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# * OMICS International

OMICS International is a pioneer and leading science event organizer, which publishes around 500 open access journals and conducts over 500 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

# THE BIOEQUIVALENCE OF CITICOLINE 500 MG FILM TABLET

**ONURSAL SAGLAM**

**BABE-2015**

**17-08-2015**

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# Title of the study:

Open-label, randomised, single oral dose, two-period, cross-over trial to assess the bioequivalence of **Ronocit 500 mg Film Tablet** in comparison with **Ceraxon 500 mg Tabletten** in healthy subjects under fasting conditions

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# Clinical Design

* **Number of subjects:** Planned: 24 Randomised: 24
* Enrolled: 31 Completed: 22
* Screened only: 7 Analysed: 22+2 (drop-out)
* Dropped out: 2 Pharmacokinetics and ANOVA: 22
* Replaced:0
* **Washout period:** 30 days.
* **Criteria for evaluation:** Plasma concentrations of uridine were used to determine the following pharmacokinetic parameters: $C_{max}$, $AUC_{0-tlast}$, $AUC_{0-\infty}$, $t_{max}$, $t_{1/2}$, MRT, $\lambda_z$.
* **Methodology:**
* This was a single centre, open-label, two treatments, two period, cross-over study.

# Cititicoline

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Animal and clinical studies indicate the potential of citicoline to improve cognitive deficits, stroke rehabilitation, brain and spinal cord injuries, neurological diseases, and eye conditions.

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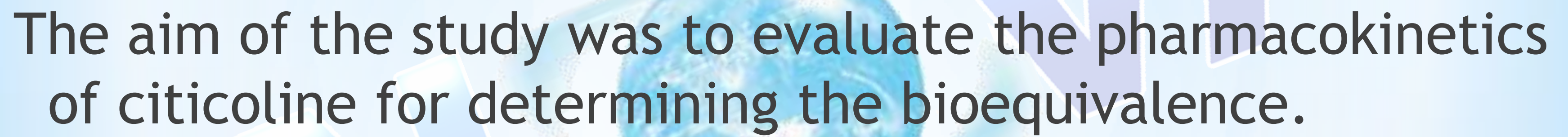

The aim of the study was to evaluate the pharmacokinetics of citicoline for determining the bioequivalence.

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# Bioanalytical Method

* A procedure for the quantitative determination of uridine in $K_2$EDTA human plasma using high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has been developed and validated at Novagenix Bioanalytical Drug R&D Centre, Ankara, Türkiye. The method validated as per EMA Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2**)
* The analysis samples were prepared with protein precipitation by using 0.1 mL of human plasma.
* The method was validated in a range of 150-2000 ng/mL for uridine.
* The lower limit of quantification was 150 ng/mL for uridine in human plasma.

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# EXTRACTION OF PLASMA SAMPLES

* **EXTRACTION OF PLASMA SAMPLES**
* The following protocol applies to validation and in study sample preparation
* Transfer 0.1 mL of blank human plasma to polypropylene tubes (MOC) labelled for zero level standards and matrix blanks
* Transfer 0.1 mL of calibration standard, QC or study samples to appropriate MOC tubes using.
* Add 50 µL of internal standard working solution (5 µg/mL URID2-IS2) to all tubes except matrix blanks.
* Add 50 µL of methanol to matrix blanks.

* Vortex 5 sec. at high speed.
* Add 300 µL of acetonitrile.
* Vortex 30 sec. at high speed.
* Centrifuge the samples at 5500 rpm and 4°C for 10 minutes.
* Take 150 µL upper phase to collection plate.
* Inject 2 µL into LC-MS/MS system.

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# INSTRUMENTATION

* Instrument labelled “LL07-SHILCMS1” system composed of:
* DGU-20AD 3R Degassing unit
* LC-20AD XR Liquid Chromatograph A
* LC-20AD XR Liquid Chromatograph B
* SIL-20AC XR Autosampler
* CTO-10AS VP Column oven
* FCV-20AH2 Valve unit
* Shimadzu 8040 Tandem Mass Spectrometer
* Shimadzu Labsolutions Software version 5.53 SP3

* COLUMN
* Analytical column: Atlantis HILIC Silica 3 µm, 4.6 x 100 mm.

