Qualitative and quantitative assessment of marketed erythropoiesis-stimulating agents by capillary electrophoresis

Sylvie Boucher, Anita Kane and Michel Girard

Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada, 251 - Sir Frederick G. Banting Driveway, Tunney’s Pasture, Ottawa (ON) K1A 0K9 CANADA.

Regulatory Needs

• Quality assessment of protein-based therapeutics
• Subsequent entry biologics
• Alternative to animal testing
• Standardization / Harmonization
Research Objectives

• Investigation of drug product

• Minimizing product handling

• Precision / Accuracy / Quantitation
Research Approach

Drug product assessment

Generally inadequate information on:

• structural integrity of API
• validation of information for length of time out of storage of final container
• Stability
Challenge in assessing structural integrity and assaying
Erythropoiesis Stimulating Agents (ESA)

**Erythropoietin (EPO)**
- 165 amino acids
- 2 disulfide bridges
- 3 N-glycosylation sites
- 1 O-glycosylation site
- Terminal sialic acid residues
- EPO-α and EPO-β differ in glycoform levels

**Darbepoetin-α (DPO)**
- Polypeptide chain analog to EPO
- 2 disulfide bridges
- 5 N-glycosylation sites
- 1 O-glycosylation site
- Increased terminal sialic acid residues: more acidic than EPO
Study Objectives

• Qualitative assessment:
  – Integrity of active ingredient
  – Glycoform patterns
  – Potential impurities

• Quantitative assessment:
  – Content of active ingredient
  – Major glycoforms distribution
Excipients in ESAs

- Polysorbate 20
- Polysorbate 80
- Human serum albumin
- Salts: sodium chloride, sodium phosphate, calcium chloride, sodium citrate
- Amino acids: glycine, leucine, threonine, glutamic acid, phenylalanine, isoleucine
- Other: urea, benzyl alcohol

Range of total amount of excipients: 10 - 35 mg/mL
Choice of Methodology

1. Ph.Eur. method for EPO glycoforms: requires removal of excipients
Choice of Methodology

2. In-house method: enables analysis of HSA-formulated drug product

# Methodology Comparison

## A. EP Method

<table>
<thead>
<tr>
<th>Pk #</th>
<th>In-House Method % Corrected Peak Area</th>
<th>EP Method % Corrected Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.36</td>
<td>1.76</td>
</tr>
<tr>
<td>2</td>
<td>16.95</td>
<td>18.57</td>
</tr>
<tr>
<td>3</td>
<td>29.23</td>
<td>30.18</td>
</tr>
<tr>
<td>4</td>
<td>27.61</td>
<td>27.6</td>
</tr>
<tr>
<td>5</td>
<td>17.13</td>
<td>16.06</td>
</tr>
<tr>
<td>6</td>
<td>5.49</td>
<td>4.55</td>
</tr>
<tr>
<td>7</td>
<td>2.23 (7+8)</td>
<td>1.28</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>Totals</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

