**STATE OF THE ART OF UNBOUND CEFTRIAXONE AS A PHARMACODYNAMIC TOOL: ARE WE READY FOR ITS IMPLEMENTATION IN CLINICAL PRACTICE?**

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**Running head:** Unbound ceftriaxone in clinical practice

**SUPPLEMENTARY MATERIALS**

1. **Supplementary Material 1: Total (CEFt) and unbound (CEFu) quantitation**
2. **Supplementary Table S1 : Comparison of between-model absolute and percentage error using ANOVA followed by Tukey Honest Significant Differences tests**
3. **Supplementary Figure S1: Bland-Altman plots for signed differences and relative differences for the 9 models**

**Total (CEFt) and unbound (CEFu) quantitation**

Briefly, 50 µL of serum (CEFt) or filtrate (CEFu) was mixed with 100 µL of methanol spiked with internal standard [13C,2H3]-Ceftriaxone (5 µg/mL). After centrifugation at 13 200 x g for 5 minutes, 100 µL of supernatant were collected for analysis. The chromatographic system consisted in a Prominence Shimadzu UFLC system (Shimadzu®, Prominence, Kyoto, Japan) in combination with a 4500QTRAP tandem mass spectrometer equipped with an electrospray ionization source operating in positive ion mode (Sciex®, Toronto, Canada). Quantitation was performed using the following precursor ion to product ion transitions: ceftriaxone m/z 554.8→395.8 / 554.8→323.8 (quantitation/qualification) and [13C,2H3]-Ceftriaxone m/z 558.8→399.9. Chromatographic separation was performed on an Alltima HP C18 HL analytical column (3 μm particle size, 150 mm length × 3 mm inner diameter; VWR international®). The auto-sampler temperature was set at 8°C, the column oven at 30°C, the injected volume was 1 μL. The mobile phase consisted in 0.2% formic acid in pure Methanol (solvent A) and 2 mM ammonium formate with 0.2% formic acid in water (solvent B). The flow rate was 600 µL/min and the following elution gradient was performed: 70% B (0–0.1 min), 70–0% B (0.1–1.5 min), 0% B (1.5–3.5 min), 0-70% B (3.5–3.6 min), and 70% B (3.6–5.5 min). Calibration curves ranged from 1 to 20 µg/mL for serum ceftriaxone quantitation. Samples above the upper limit of quantification were 20-fold diluted in ceftriaxone-free serum. Chromatographic data acquisition and processing were performed using the Analyst v1.6.3 software (AB Sciex®). The analytical procedure used in this study met international analytical requirements according to ISO 15189, EMA and FDA guidelines on bioanalytical method validation [1,2]. The lower limit of quantification of the method was 1 µg/mL, the accuracy ranged from 85 to 115% and the intraday and interday precision were lower than 15%.

[1] EMA Guideline on Bioanalytical Method Validation. 2011.

[2] FDA Guidance for Industry Bioanalytical Method Validation. 2001.

**Supplementary Table S1 : Comparison of between-model absolute and percentage error using ANOVA followed by Tukey Honest Significant Differences tests**

