Simultaneous determination of alendronate, pamidronate, ibandronate and risedronate using ion chromatography with integrated pulsed amperometric detection

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Abstract A method for the simultaneous determination of alendronate, pamidronate, ibandronate and risedronate using ion chromatography with integrated pulsed amperometric detection (IPAD) has been developed. The electrochemical behavior showed the catalytic currents of these bisphosphonates are based on the oxidation of amines in their structures. Because the bisphosphonates are polar compounds and present as anions under alkaline condition, therefore, they can be separated by anion exchange chromatography. A Dionex AS18 column*250 mm x 2 mm* and an AG18 column*50 mm x 2 mm* and 24 mmol/L NaOH solution were used for the separation. Multi-step potential waveform parameters were optimized to maximize the signal-to-noise ratio S/N which exhibited adsorption/desorption catalytic features at the gold electrode surface in alkaline solution. Utilizing the optimized waveform method showed good linearity \( r^2 = 0.997 \) with satisfactory repeatability relative standard deviations RSDs of the peak areas in the range of 0.84% – 1.37% and sufficient sensitivity limits of detection of 0.061 – 0.18 \( \mu g/mL \) for the identification of the four bisphosphonates. The recoveries were 80.81% – 97.32% with the RSDs of 1.46% – 3.02%. It is demonstrated that this method is a rapid and simple one for the determination of the four bisphosphonates in human plasma.
Key words: ion chromatography, IC, integrated pulsed amperometric detection, IPAD, alendronate, pamidronate, ibandronate, risedronate, plasma

Fig. 1 Chemical structures of bisphosphonates

1 1.1

DX-600 GP50 LC25
ED50A Dionex Tedia
Chrome 6.8 Milli-Q Organomation N-EVAP-12
Dionex OnGuard II RP 2.5 mL Dionex

1.2

Dionex AS18 AG18 50 mm × 2 mm NaOH 24 mmol/L pH-Ag/AgCl
250 mm × 2 mm AG18 50 mm × 2 mm NaOH 24 mmol/L

IC 12 - 14 d 4 - 7 HPLC 12 - 14 d 4 - 7
IPC 12 - 14 d 4 - 7

1 - 5 ng/mL FMOC 4 - 7 IC P - C - P

Fe3 12 - 14 d

AEC
2.1

Table 1  Waveform specifications of IPAD for the determination of alendronate, pamidronate, ibandronate and risendronate

<table>
<thead>
<tr>
<th>Time/s</th>
<th>Potential vs Ag/AgCl/V</th>
<th>Integration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>-0.30</td>
<td></td>
</tr>
<tr>
<td>0.04</td>
<td>-0.30</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>-0.15</td>
<td></td>
</tr>
<tr>
<td>0.21</td>
<td>-0.15</td>
<td>begin</td>
</tr>
<tr>
<td>0.22</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>0.46</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>0.47</td>
<td>-0.15</td>
<td></td>
</tr>
<tr>
<td>0.56</td>
<td>-0.15</td>
<td>end</td>
</tr>
<tr>
<td>0.57</td>
<td>-2.00</td>
<td></td>
</tr>
<tr>
<td>0.58</td>
<td>-2.00</td>
<td></td>
</tr>
<tr>
<td>0.59</td>
<td>0.60</td>
<td></td>
</tr>
</tbody>
</table>

2.2 IPAD

Johnson  La-
Course 15, 16

Au-OH  4

2.3

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Calibration curve</th>
<th>Linear range/ $\mu g/mL$</th>
<th>$r^2$</th>
<th>LOQ/ $ng/mL$</th>
<th>LOD/ $ng/mL$</th>
<th>Intra-day RSD/%</th>
<th>Inter-day RSD/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pamidronate</td>
<td>$Y = 0.9919X + 0.0028$</td>
<td>0.01 - 0.80</td>
<td>0.9989</td>
<td>5.48</td>
<td>1.66</td>
<td>1.03</td>
<td>3.34</td>
</tr>
<tr>
<td>Alendronate</td>
<td>$Y = 0.9928X + 0.0028$</td>
<td>0.01 - 0.80</td>
<td>0.9995</td>
<td>10.01</td>
<td>3.02</td>
<td>0.84</td>
<td>1.87</td>
</tr>
<tr>
<td>Ibandronate</td>
<td>$Y = 0.9967X + 0.0014$</td>
<td>0.02 - 1.00</td>
<td>0.9972</td>
<td>8.12</td>
<td>2.46</td>
<td>1.37</td>
<td>3.65</td>
</tr>
<tr>
<td>Risedronate</td>
<td>$Y = 0.9717X + 0.0099$</td>
<td>0.02 - 1.00</td>
<td>0.9991</td>
<td>17.21</td>
<td>5.21</td>
<td>1.07</td>
<td>2.94</td>
</tr>
</tbody>
</table>

$Y$ peak area; $X$ mass concentration $\mu g/mL$.

![Graphs showing calibration curves and chromatograms](image)

**Fig. 4** Typical AEC-IPAD chromatogram of a mixed standard solution of 0.1 $\mu g/mL$.


Peaks [1]: pamidronate [2]: alendronate [3]: ibandronate [4]: risedronate.

![Graphs showing effects of mass concentrations of the four bisphosphonates on the signal-to-noise ratio](image)

**Fig. 3** Effects of $E_1$ [a] $E_2$ [b] $E_3$ [c] and $E_4$ [d] on the signal-to-noise ratio.

**Notes:** Mass concentrations of the four bisphosphonates 0.2 $\mu g/mL$.

**Fig. 5** Typical AEC-IPAD chromatogram of human plasma spiked with 4 bisphosphonates at 0.5 $\mu g/mL$.

For peak identifications see Fig. 4.
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Table 3  Recoveries of pamidronate, alendronate, ibandronate, and risedronate spiked in a plasma sample \( n = 5 \)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Added/Found /( \mu g/mL )</th>
<th>Recovery %</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pamidronate</td>
<td>0.50 / 0.46</td>
<td>92.01</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>0.1 / 0.091</td>
<td>91.02</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>0.05 / 0.043</td>
<td>86.03</td>
<td>1.83</td>
</tr>
<tr>
<td>Alendronate</td>
<td>0.50 / 0.45</td>
<td>90.28</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>0.1 / 0.088</td>
<td>88.04</td>
<td>2.01</td>
</tr>
<tr>
<td></td>
<td>0.05 / 0.041</td>
<td>82.07</td>
<td>2.40</td>
</tr>
<tr>
<td>Ibandronate</td>
<td>0.50 / 0.48</td>
<td>97.32</td>
<td>1.54</td>
</tr>
<tr>
<td></td>
<td>0.1 / 0.095</td>
<td>95.32</td>
<td>1.94</td>
</tr>
<tr>
<td></td>
<td>0.05 / 0.042</td>
<td>84.54</td>
<td>2.24</td>
</tr>
<tr>
<td>Risedronate</td>
<td>0.50 / 0.44</td>
<td>88.37</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td>0.1 / 0.083</td>
<td>83.26</td>
<td>2.57</td>
</tr>
<tr>
<td></td>
<td>0.05 / 0.040</td>
<td>80.81</td>
<td>3.02</td>
</tr>
</tbody>
</table>

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AEC-IPAD

1. Coleman R E. Oncologist 2004; 4 14