## B. Bietlot-Girard Method
EPO-α vs EPO-β with in-house methodology
# ESAs

<table>
<thead>
<tr>
<th>Product</th>
<th>Dosage strength (per vial)</th>
<th>Lots</th>
<th>Excipient</th>
<th>Active Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>3,000 IU</td>
<td>A1 A2 A3</td>
<td>Human serum albumin</td>
<td>EPO-α</td>
</tr>
<tr>
<td></td>
<td>10,000 IU</td>
<td>A4 A5 A6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20,000 IU</td>
<td>A7 A8 A9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>2,000 IU</td>
<td>B1 B2 B3</td>
<td>Polysorbate 80</td>
<td>EPO-α</td>
</tr>
<tr>
<td></td>
<td>4,000 IU</td>
<td>B4 B5 B6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10,000 IU</td>
<td>B7 B8 B9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40,000 IU</td>
<td>B10 B11 B12</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>10,000 IU</td>
<td>C1 C2</td>
<td>Polysorbate 20</td>
<td>EPO-β</td>
</tr>
<tr>
<td></td>
<td>20,000 IU</td>
<td>C3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100,000 IU</td>
<td>C4 C5 C6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Da</strong></td>
<td>0.04 mg/mL</td>
<td>Da1 Da2 Da3</td>
<td>Human serum albumin</td>
<td>DPO-α</td>
</tr>
<tr>
<td></td>
<td>0.20 mg/mL</td>
<td>Da4 Da5 Da6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.50 mg/mL</td>
<td>Da7 Da8 Da9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Db</strong></td>
<td>0.04 mg/mL</td>
<td>Db10 Db11 Db12</td>
<td>Polysorbate 80</td>
<td>DPO-α</td>
</tr>
<tr>
<td></td>
<td>0.20 mg/mL</td>
<td>Db13 Db14 Db15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.50 mg/mL</td>
<td>Db16 Db17 Db18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Product A

A4

I1

I2

I3

I4

I5

I6

DS A

0.0020

0.0015

0.0010

0.0005

0.0000

0.0005

0.0010

0.0015

0.0020

AU

-0.01

0.00

0.01

0.02

0.03

0.04

0.05

AU

-0.01

0.00

0.01

0.02

0.03

0.04

0.05

A

HSA

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Health Canada    Santé Canada
Product B
Product C
### Qualification Parameters

<table>
<thead>
<tr>
<th>Drug Substance</th>
<th>Repeatability</th>
<th>Intermediate Precision</th>
<th>Limit of detection(^d)</th>
<th>Limit of quantification(^d)</th>
<th>Linearity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MT(^a) (Avg %RSD(^b))</td>
<td>CPA(^a) (Avg %RSD(^b))</td>
<td>MT (Avg %RSD(^c))</td>
<td>CPA (Avg %RSD(^c))</td>
<td>R(^2)</td>
</tr>
<tr>
<td>A</td>
<td>0.11</td>
<td>5.54</td>
<td>1.67</td>
<td>10.64</td>
<td>2000 IU/mL</td>
</tr>
<tr>
<td>B</td>
<td>0.62</td>
<td>4.21</td>
<td>4.62</td>
<td>10.36</td>
<td>2000 IU/mL</td>
</tr>
<tr>
<td>C</td>
<td>0.18</td>
<td>6.47</td>
<td>1.05</td>
<td>4.26</td>
<td>2500 IU/mL</td>
</tr>
<tr>
<td>D</td>
<td>0.17</td>
<td>6.53</td>
<td>1.82</td>
<td>4.56</td>
<td>0.010 mg/mL</td>
</tr>
</tbody>
</table>

- MT = Migration time; CPA = corrected peak area.
- Avg %RSD = average %RSD from replicate injections (n=5) of standards at three concentrations in the same sequence (see text).
- Avg %RSD = average %RSD from replicate injections (n=5) of standards at three concentrations in different sequences (see text).
- Limit of detection was set for the lowest concentration giving a S/N ≥ 3 (n=5); limit of quantification was for the lowest concentration giving a RSD < 10 % (n=5).
Matrix Effect on Peak Area

EPO-α + HSA
Average Corrected Peak Area: 52041 (±3.9%)

EPO-α
Average Corrected Peak Area: 55610 (±4.3%)

Variation: - 6.9%

No significant matrix effect
In-House Method Pros and Cons

• Pros:
  – No pre-analysis workup
  – Works for all EPO products tested
  – All formulation types tested
  – Most dosage strengths
  – Quantitative

• Cons:
  – Specific capillary and regeneration solution – high cost
  – Analysis time relatively long
  – Quantitative assessment of small glycoforms difficult
Product Evaluation:

- Drug products and drug substances obtained from Europe and North America
- Drug substance lots not necessarily those used for drug products
Analysis Design

Product

Dosage Strength

Lot

Vial

Injection
2-Point Calibration Curves for DS A from several days

Drug Substance A

Sample concentration (IU/ml)