| Difference | Lower CI | Upper CI | Adjusted P-value | Error Type | Comparison |
| --- | --- | --- | --- | --- | --- |
| **13.69** | **5.24** | **22.15** | **2.19e-05** | **Signed Error** | **Dreesen-Bos** |
| **15.80** | **7.35** | **24.26** | **3.56e-07** | **Signed Error** | **Gijsen-Bos** |
| **24.95** | **16.50** | **33.41** | **4.10e-10** | **Signed Error** | **Gregoire-Bos** |
| **18.45** | **10.00** | **26.91** | **1.41e-09** | **Signed Error** | **Hartmann-Bos** |
| **21.53** | **13.08** | **29.98** | **4.11e-10** | **Signed Error** | **Heffernan-Bos** |
| **9.56** | **1.10** | **18.01** | **1.37e-02** | **Signed Error** | **Leegwater-Bos** |
| **-10.46** | **-18.92** | **-2.01** | **4.09e-03** | **Signed Error** | **Standing-Bos** |
| 2.39 | -6.07 | 10.84 | 9.94e-01 | Signed Error | Ulldemolins-Bos |
| 2.11 | -6.34 | 10.56 | 9.97e-01 | Signed Error | Gijsen-Dreesen |
| **11.26** | **2.81** | **19.72** | **1.28e-03** | **Signed Error** | **Gregoire-Dreesen** |
| 4.76 | -3.69 | 13.22 | 7.12e-01 | Signed Error | Hartmann-Dreesen |
| 7.84 | -0.62 | 16.29 | 9.40e-02 | Signed Error | Heffernan-Dreesen |
| -4.13 | -12.59 | 4.32 | 8.45e-01 | Signed Error | Leegwater-Dreesen |
| **-24.16** | **-32.61** | **-15.70** | **4.10e-10** | **Signed Error** | **Standing-Dreesen** |
| **-11.30** | **-19.76** | **-2.85** | **1.20e-03** | **Signed Error** | **Ulldemolins-Dreesen** |
| **9.15** | **0.70** | **17.60** | **2.26e-02** | **Signed Error** | **Gregoire-Gijsen** |
| 2.65 | -5.80 | 11.10 | 9.88e-01 | Signed Error | Hartmann-Gijsen |
| 5.73 | -2.73 | 14.18 | 4.68e-01 | Signed Error | Heffernan-Gijsen |
| -6.24 | -14.70 | 2.21 | 3.44e-01 | Signed Error | Leegwater-Gijsen |
| **-26.27** | **-34.72** | **-17.81** | **4.10e-10** | **Signed Error** | **Standing-Gijsen** |
| **-13.41** | **-21.87** | **-4.96** | **3.61e-05** | **Signed Error** | **Ulldemolins-Gijsen** |
| -6.50 | -14.95 | 1.95 | 2.89e-01 | Signed Error | Hartmann-Gregoire |
| -3.42 | -11.88 | 5.03 | 9.42e-01 | Signed Error | Heffernan-Gregoire |
| **-15.40** | **-23.85** | **-6.94** | **8.20e-07** | **Signed Error** | **Leegwater-Gregoire** |
| **-35.42** | **-43.87** | **-26.96** | **4.10e-10** | **Signed Error** | **Standing-Gregoire** |
| **-22.57** | **-31.02** | **-14.11** | **4.10e-10** | **Signed Error** | **Ulldemolins-Gregoire** |
| 3.08 | -5.38 | 11.53 | 9.69e-01 | Signed Error | Heffernan-Hartmann |
| **-8.90** | **-17.35** | **-0.44** | **3.05e-02** | **Signed Error** | **Leegwater-Hartmann** |
| **-28.92** | **-37.37** | **-20.46** | **4.10e-10** | **Signed Error** | **Standing-Hartmann** |
| **-16.07** | **-24.52** | **-7.61** | **2.06e-07** | **Signed Error** | **Ulldemolins-Hartmann** |
| **-11.97** | **-20.43** | **-3.52** | **4.22e-04** | **Signed Error** | **Leegwater-Heffernan** |
| **-31.99** | **-40.45** | **-23.54** | **4.10e-10** | **Signed Error** | **Standing-Heffernan** |
| **-19.14** | **-27.60** | **-10.69** | **6.03e-10** | **Signed Error** | **Ulldemolins-Heffernan** |
| **-20.02** | **-28.48** | **-11.57** | **4.32e-10** | **Signed Error** | **Standing-Leegwater** |
| -7.17 | -15.62 | 1.28 | 1.72e-01 | Signed Error | Ulldemolins-Leegwater |
| **12.85** | **4.40** | **21.31** | **9.71e-05** | **Signed Error** | **Ulldemolins-Standing** |
| **54.42** | **2.34** | **106.50** | **3.28e-02** | **Percentage Error** | **Dreesen-Bos** |