* MOBILE PHASE
* Methanol: 10 mM ammonium formate in water (90:10, v/v)

* MRM of the Ions:

* Uridine Uridine-d2
* 245.10>113.00 246.90>115.00

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# RESULT

* For the assessment of bioequivalence, since the uridine occurs endogenously in the human body, the pharmacokinetic profiles had been baseline adjusted. $t_0$ has been used as baseline adjustment per period. Negative adjusted concentrations obtained in the adjustment process has been set to zero. Then pharmacokinetic variables have been determined.

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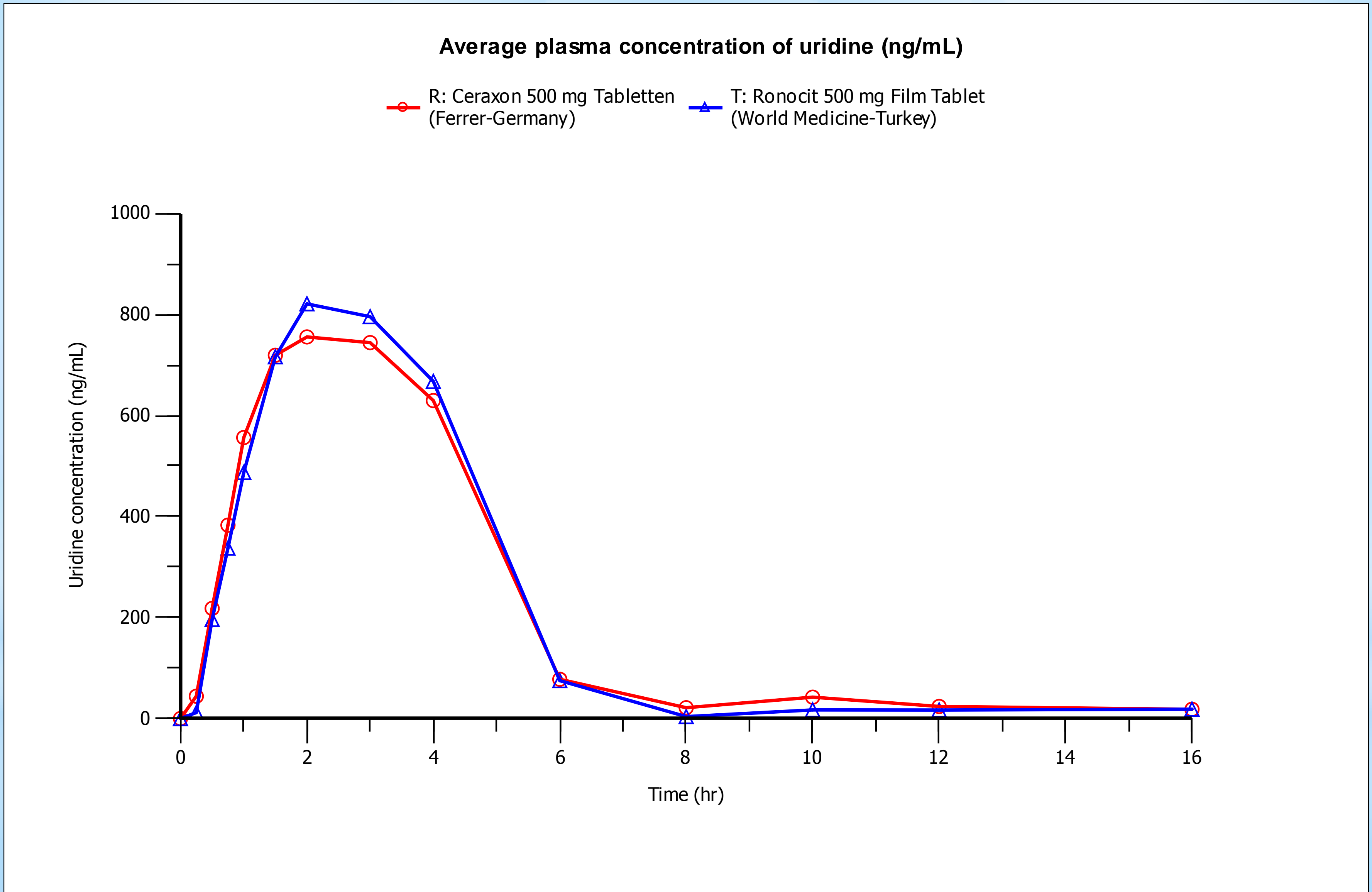
# Average plasma concentration of uridine (Subjects 1 - 22)



