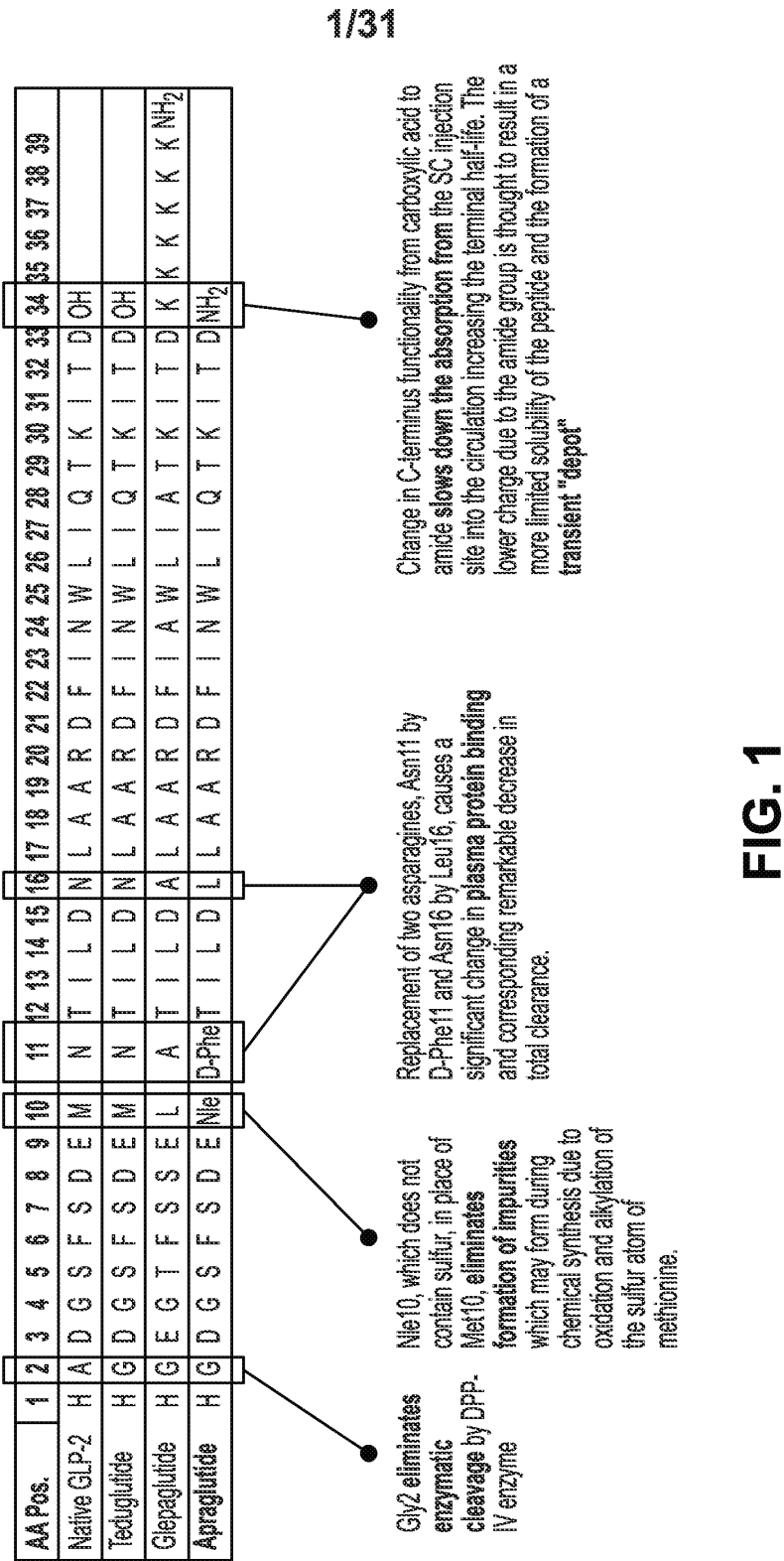




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Apraglutide Manufacturing Process Flow Diagram

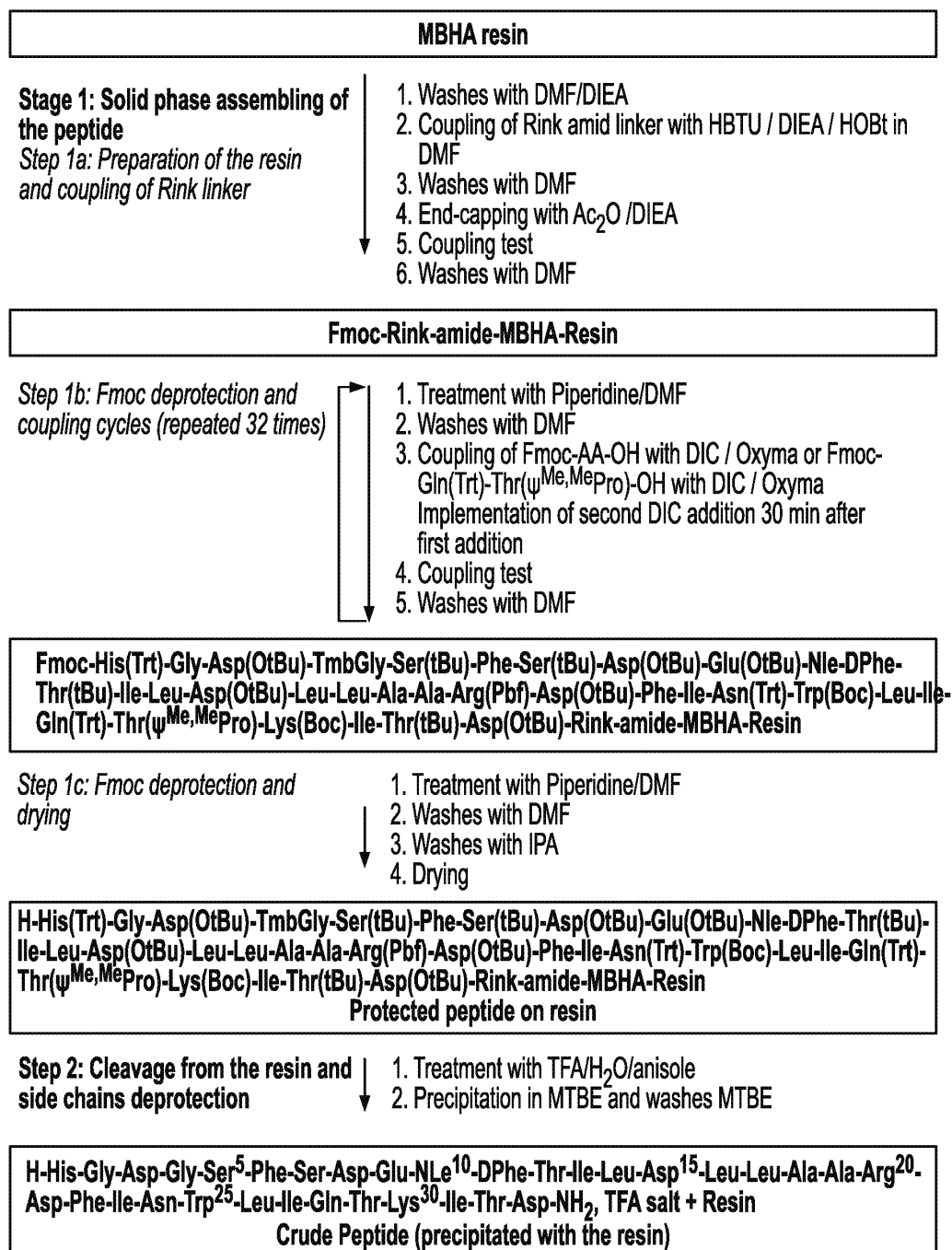


FIG. 2A

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Stage 3: First purification by preparative RP-HPLC

1. Extraction of crude peptide from cleaved peptide resin mixture in H₂O/CH₃CN/NH₄OH and filtration
2. Purification by preparative RP-HPLC in 0.05% TFA (H₂O/acetonitrile)
3. pH adjustment of fractions using 0.6M NaHCO₃
4. Pooling
5. IPC: purity of the pool by RP-HPLC ≥90%

H-His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-NLe-DPhe-Thr-Ile-Leu-Asp-Leu-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-NH₂
RP-HPLC Semi Purified Peptide in solution

Stage 4: Second purification by preparative RP-HPLC

1. Dilution of previous solution with H₂O
2. Purification by RP-HPLC in 0.05M NaHCO₃/H₂O/ acetonitrile
3. Pooling
4. IPC: purity of the pool by RP-HPLC ≥97%

H-His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-NLe-DPhe-Thr-Ile-Leu-Asp-Leu-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-NH₂
RP-HPLC Purified Peptide in solution

Stage 5: Desalting/partial ion exchange by preparative RP-HPLC

1. Dilution of previous solution with H₂O
2. Salt exchange by RP-HPLC with 1.5mM NaOAc/water/ acetonitrile eluents
3. Pooling
4. IPC: purity of the pool ≥97% by RP-HPLC
5. Pool volume reduction by evaporation
6. Addition of 0.1% AcOH in water to reach pH 7.85-7.90

H-His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-NLe-DPhe-Thr-Ile-Leu-Asp-Leu-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-NH₂, Sodium salt
RP-HPLC Purified Peptide in solution

