: HPLC grade, Milli-Q (Millipore).

CHROMATOGRAPHIC SYSTEM AND CONDITIONS
- 1200 LC-MS-MS (Varian) constituted of:
  - Binary pump: Model Proline 210
  - Autosampler: Model ProStar 410 working at 10°C temperature
  - Column oven: 30°C
  - Detector: Mass spectrometer 1200L, turbo ion-spray source.
  - Mobile phase A: Purified water – Acetonitrile - Formic Acid (10:90:0.1 v/v).
  - Mobile phase B: Purified water – Acetonitrile - Formic Acid (50:90:0.1 v/v).
  - Run time: 6 min

RESULTS

SELECTIVITY

Sensitivity was assessed by checking chromatograms of blank controls (plasma and reagents), of pure and extracted plasma standards to determine whether any interfering peak was present at the retention time of each analyte. Selectivity included: Blank plasma, blank reagent, blank plasma and zero-level sample (IS).

Representative chromatograms included blank plasma sample (B), a blank reagent (R), a blank plasma (BP), a zero-level sample (IS), a plasma standard at 5.275 ng/mL (LLOQ). No interfering peaks were observed at the retention time of the analytes.

LINEARITY: CURVE PARAMETERS

The concentration range studied was from 5.275 to 527.5 ng/mL. A linear correlation was found between the CURS peak area ratio and the corresponding concentrations of Curcumin in this range.

FACTORs
\[ y = a + bx \]

\[ y \] is peak area Curve ratio
\[ a \] and \[ b \] are intercept and slope, respectively

The method was validated with regard to the following parameters:
- Selectivity
- Linearity
- Limit of quantification
- Accuracy
- Recovery
- Precision
- Stability

WORKING STANDARD SOLUTION STABILITY

Working standard solutions of Curcumin and IS were prepared on April 11, 2007 and stored at -40°C. On April 26, 2007, pure standards at concentration corresponding to LLOQ, MQC and HQC were prepared and injected into the system.

The results of this study indicate that:
- Spectrally no acceptable interference was present in blank plasma samples: the calibration curves resulted to be linear in the range 5.275-527.5 ng/mL with a r² of 0.9988.