# Average cumulative plasma concentration of uridine (Subjects 1 - 24)

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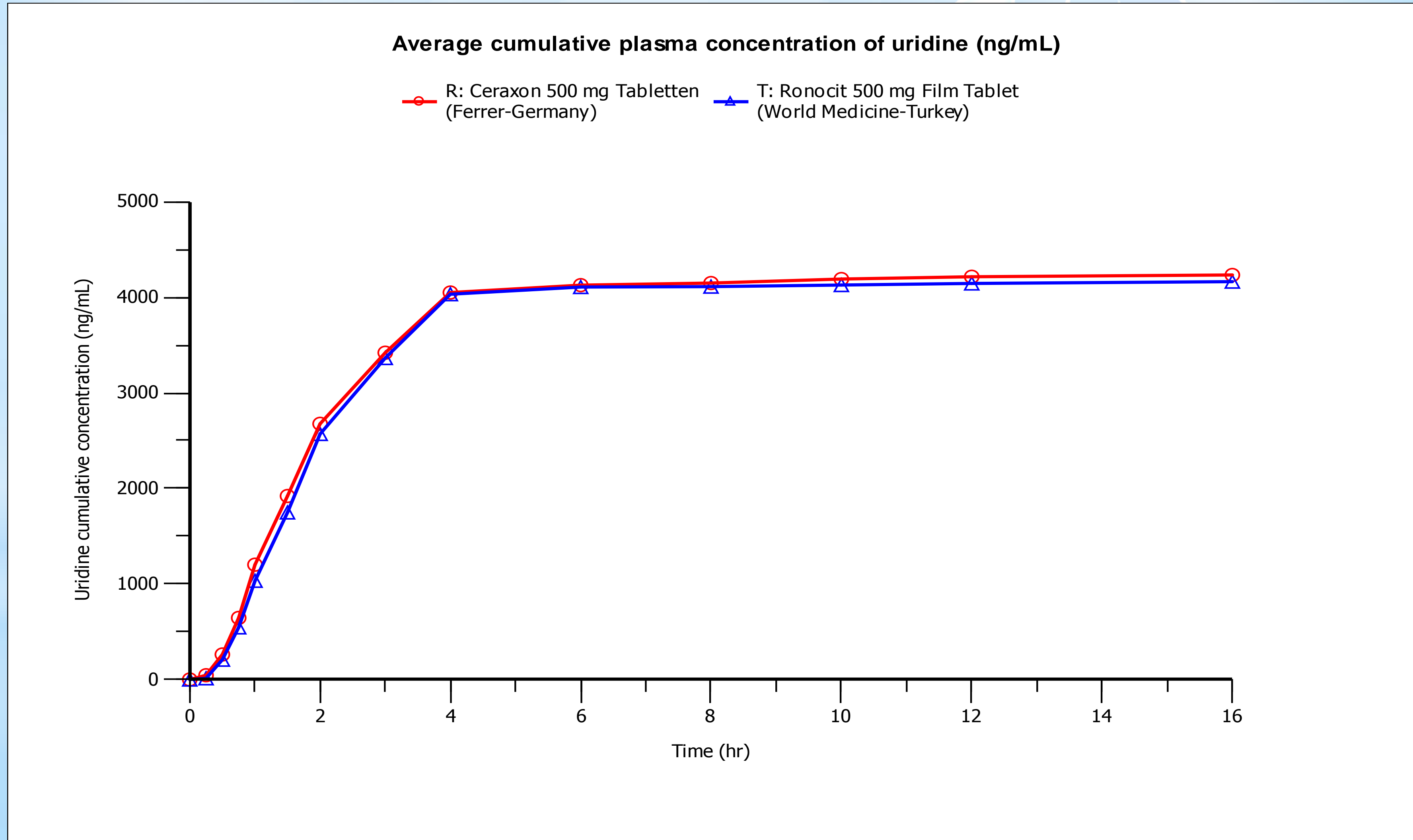
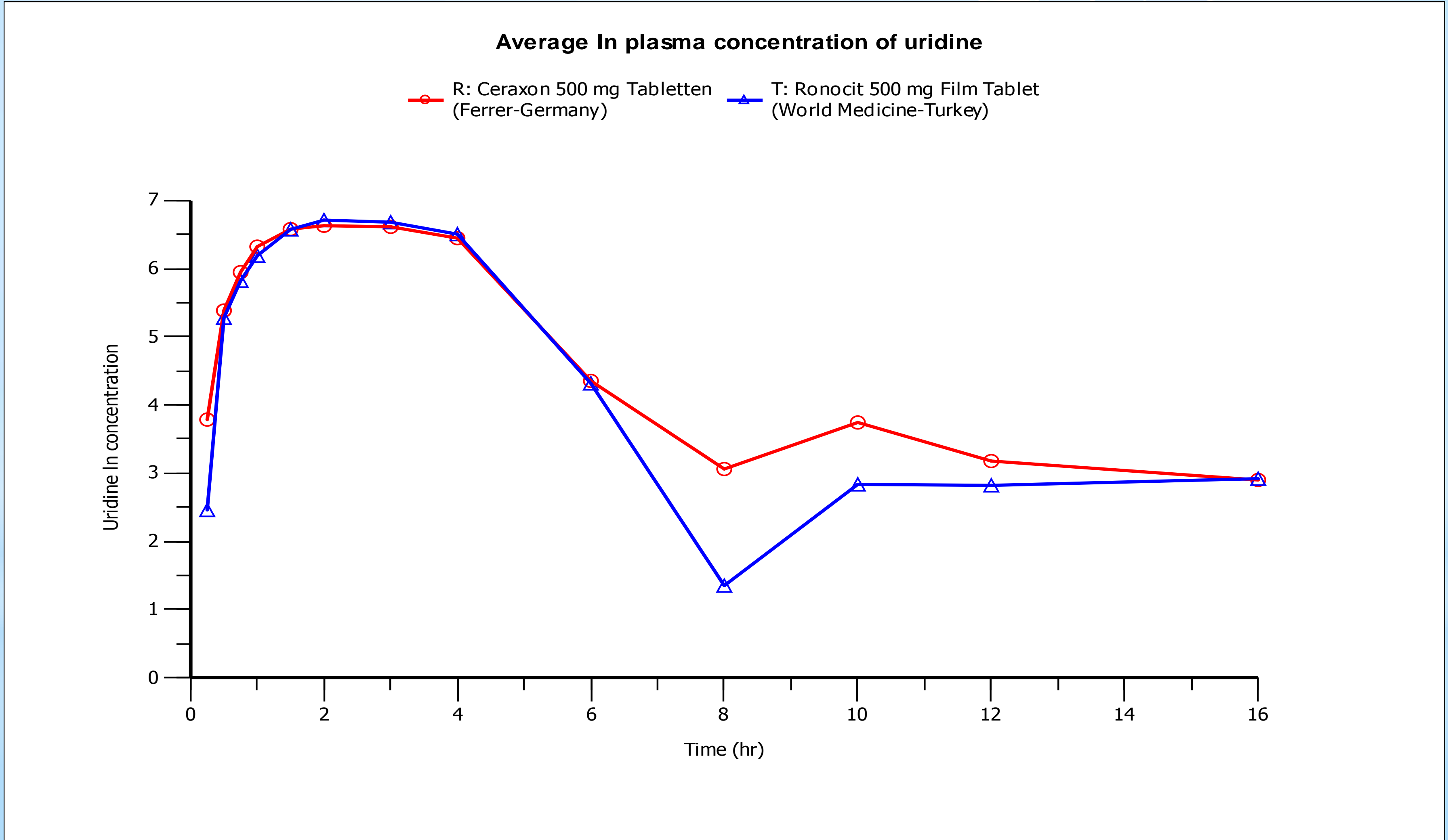