Step 6: Freeze drying and packaging

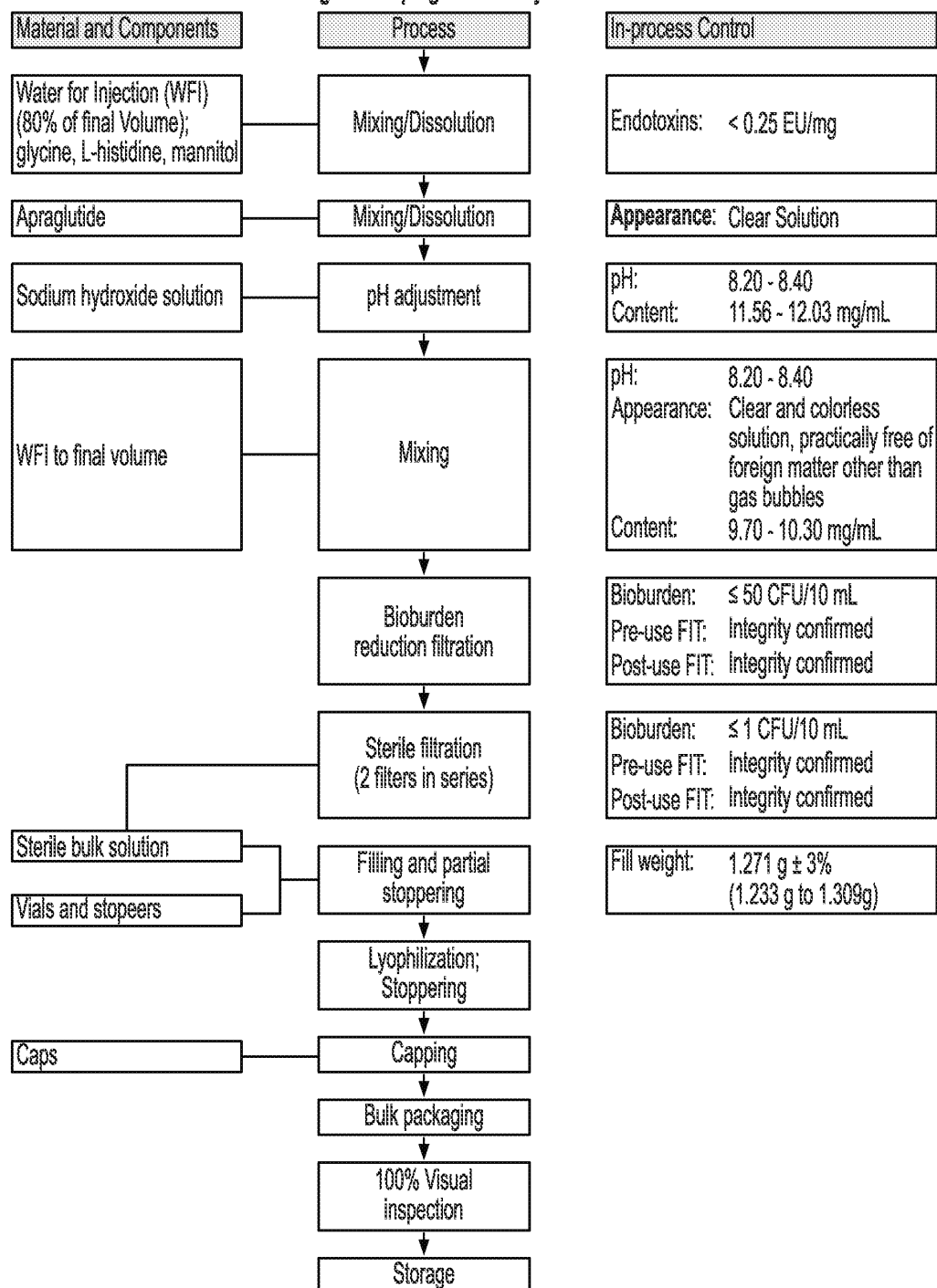
1. Filtration through 0.2 micrometer sterile filter
2. Lyophilization
3. Packaging under Argon

H-His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-NLe-DPhe-Thr-Ile-Leu-Asp-Leu-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-NH₂, 3Na
Lyophilized API: Apraglutide

FIG. 2B

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Flow Diagram of Apraglutide for Injection Manufacture



FIT = Filter integrity test

FIG. 3

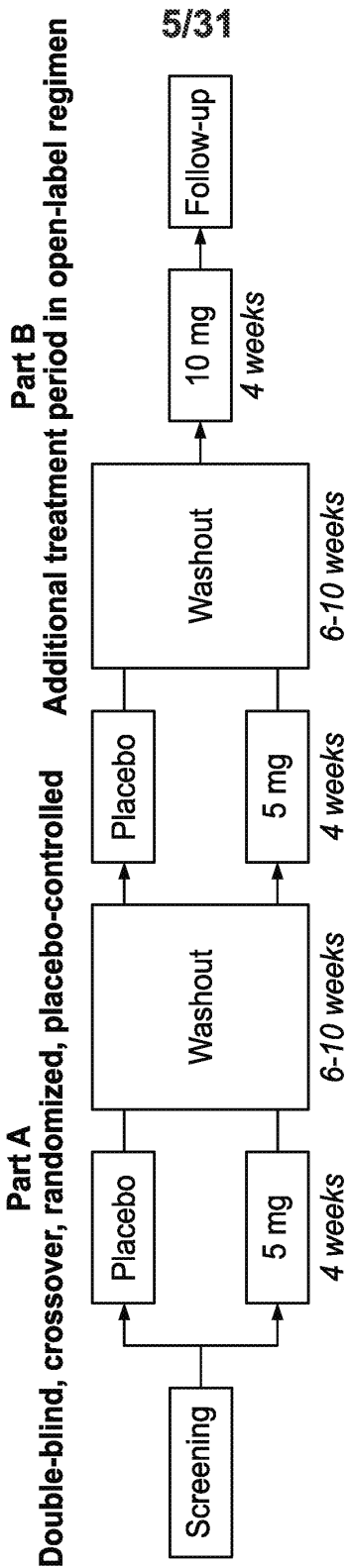


FIG. 4

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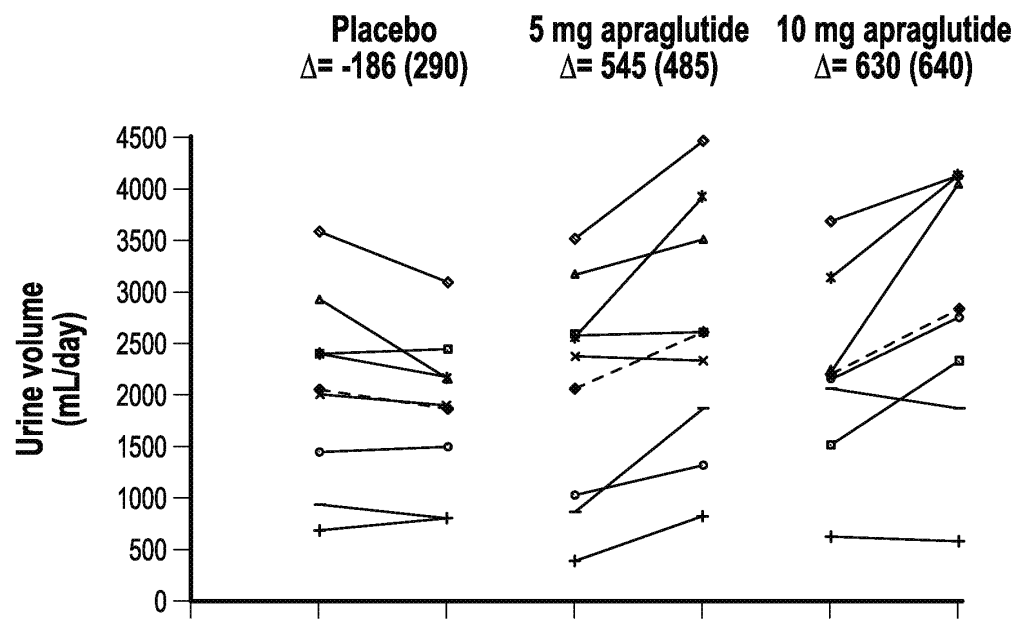


FIG. 5

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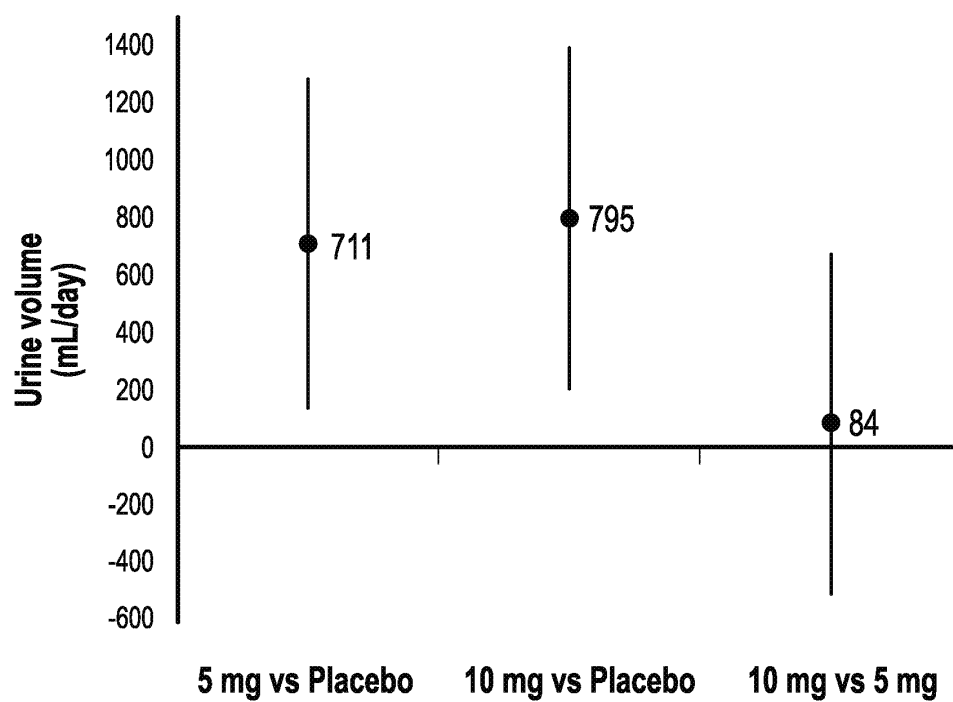