Area (200nm)

\[ y = 9.529x - 6475.993 \]
\[ R^2 = 1.000 \]

\[ y = 9.8535x - 7451.5 \]
\[ y = 12.918x - 18133 \]

\[ y = 9.6465x - 7451.5 \]
\[ y = 10.625x - 12073 \]
\[ y = 9.6465x - 7451.5 \]
\[ y = 12.918x - 18133 \]
Content in Vials from Lots of Same Dosage Strength – Product A

**Product A (3000 IU)**

- Lot 1: Avg = 2927 (± 2.6%)
- Lot 2: Avg = 2756 (± 5.8%)
- Lot 3: Avg = 2959 (± 6.7%)

**Product A (10000 IU)**

- Lot 1: Avg = 9553 (± 7.3%)
- Lot 2: Avg = 9459 (± 3.1%)
- Lot 3: Avg = 9754 (± 5.5%)

**Product A (20000 IU)**

- Lot 1: Avg = 22052 (± 5.0%)
- Lot 2: Avg = 22223 (± 4.7%)
- Lot 3: Avg = 18319 (± 4.1%)
Average Content in Lots of Same Dosage Strength – Product A

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Average Content</th>
<th>Percentage Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000 IU</td>
<td>2875 IU</td>
<td>±3.6%</td>
</tr>
<tr>
<td>10000 IU</td>
<td>9293 IU</td>
<td>±1.6%</td>
</tr>
<tr>
<td>20000 IU</td>
<td>20221 IU</td>
<td>±10.6%</td>
</tr>
</tbody>
</table>
Content of Products A-D

- **Product A**
  - Avg = 2875 (±3.6%)
  - Avg = 9293 (±1.6%)
  - Avg = 20221 (±10.6%)

- **Product B**
  - Avg = 36920 (±9.0%)
  - Avg = 8346 (±8.1%)
  - Avg = 0.587 (±7.1%)

- **Product C**
  - Avg = 0.049 (±8.9%)
  - Avg = 0.222 (±10.5%)
  - Avg = 0.048 (±8.5%)

- **Product D**
  - Avg = 0.531 (±1.4%)
  - Avg = 0.215 (±8.5%)
  - Avg = 0.215 (±8.5%)

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*Health Canada    Santé Canada*
Isoform Distribution – Product A

A. Isoform Distribution
Drug Substance A

B. Isoform Distribution
Product A4, Vial 1

C. Isoform Distribution
Product A4, Vials 1-3

D. Isoform Distribution
Product A, lots A4-A6
Isoform Distribution – Product B

A  Isoform Distribution
Drug Substance B

B  Isoform Distribution
Product B7, Vial 1

C  Isoform Distribution
Product B8, Vials 1-3

D  Isoform Distribution
Product B, lots B7- B9

Reps

Vials

Lots
Isoform Distribution – Product C

A

Isoform Distribution
Drug Substance C

% Peak Area

Reps

B

Isoform Distribution
Product C1, Vial 1

% Peak Area

Reps

C

Isoform Distribution
Product C, Vials 1-3

% Peak Area

Vials

D

Isoform Distribution
Product C, Lots C1-C3

% Peak Area

Lots
Isoform Distribution – Product Db

A. Isoform Distribution
   Drug Substance D
   Reps

B. Isoform Distribution
   Product Db13, Vial 1
   Reps

C. Isoform Distribution
   Product Db13, Vials 1-3
   Vials

D. Isoform Distribution
   Product Db13-15
   Lots
Conclusion

• CE gave satisfactory results for the qualitative and quantitative assessment of finished products without pre-fractionation.
• Method worked for all products and most dosage forms currently on the market.
• Method may be useful for lot release, specification setting or post-marketing evaluation.
• Results showed that products evaluated met specifications and were produced in a consistent manner.
Acknowledgments

We would like to acknowledge the following manufacturers for their generosity in providing samples:

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