# Average ln plasma concentration of uridine (Subjects 1 - 24)



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**Plasma concentration of uridine (ng/mL)**

1 3 5 7 9 13 15 17 19 20 21 22 23 24
2 4 6 8 11 14 16 18

TREATMENT=R

Uridine concentration (ng/mL)

2000
1500
1000
500
0

0 2 4 6 8 10 12 14 16

TIME (hr)

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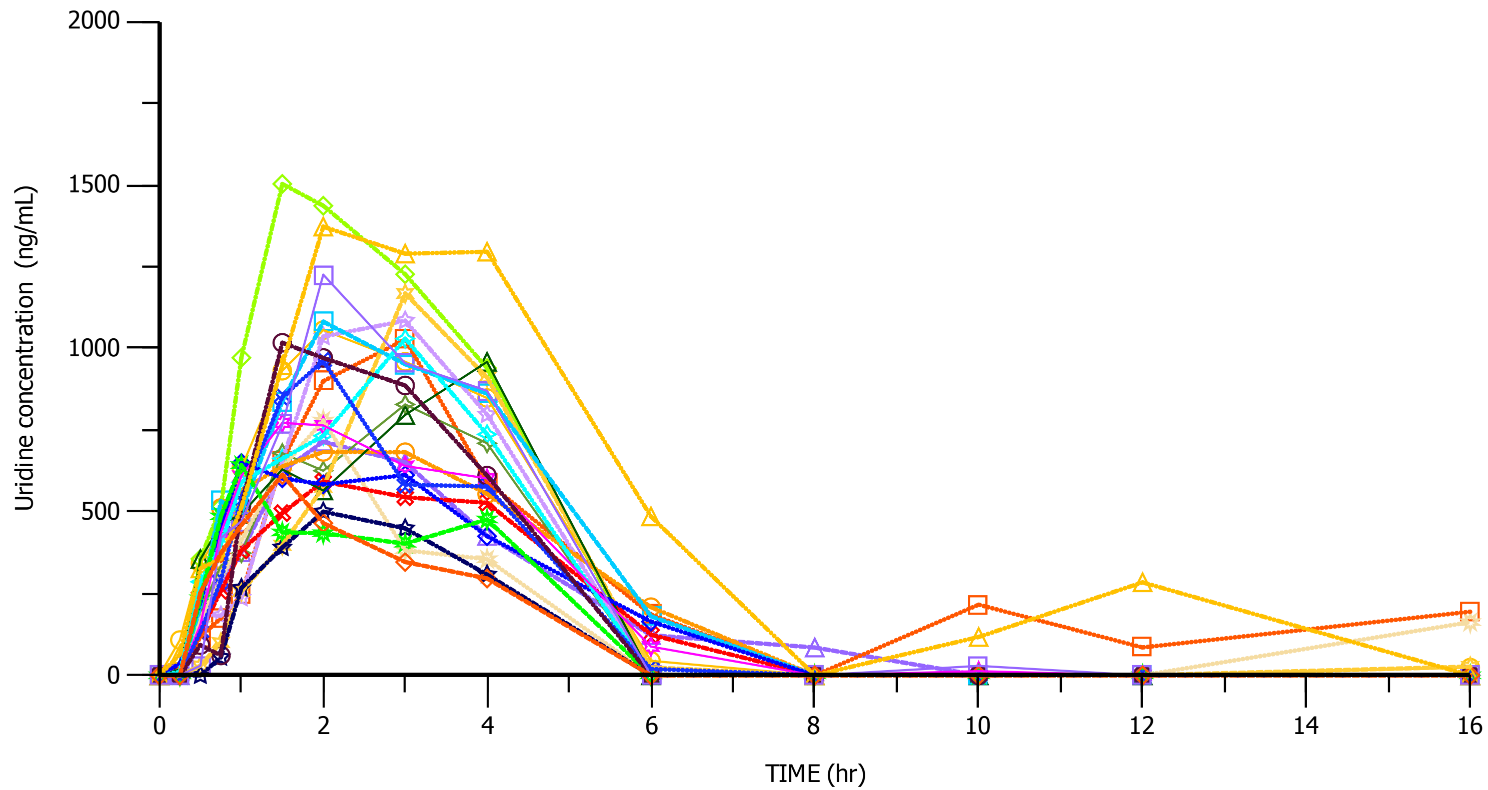

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# RESULT

| Parameter | Difference | DiffSE | TESTLSM | REFLSM | Ratio | 90% CI | CV% |
|---|---|---|---|---|---|---|---|
| $\ln(C_{max})$ | 0.0816 | 0.0756 | 6.7906 | 6.7090 | 1.0850 | 0.9524 - 1.2361 | 25.4 |
| $\ln(AUC_{0\text{-}tlast})$ | -0.0020 | 0.0868 | 7.9276 | 7.9296 | 0.9980 | 0.8592 - 1.1592 | 29.3 |
| $\ln(AUC_{0\text{-}\infty})$ | -0.0246 | 0.1594 | 8.3405 | 8.3650 | 0.9757 | 0.7285 - 1.3068 | 40.9 |
| $t_{max}$ (hr) | -0.2042 | 0.2570 | 2.1125 | 2.3167 | 0.9119 | 0.7205 - 1.1032 | |
| $t_{½}$ (hr) | -6.1432 | 3.9151 | 1.6334 | 7.7766 | 0.2100 | -0.7120 - 1.1329 | |
| $\lambda_z$ (1/hr) | -0.0364 | 0.1217 | 0.3905 | 0.4269 | 0.9148 | 0.3921 - 1.4374 | |
| MRT (hr) | -7.9654 | 5.9064 | 2.9096 | 10.8750 | 0.2676 | -0.7280 - 1.2631 | |