FIG. 6

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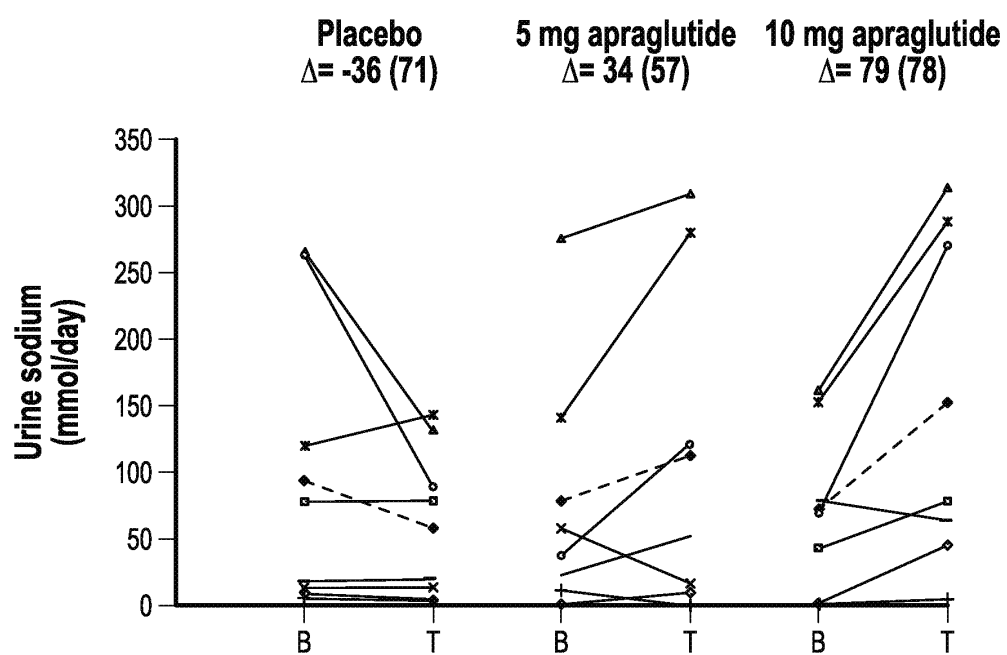


FIG. 7

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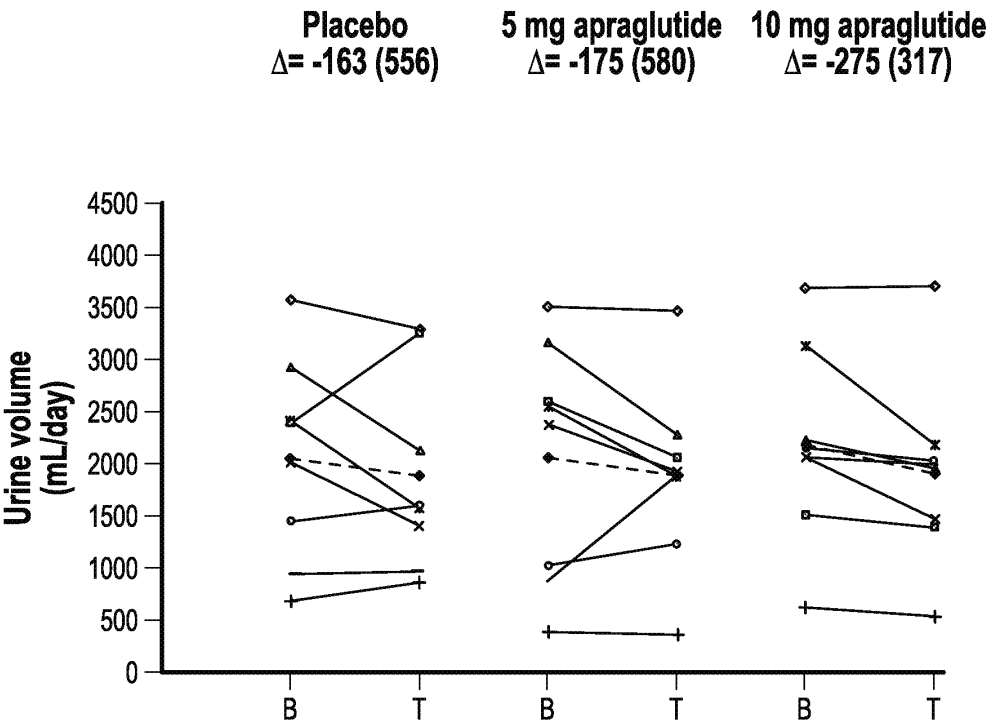


FIG. 8

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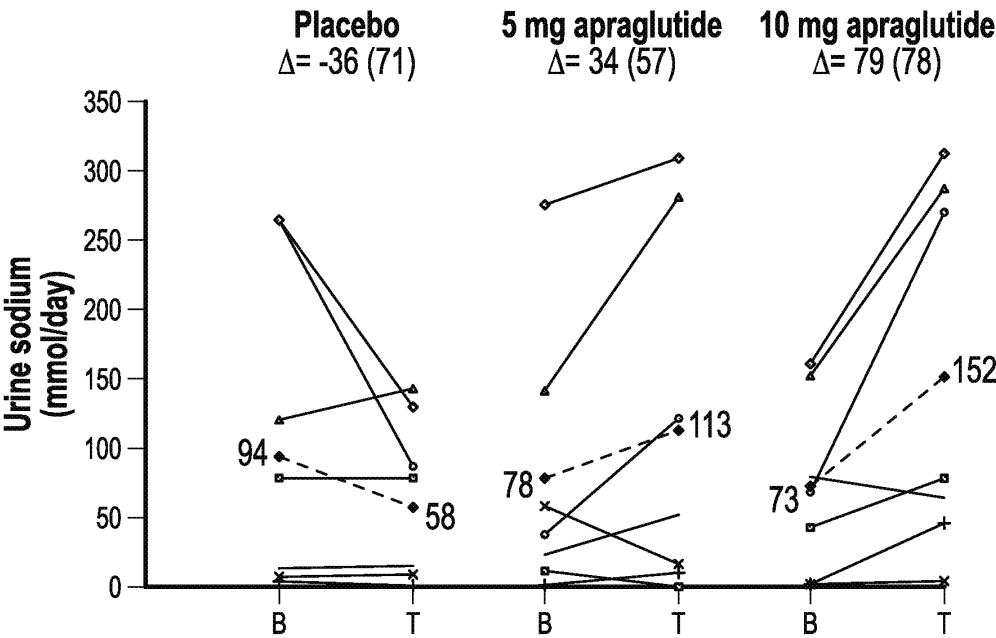


