The feasibility of the simultaneous quantitative analysis of ibuprofen and indomethacin extracted from serum was studied by reversed phase HPLC on Discovery C18 columns. The results showed a wide linear range, good resolution, excellent column stability and reproducibility for columns from different batches.

Key Words:
- ibuprofen
- indomethacin
- reversed phase HPLC
- Discovery C18 column

Ibuprofen is a common nonsteroidal anti-inflammatory drug; indomethacin is a drug commonly used to treat newborns who have patent ductus arteriosus. (Chemical structures are shown in Figure A.) Measuring serum concentrations is useful in assessing compliance, detecting possible abuse, and monitoring suspected toxicity (1,2).

We developed a method to quantitate ibuprofen and indomethacin extracted from serum using Discovery® C18 reversed phase HPLC columns (5cm x 4.6mm ID, 5µm particles). Wavelengths of 220nm and 254nm were selected to monitor the analytes simultaneously.

Proteins are precipitated from serum with acetonitrile. Following centrifugation, the supernatant is analyzed by HPLC. In our initial isocratic analyses, we used a mobile phase consisting of a 50:50 mix of 50mM phosphate buffer (pH 4):acetonitrile at a flow rate of 1mL/min. The internal standard used in this analysis was chlorimipramine.

When we used a mobile phase mix of 60:40 phosphate buffer (pH 4.0):acetonitrile, and a flow rate of 2mL/min, the internal standard peak was well removed from the serum matrix peak (Figure B). The peaks were detected at UV 254nm.

The linearity range for ibuprofen in serum is 0-2000µg/mL; for indomethacin in serum, the linearity range is 0.1-10µg/mL. We repeated studies on three Discovery C18 columns from different batches. The correlation coefficient (R²) of the data was 0.999. The linearity curves shown in Figure C were obtained based on the following procedure:

1. Using the internal standard quantitation method, we plotted the ratios of the standard peak height to the internal standard peak height versus the concentrations of standard solutions. The best-fit linear curve was generated.
2. Using the peak height ratio and calibration curve, the actual concentrations for each prepared solution were calculated.
3. By plotting each actual concentration versus its target concentration, a linearity curve was produced.

Figure B. Ibuprofen and Indomethacin Extracted from Bovine Serum

- Column: Discovery C18, 5cm x 4.6mm ID, 5µm particles
- Cat. No.: 504947
- Mobile Phase: 50mM phosphate buffer, pH 4.0:acetonitrile, 60:40
- Flow Rate: 2mL/min
- Temp.: 35°C
- Det.: UV, 254nm
- Inj.: 15µL

Figure A. Ibuprofen and Indomethacin

![Chemical structures of Ibuprofen and Indomethacin](image)
We examined three different bonding lots on two different batches of silica. A comparison of the retention time among the columns revealed that the coefficient of variation was between 1.34% and 2.6% for each compound (Table 1). This indicates good robustness of the method using the Discovery columns. Excellent retention time reproducibility was observed.

We continue to evaluate other robustness characteristics of this method, while method ruggedness testing is being conducted in an independent laboratory.

Discovery C18 columns are excellent for the quantitative analysis of ibuprofen and indomethacin in serum. These columns yield excellent resolution and retention time reproducibility.

References

Ordering Information:

Table 1. Inter-Column Comparison of Retention Time Variations (Columns from Different Batches)

<table>
<thead>
<tr>
<th>Column Batch No.</th>
<th>Retention of Indomethacin</th>
<th>Retention of Ibuprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1 (3142, PS73)</td>
<td>1.21</td>
<td>2.2198</td>
</tr>
<tr>
<td>Column 2 (3167, PS77)</td>
<td>1.228</td>
<td>2.266</td>
</tr>
<tr>
<td>Column 3 (3172, PS77)</td>
<td>1.243</td>
<td>2.314</td>
</tr>
<tr>
<td>Average</td>
<td>1.227</td>
<td>2.259</td>
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<tr>
<td>STD</td>
<td>0.016</td>
<td>0.058</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.34</td>
<td>2.57</td>
</tr>
</tbody>
</table>

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