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

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SUPPLEMENTARY MATERIALS

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Supplementary Material S1: Total (CEF_{tot}) and unbound (CEF_u) quantitation

Briefly, 50 µL of serum (CEF_{tot}) or filtrate (CEF_u) was mixed with 100 µL of methanol spiked with internal standard [^{13}C,$^{2}H_3$]-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 [^{13}C,$^{2}H_3$]-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**

Difference	Lower CI	Upper CI	Adjusted P-value	Error Type	Comparison
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**

Difference	Lower CI	Upper CI	Adjusted P-value	Error Type	Comparison
-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**

Difference	Lower CI	Upper CI	Adjusted P-value	Error Type	Comparison
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**

Difference	Lower CI	Upper CI	Adjusted P-value	Error Type	Comparison
-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