FIG. 9

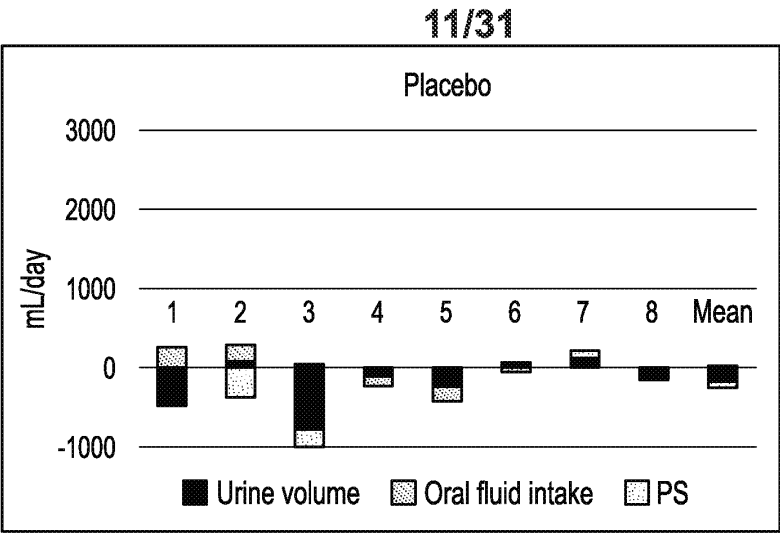


FIG. 10A

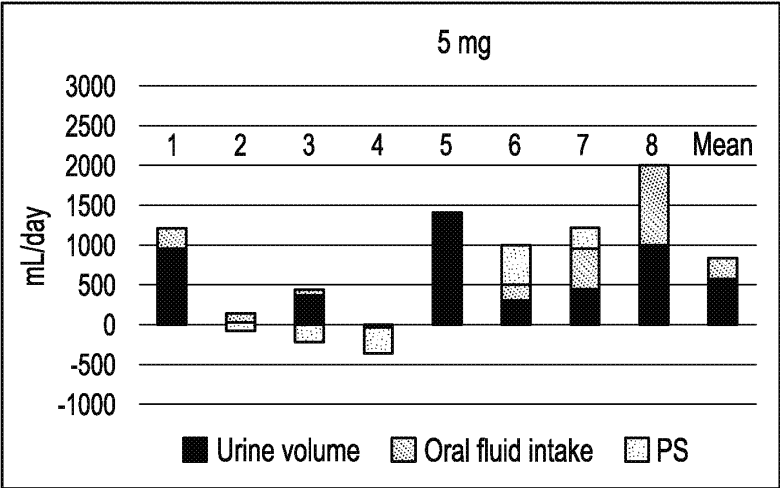


FIG. 10B

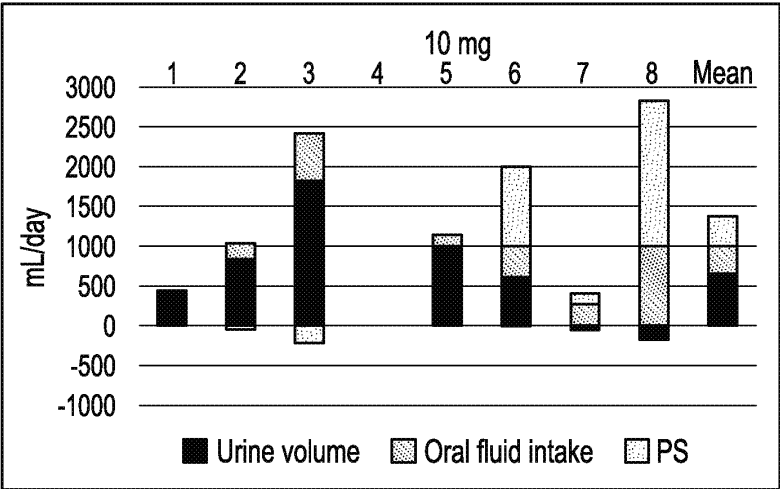


FIG. 10C

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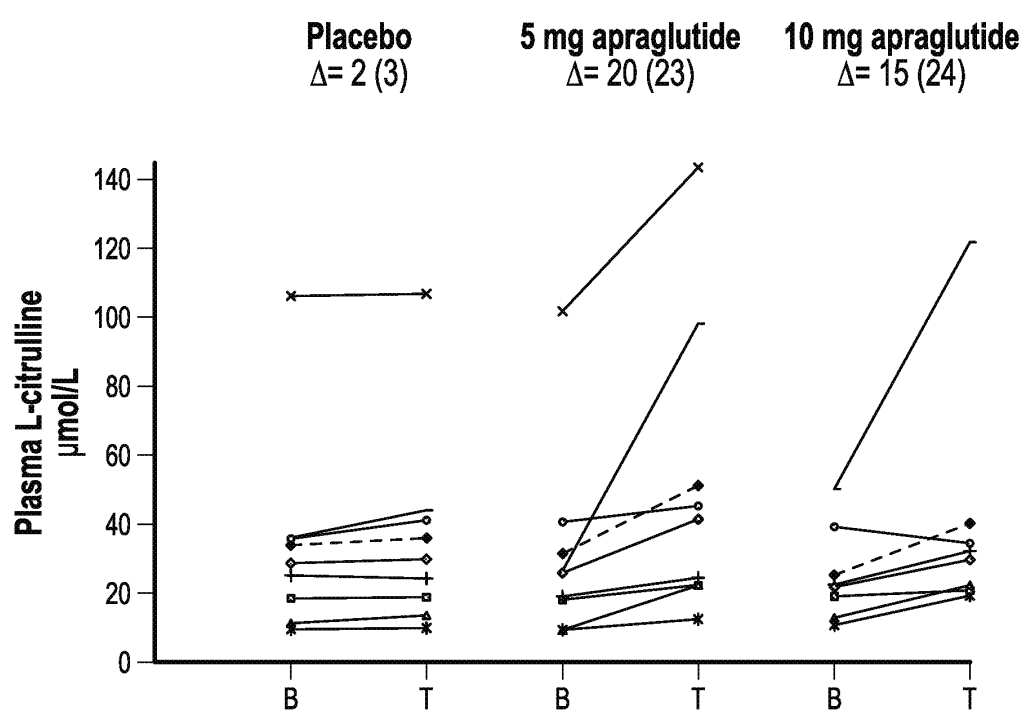
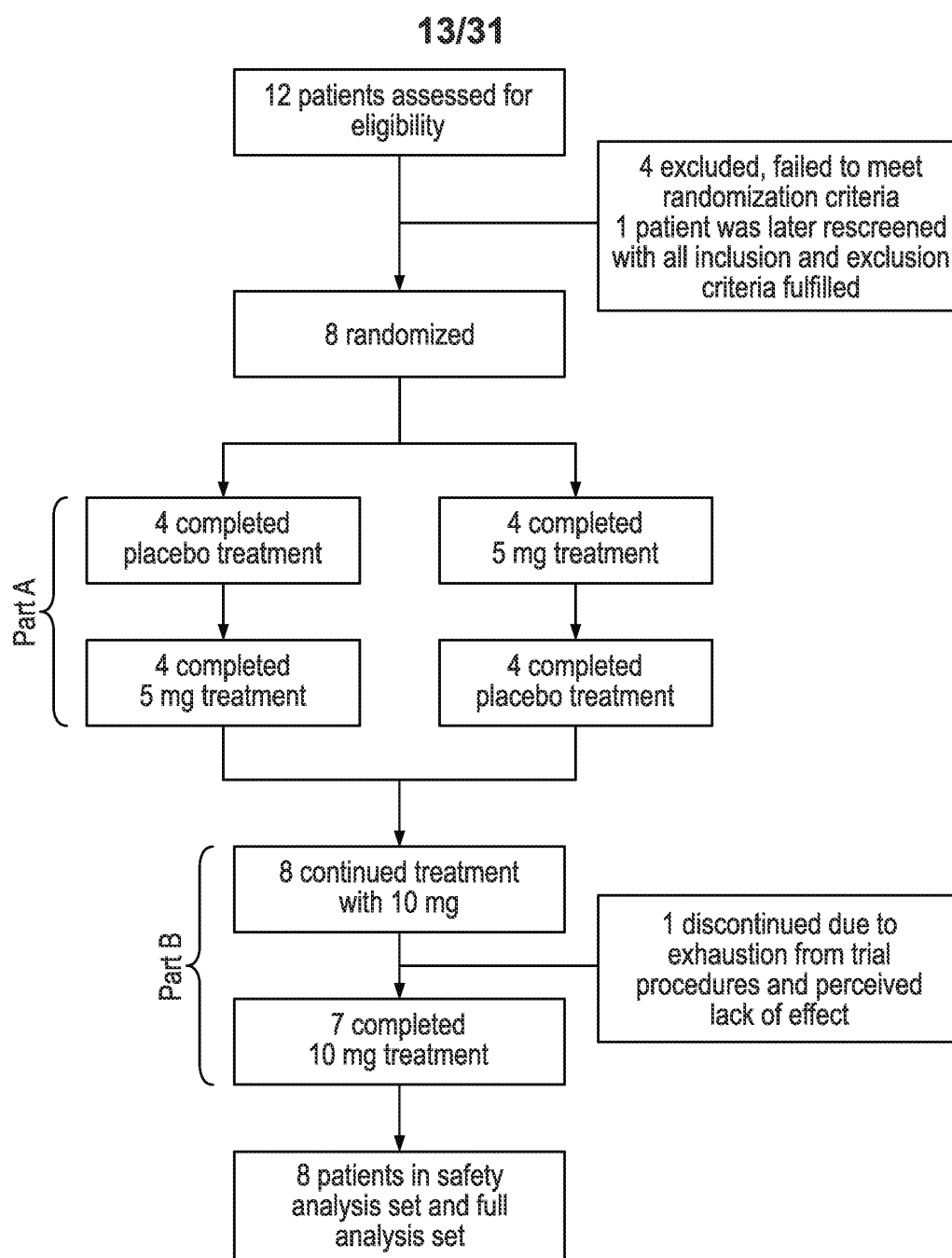
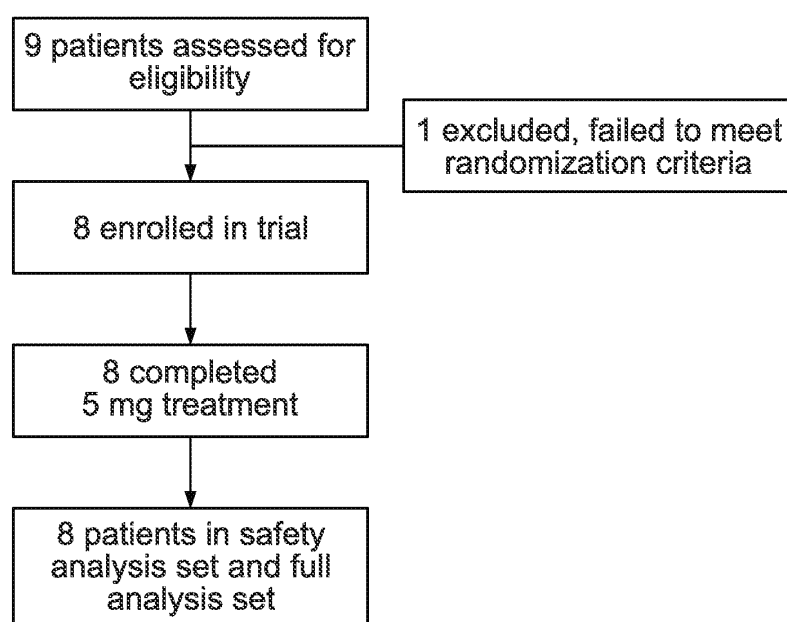


FIG. 11

**FIG. 12**

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**FIG. 13**

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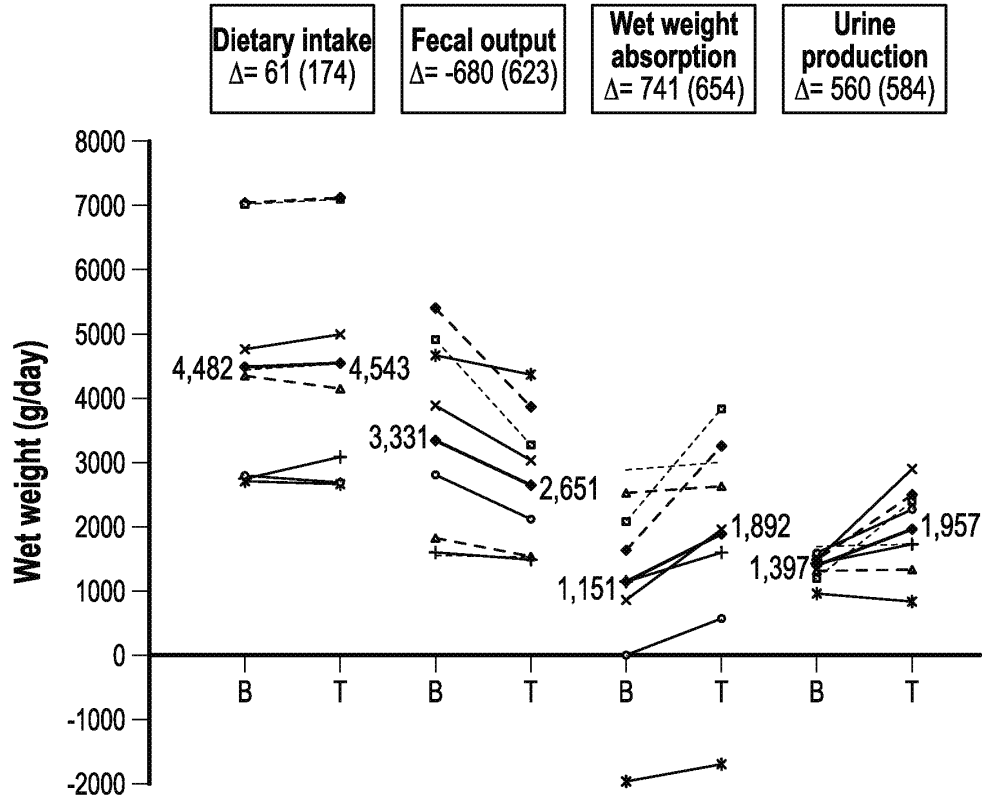