| **72.70** | **20.62** | **124.78** | **5.54e-04** | **Percentage Error** | **Gijsen-Bos** |
| **142.10** | **90.02** | **194.18** | **4.10e-10** | **Percentage Error** | **Gregoire-Bos** |
| **88.89** | **36.81** | **140.97** | **5.50e-06** | **Percentage Error** | **Hartmann-Bos** |
| **125.36** | **73.28** | **177.45** | **4.20e-10** | **Percentage Error** | **Heffernan-Bos** |
| 51.61 | -0.47 | 103.69 | 5.43e-02 | Percentage Error | Leegwater-Bos |
| **-144.27** | **-196.35** | **-92.19** | **4.10e-10** | **Percentage Error** | **Standing-Bos** |
| **-95.14** | **-147.22** | **-43.06** | **7.43e-07** | **Percentage Error** | **Ulldemolins-Bos** |
| 18.29 | -33.79 | 70.37 | 9.75e-01 | Percentage Error | Gijsen-Dreesen |
| **87.68** | **35.60** | **139.77** | **7.99e-06** | **Percentage Error** | **Gregoire-Dreesen** |
| 34.47 | -17.61 | 86.56 | 5.01e-01 | Percentage Error | Hartmann-Dreesen |
| **70.95** | **18.87** | **123.03** | **8.66e-04** | **Percentage Error** | **Heffernan-Dreesen** |
| -2.81 | -54.89 | 49.27 | 1.00e+00 | Percentage Error | Leegwater-Dreesen |
| **-198.69** | **-250.77** | **-146.61** | **4.10e-10** | **Percentage Error** | **Standing-Dreesen** |
| **-149.56** | **-201.64** | **-97.48** | **4.10e-10** | **Percentage Error** | **Ulldemolins-Dreesen** |
| **69.40** | **17.32** | **121.48** | **1.27e-03** | **Percentage Error** | **Gregoire-Gijsen** |
| 16.19 | -35.89 | 68.27 | 9.89e-01 | Percentage Error | Hartmann-Gijsen |
| **52.66** | **0.58** | **104.74** | **4.51e-02** | **Percentage Error** | **Heffernan-Gijsen** |
| -21.10 | -73.18 | 30.98 | 9.42e-01 | Percentage Error | Leegwater-Gijsen |
| **-216.97** | **-269.06** | **-164.89** | **4.10e-10** | **Percentage Error** | **Standing-Gijsen** |
| **-167.85** | **-219.93** | **-115.77** | **4.10e-10** | **Percentage Error** | **Ulldemolins-Gijsen** |
| **-53.21** | **-105.29** | **-1.13** | **4.09e-02** | **Percentage Error** | **Hartmann-Gregoire** |
| -16.74 | -68.82 | 35.34 | 9.86e-01 | Percentage Error | Heffernan-Gregoire |
| **-90.50** | **-142.58** | **-38.41** | **3.33e-06** | **Percentage Error** | **Leegwater-Gregoire** |
| **-286.37** | **-338.45** | **-234.29** | **4.10e-10** | **Percentage Error** | **Standing-Gregoire** |
| **-237.25** | **-289.33** | **-185.16** | **4.10e-10** | **Percentage Error** | **Ulldemolins-Gregoire** |
| 36.47 | -15.61 | 88.55 | 4.20e-01 | Percentage Error | Heffernan-Hartmann |
| -37.29 | -89.37 | 14.80 | 3.88e-01 | Percentage Error | Leegwater-Hartmann |
| **-233.16** | **-285.24** | **-181.08** | **4.10e-10** | **Percentage Error** | **Standing-Hartmann** |
| **-184.04** | **-236.12** | **-131.95** | **4.10e-10** | **Percentage Error** | **Ulldemolins-Hartmann** |
| **-73.76** | **-125.84** | **-21.68** | **4.21e-04** | **Percentage Error** | **Leegwater-Heffernan** |
| **-269.64** | **-321.72** | **-217.55** | **4.10e-10** | **Percentage Error** | **Standing-Heffernan** |
| **-220.51** | **-272.59** | **-168.43** | **4.10e-10** | **Percentage Error** | **Ulldemolins-Heffernan** |
| **-195.88** | **-247.96** | **-143.80** | **4.10e-10** | **Percentage Error** | **Standing-Leegwater** |
| **-146.75** | **-198.83** | **-94.67** | **4.10e-10** | **Percentage Error** | **Ulldemolins-Leegwater** |
| 49.13 | -2.95 | 101.21 | 8.22e-02 | Percentage Error | Ulldemolins-Standing |

**Supplementary Figure S1: Bland-Altman plots for signed differences and relative differences for the 9 models**