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# RESULT (without baseline correction)

| Parameter | Difference | DiffSE | TESTLSM | REFLSM | Ratio | 90% CI | CV% |
|---|---|---|---|---|---|---|---|
| $\ln(C_{max})$ | 0.0444 | 0.0753 | 7.1388 | 7.0944 | 1.0454 | 0.9180 - 1.1903 | 25.3 |
| $\ln(AUC_{0-tlast})$ | -0.0080 | 0.1112 | 8.7505 | 8.7586 | 0.9920 | 0.8188 - 1.2018 | 38.0 |
| $\ln(AUC_{0-\infty})$ | -0.0791 | 0.1440 | 9.1847 | 9.2638 | 0.9240 | 0.7148 - 1.1942 | 41.1 |
| $t_{max}$ (hr) | -0.2042 | 0.2570 | 2.1125 | 2.3167 | 0.9119 | 0.7205 - 1.1032 | |
| $t_{1/2}$ (hr) | -6.6895 | 6.6181 | 3.2460 | 9.9355 | 0.3267 | -0.860 - 1.5138 | |
| $\lambda_z$ (1/hr) | -0.0187 | 0.0382 | 0.1619 | 0.1807 | 0.8963 | 0.5191 - 1.2734 | |
| MRT (hr) | -9.0675 | 9.5243 | 5.4444 | 14.5119 | 0.3752 | -0.794 - 1.5448 | |

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# CONCLUSION

* After baseline correction, since the 90% confidence intervals of the test/reference mean ratios for $C_{max}$ and $AUC_{0\text{-}tlast}$ of **uridine** were contained within the conventional acceptance limits preset in the Clinical Study Protocol as 0.80-1.25; according to the applied bioequivalence study, **it is concluded that the test and reference citicoline products are bioequivalent.**

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# DISCUSSION

* In the Clinical Study Protocol, it has been declared that choline will be determined in human plasma. But, the choline amount in the human plasma as endogenous is high. Although, the drug's choline amount is too low to determine the lowest amounts in the calibration curve and the repeatability is highly affected. In the literatures and in our uridine quantification process shows that these limitations are not occurred.
* Therefore, to measure the citicoline drug affect, the quantification of uridine is chosen instead of choline. Validation process of the quantification of uridine in the human plasma has been done in accordance with the actual guidance of EMA and FDA.
* During predose sampling points, there has been a doubt about subjects would be receiving different meals (breakfast, lunch, snacks, dinner) because of being out of clinic. Since food effects the endogenous uridine levels and hence effects the pre-dose concentrations for baseline correction, in statistical analysis only $t_0$ sampling point has been taken as baseline and sampling points that subjects do not depart from the clinic have been taken as $t_0$-$t_{16.00}$.

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* There have been two drop-outs (Subject 10 and 12 in Period I and II, respectively). Dropped out subjects did not want to continue the study by their freewill and have not been replaced. As a result, 22 subjects completed the clinical phase of the study.
* There has been only one adverse event in all two periods and this adverse event has been evaluated as possible drug related and fully recovered. The overall tolerability of the products found to be very good.
* The mean of $C_{max}$ were 921.424 ng/mL (for test product) and 853.339 ng/mL (for reference product).
* The mean of $AUC_{0\text{-}tlast}$ were 2946.923 hr.ng/mL (for test product) and 3039.192 hr.ng/mL (for reference product).
* The mean times to reach the maximal concentration were 2.136 hours (for test product) and 2.341 hours (for reference product).
* The mean terminal half-lives of uridine were 2.965 hours (for test product) and 7.885 hours (for reference product) after drug administrations.

# Thank you…

# *Let us meet again..

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We welcome you all to our future conferences of

OMICS International

**7th World Congress on**

**Bioavailability & Bioequivalence: BA/BE Studies Summit**

On

**August 29 - 31, 2016** at **Atlanta, USA**

http://bioavailability-bioequivalence.pharmaceuticalconferences.com/