FIG. 14

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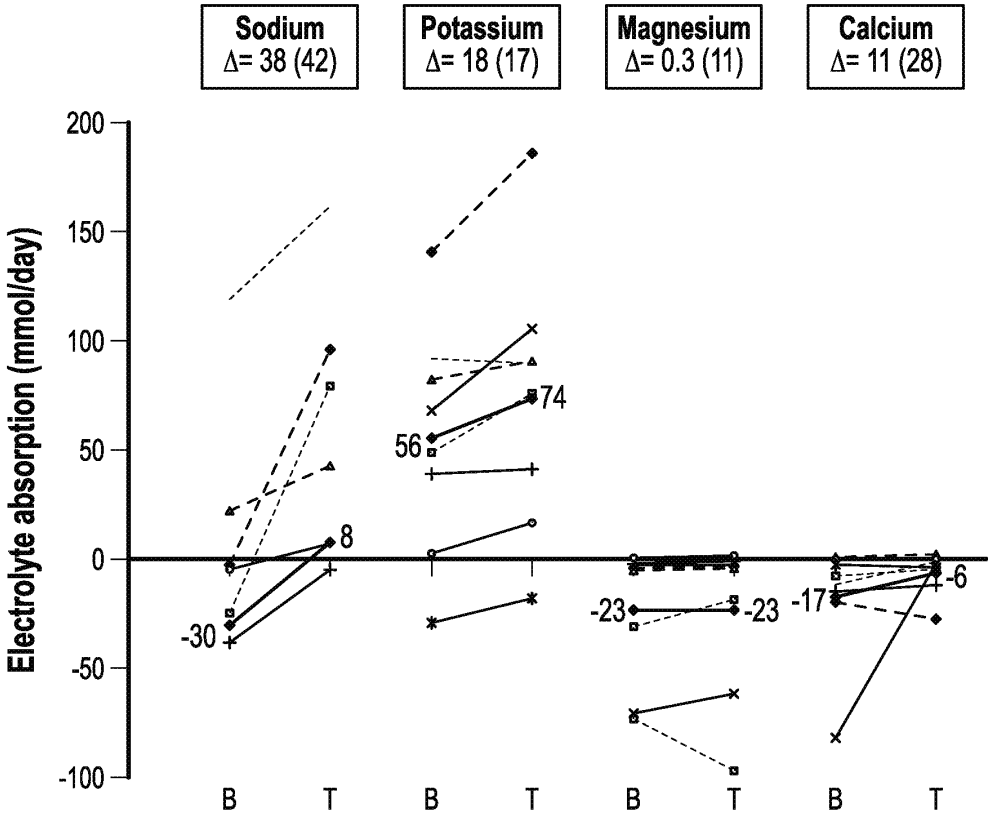


FIG. 15

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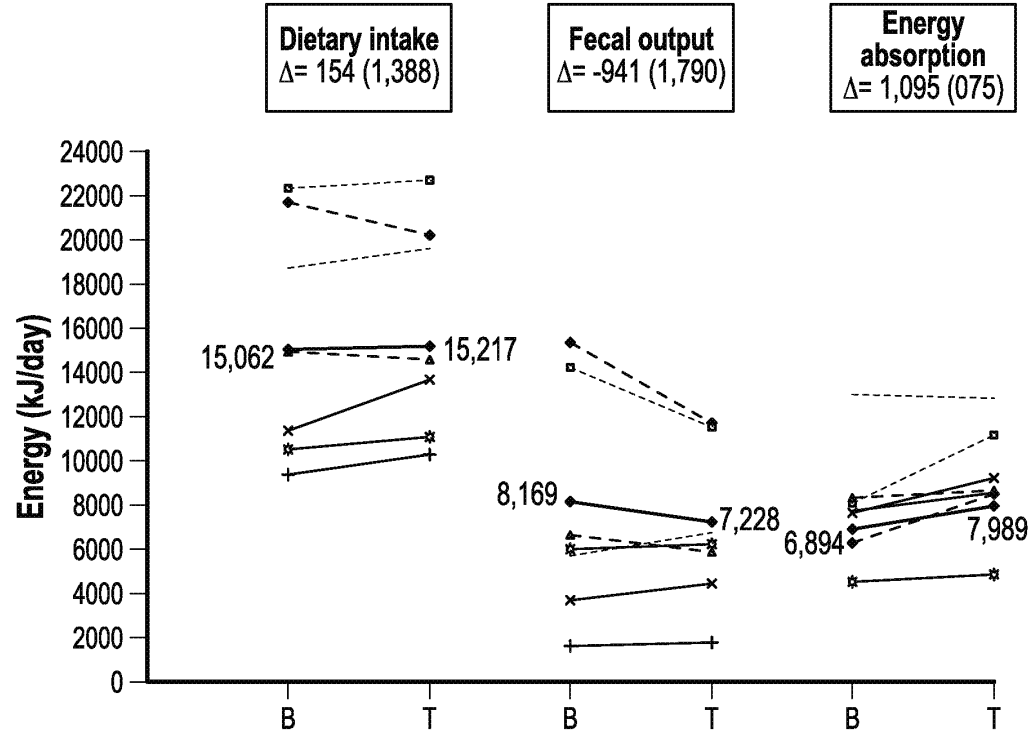


FIG. 16

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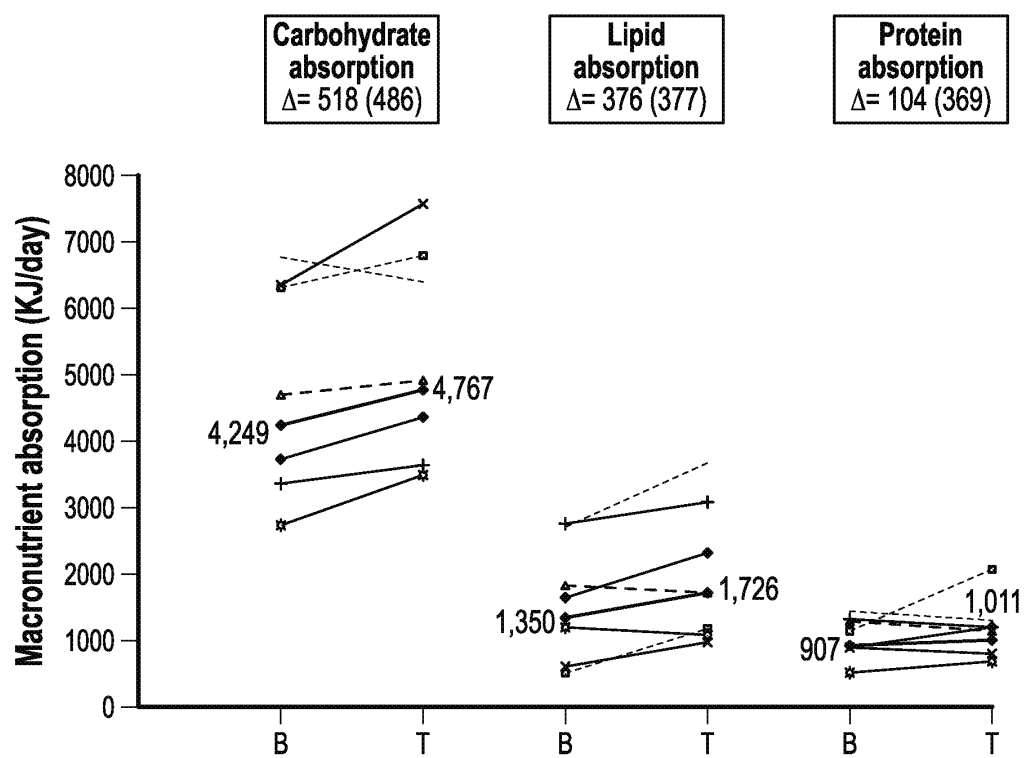


