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**HT Quantification for a Drug Mixture in Rat Plasma: A Comparison of UPLC™/MS/MS and HPLC/MS/MS**

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**Introduction**

The signal intensities were unaffected by the change of the dwell time in HPLC/MS/MS, whereas in UPLC/MS/MS a small decrease was observed.

**Methodology**

- **UPLC™/MS/MS**:
  - System parameters: 0.7 µm column, 50°C column temperature, 240 nm detection, 10000 µl sample volume.
  - Precursor ions of diphenhydramine, prednisolone and alprazolam were 256.3, 229.3 and 289.8, respectively.
  - Neutral loss of 307.2, 161.1 and 322.3, respectively.
  - Reaction type: External Std., Area.
  - Response type: Linear.
  - Calibration curves for both UPLC/MS/MS and HPLC/MS/MS were linear (R² > 0.996).

**Experimental Conditions**

- **UPLC Conditions**:
  - Waters X-Select C18 column (100 x 2.1 mm, 1.7 µm).
  - Mobile Phase: A: 0.1% formic acid, B: acetonitrile.
  - Flow Rate: 0.6 ml/min.
  - Source Temperature: 130°C.

- **HPLC Conditions**:
  - Waters Nova-Pak C18 column (150 x 4.6 mm, 3.5 µm).
  - Mobile Phase: A: 0.1% formic acid, B: acetonitrile.
  - Flow Rate: 0.6 ml/min.
  - Source Temperature: 130°C.

**Overview**

- LC/MS/MS for Drug Analysis
- Comparison of UPLC/MS/MS vs. HPLC/MS/MS
- Quantification performed in crashed rat Plasma
- Complete method evaluation for UPLC/MS/MS
- System calibration and carryover in crashed rat plasma

**Results**

**UPLC Calibration Curves**

3 injections per Concentration

**HPLC Calibration Curves**

3 injections per Concentration

**Sensitivity**

- UPLC/MS/MS: Picograms per liter
- HPLC/MS/MS: Nanograms per liter

**The Comparative Advantages of UPLC™/MS/MS**

**Higher Speed**

- Cycle time of an MS detection is the summation of the dwell times for all ions detected.
  - For the whole experiment, the above sequence was run 3 times. 5 minutes in total.
  - Consistent peak shape and peak area indicating the stability of the system.

**Model Analysis**

**Quantification Results**

**UPLC/MS/MS**

- For the UPLC/MS/MS in the next time period.
  - Response time: 2.06 s, 21 points
  - RSD%: 1.39, 0.998
  - Bias%: 0.109, 0.998
  - LOD: 0.00136 ng/mL
  - R² = 0.996
  - Points Across Peak = 60
  - Peak Area = 16262

**HPLC/MS/MS**

- For the HPLC/MS/MS for the next time period.
  - Response time: 2.4s, 20 points
  - RSD%: 2.54, 0.996
  - Bias%: 4.71, 0.996
  - LOD: 0.00136 ng/mL
  - R² = 0.996
  - Points Across Peak = 60
  - Peak Area = 5249.22

**Conclusion**

- The shorter the cycle time, the more challenging to the MS in terms of stability, backpressure and system performance.

- The carryover for UPLC/MS/MS was studied.
  - For 300 injections of QC sample in crashed rat plasma over 24 hours, the chromatograms with red labels were the LC results for ibuprofen and naproxen.

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