FIG. 17

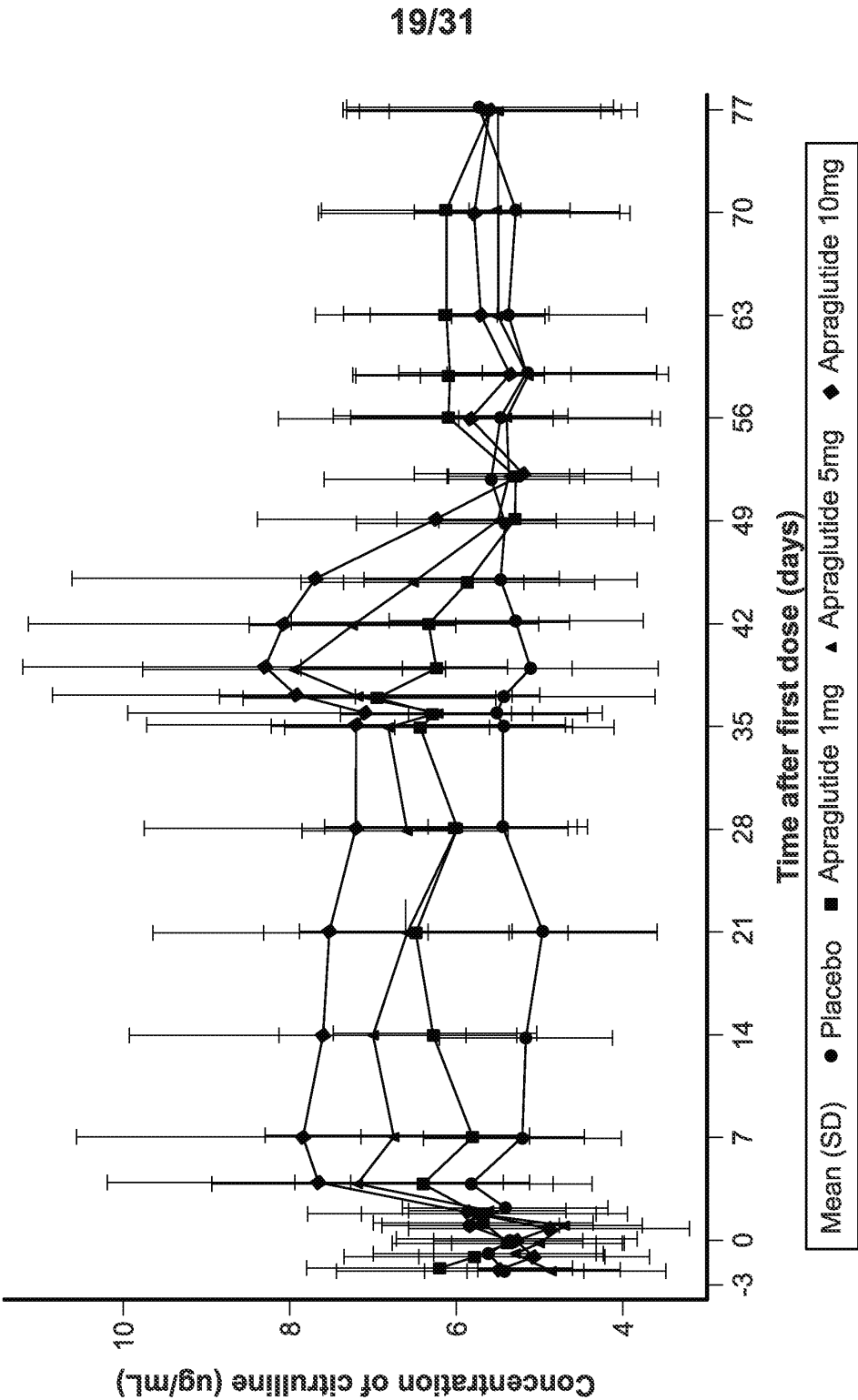


FIG. 18

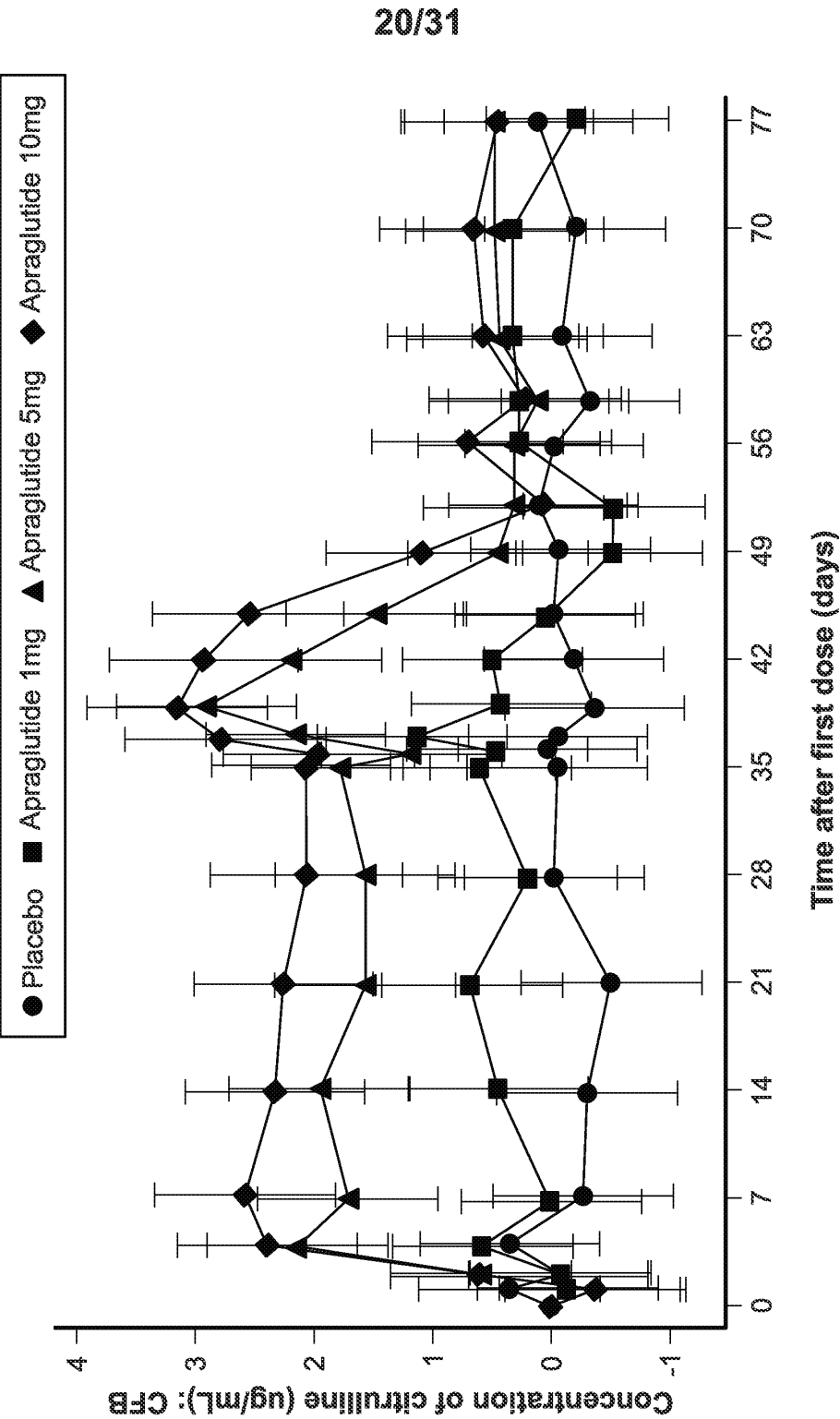


FIG. 19

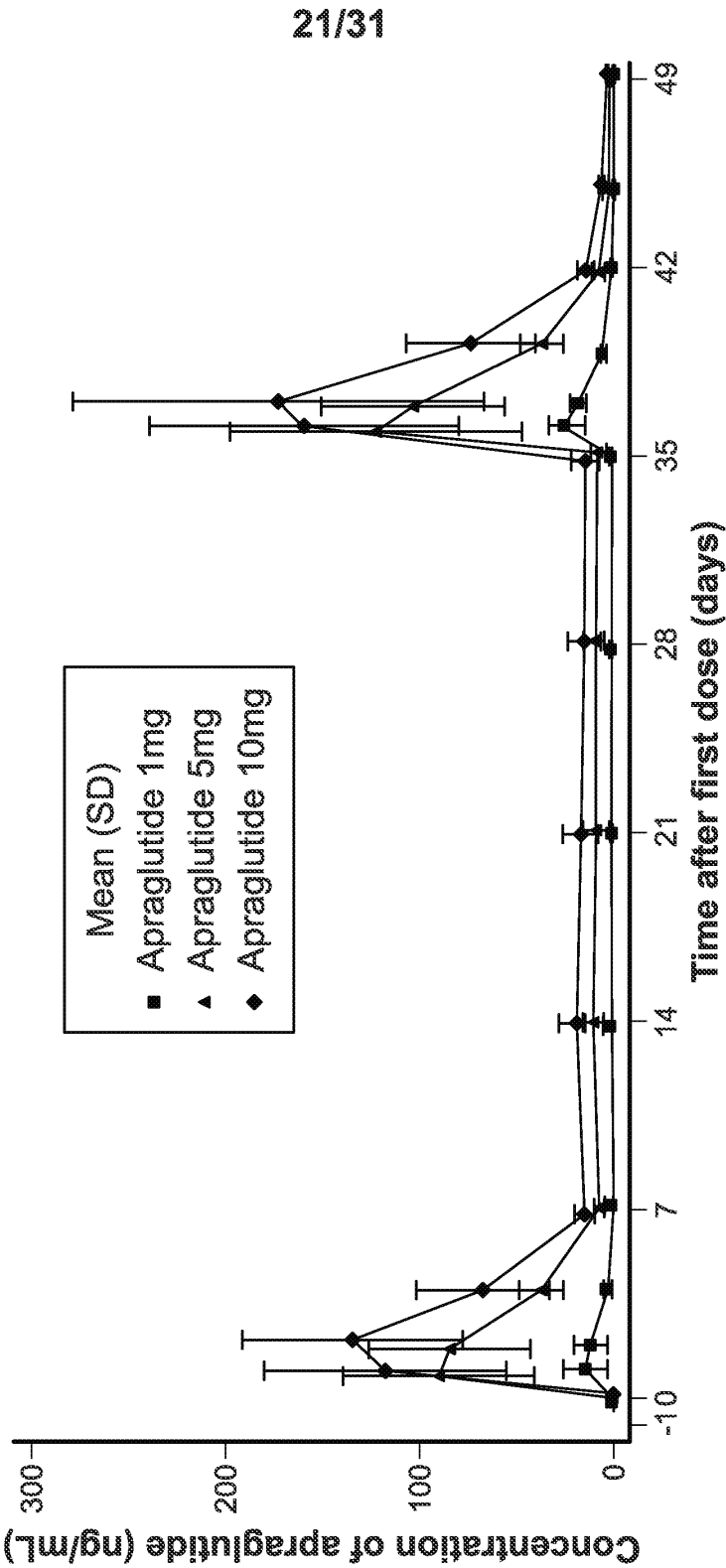


FIG. 20A

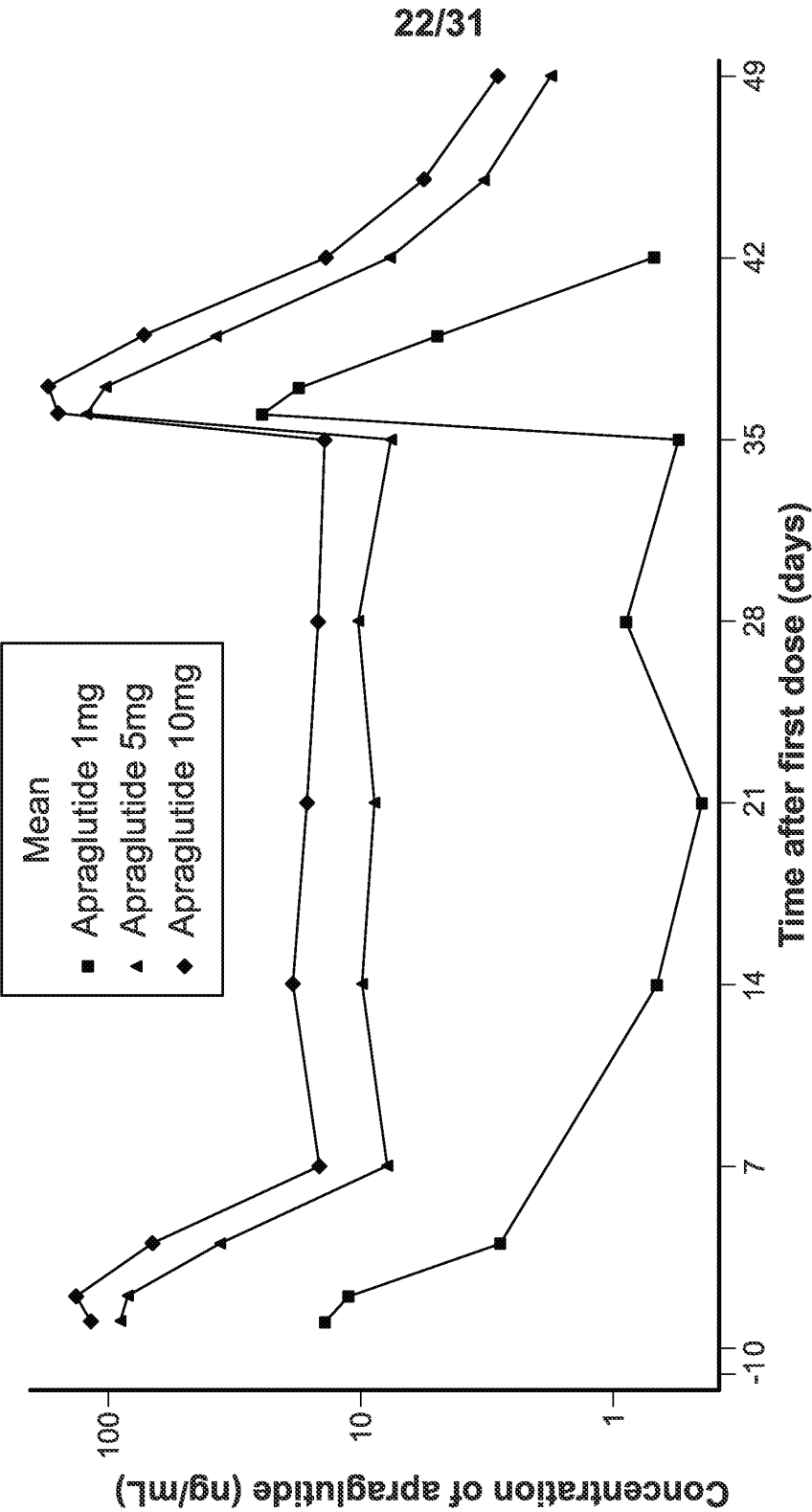


FIG. 20B

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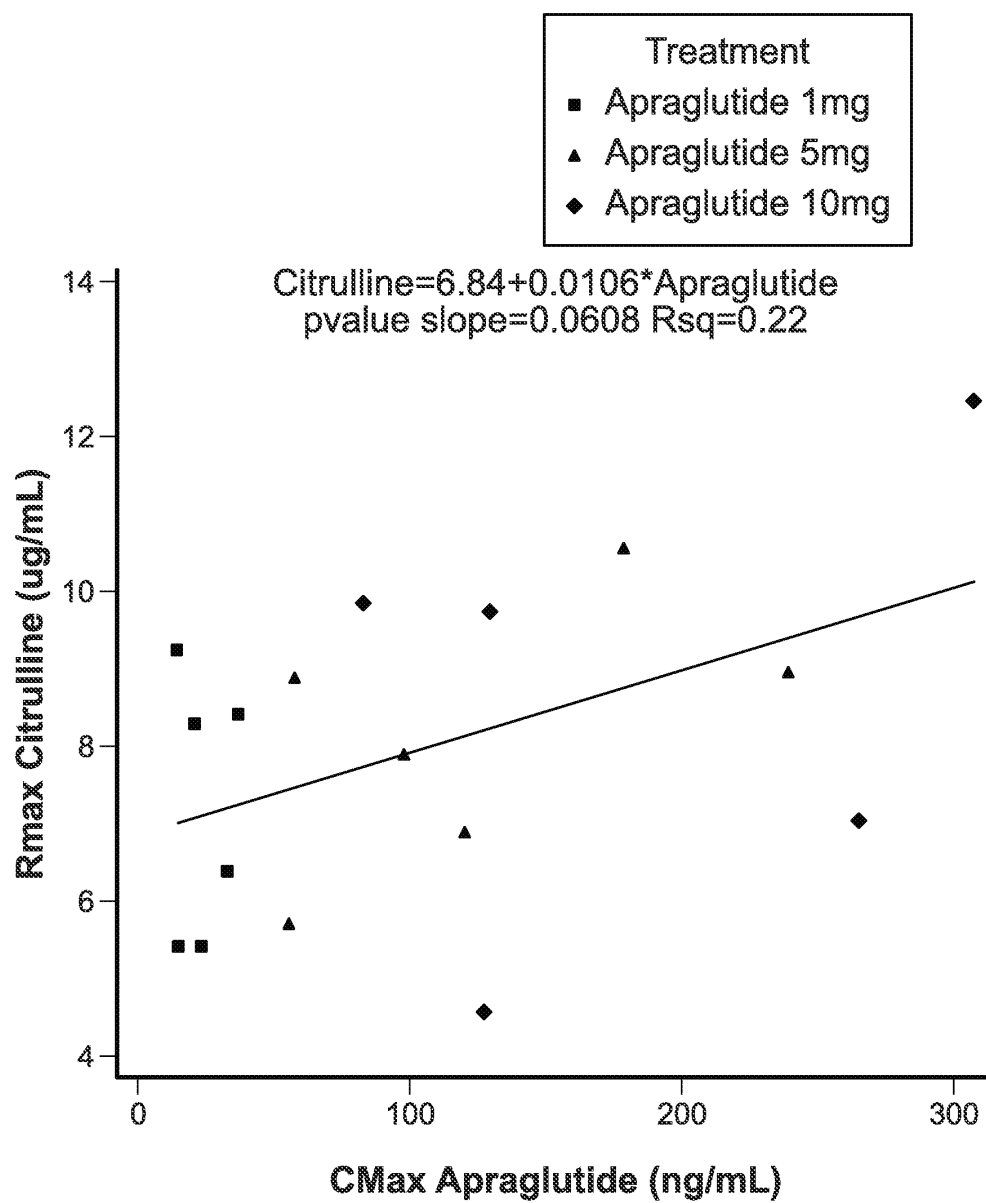


FIG. 21

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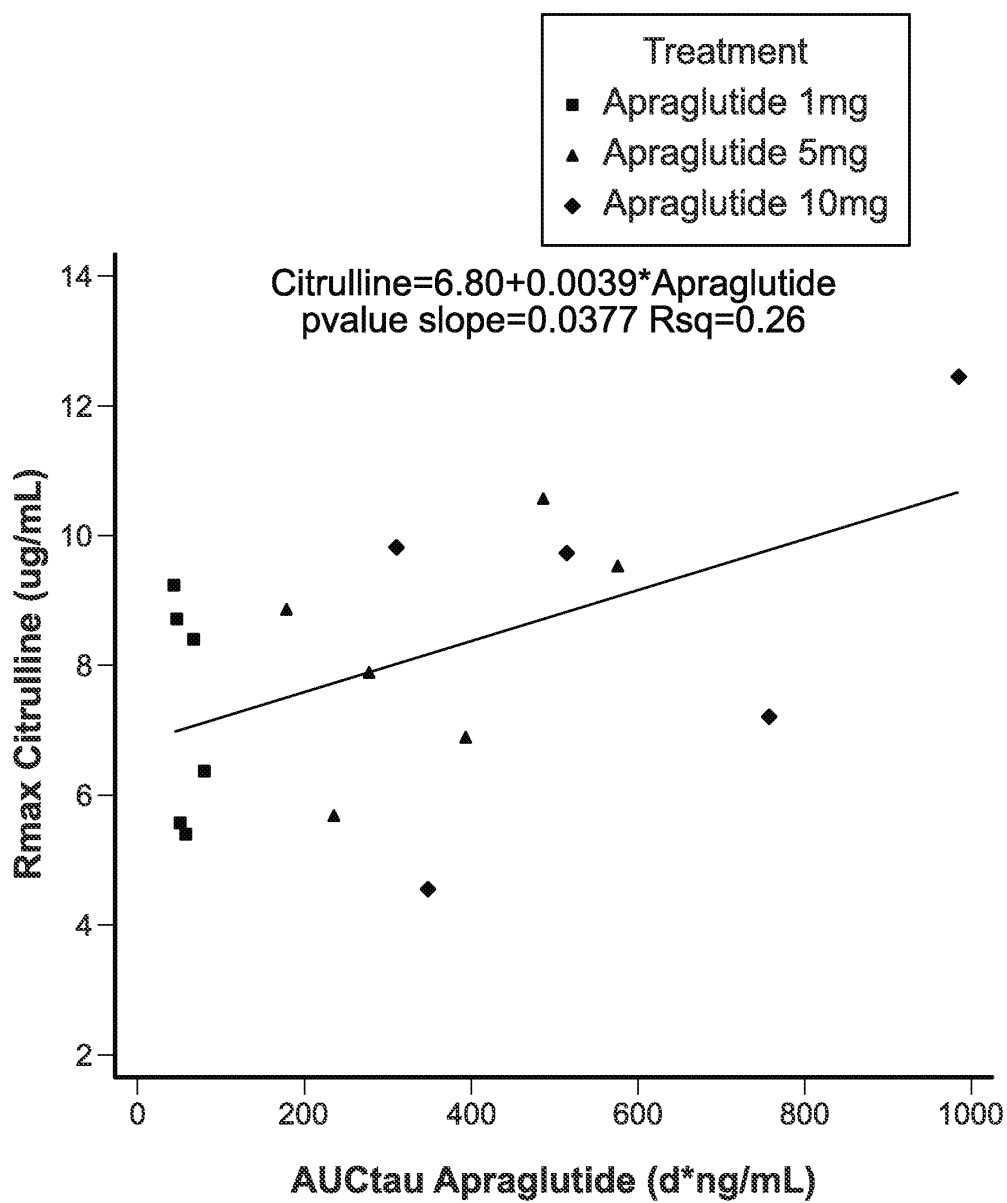


FIG. 22

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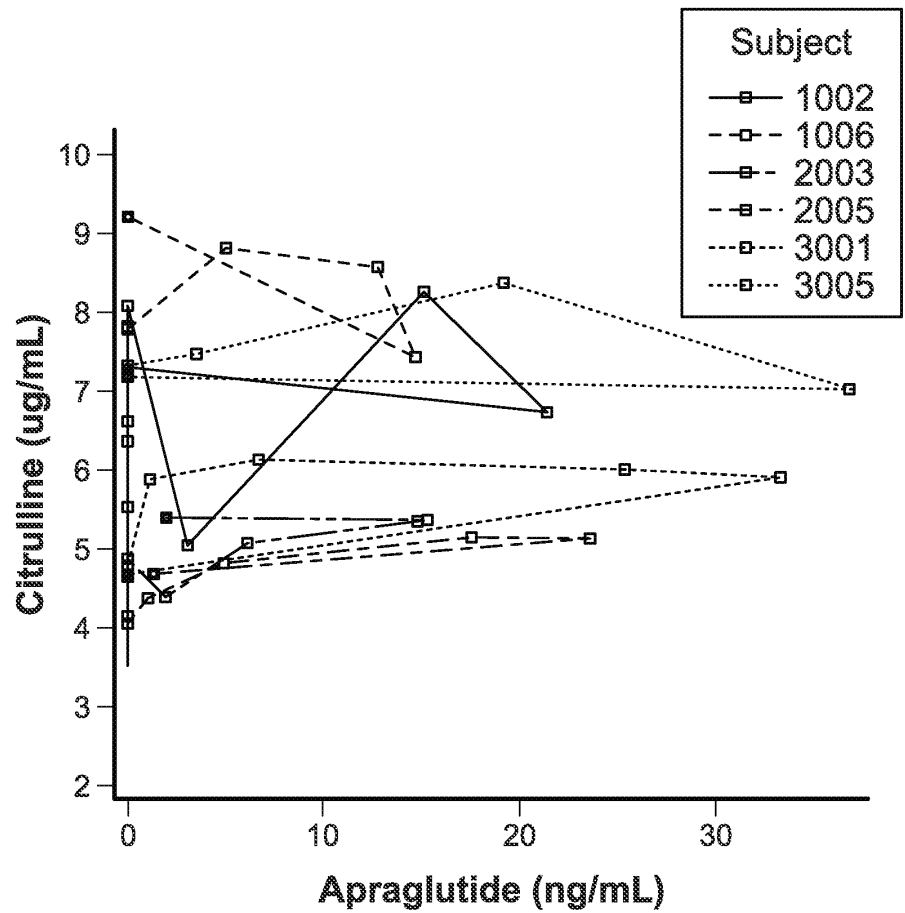
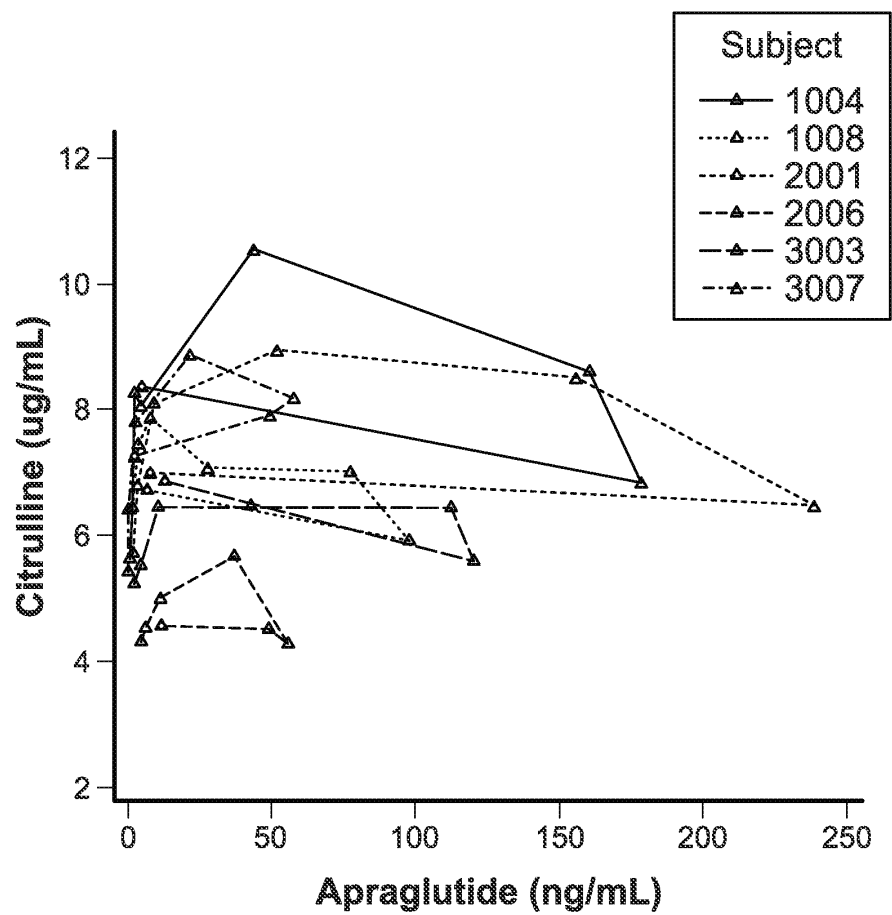
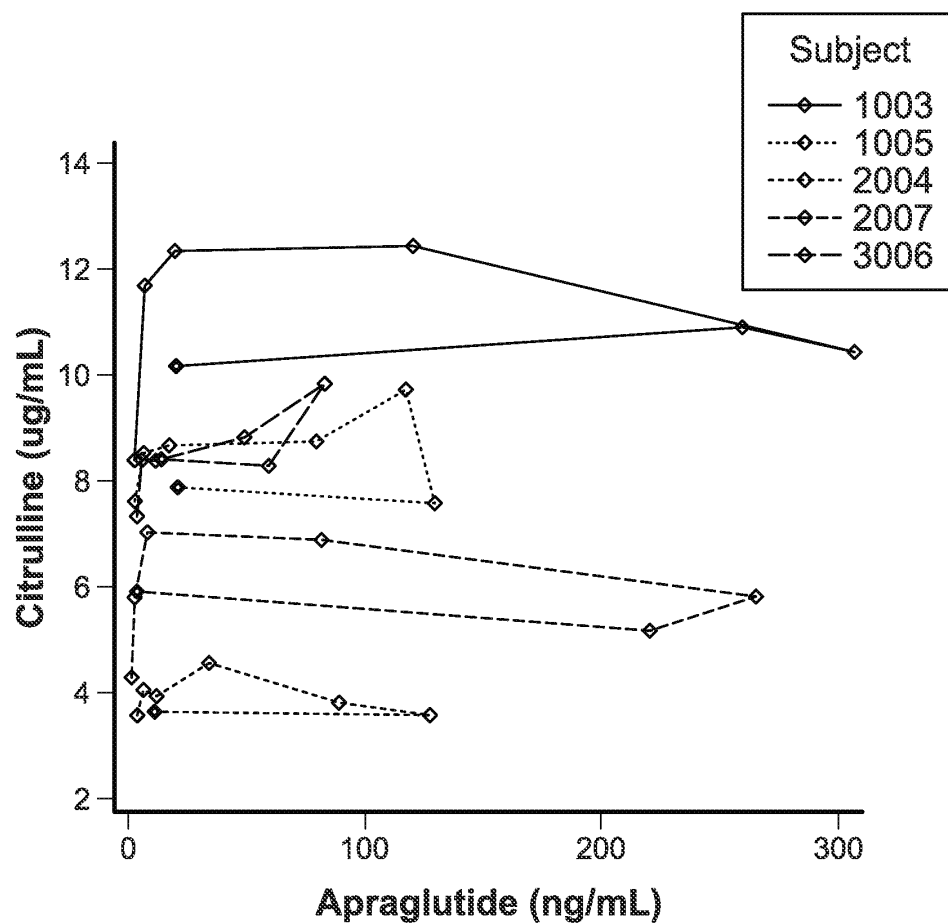


FIG. 23

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**FIG. 24**

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**FIG. 25**

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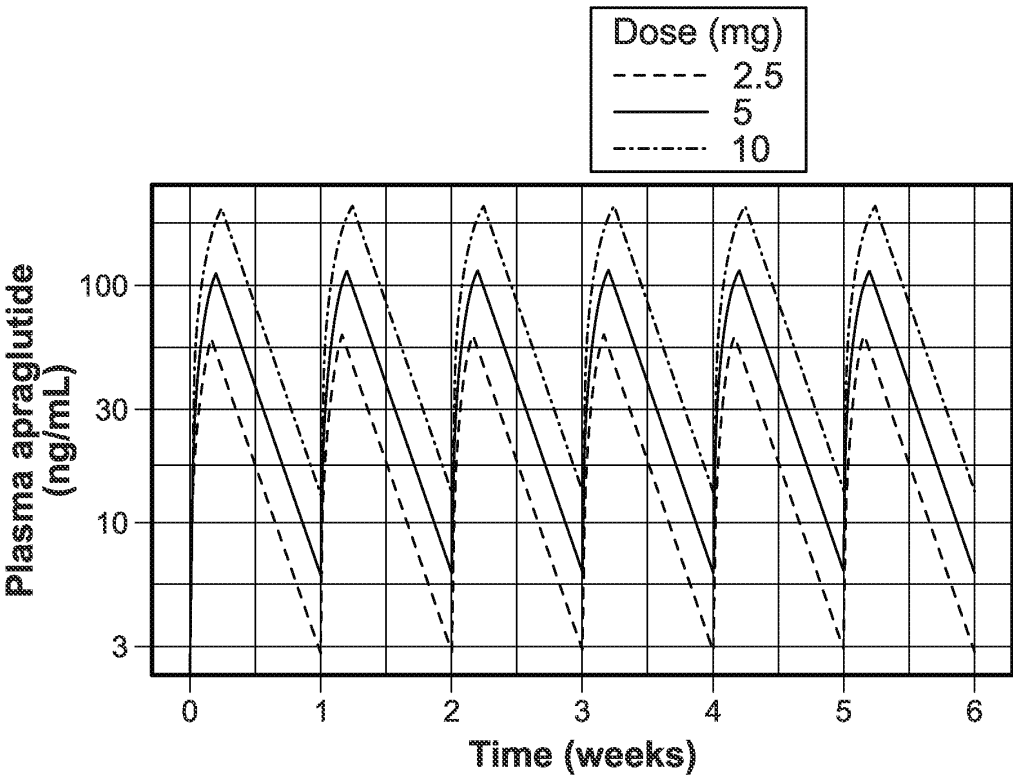


FIG. 26A

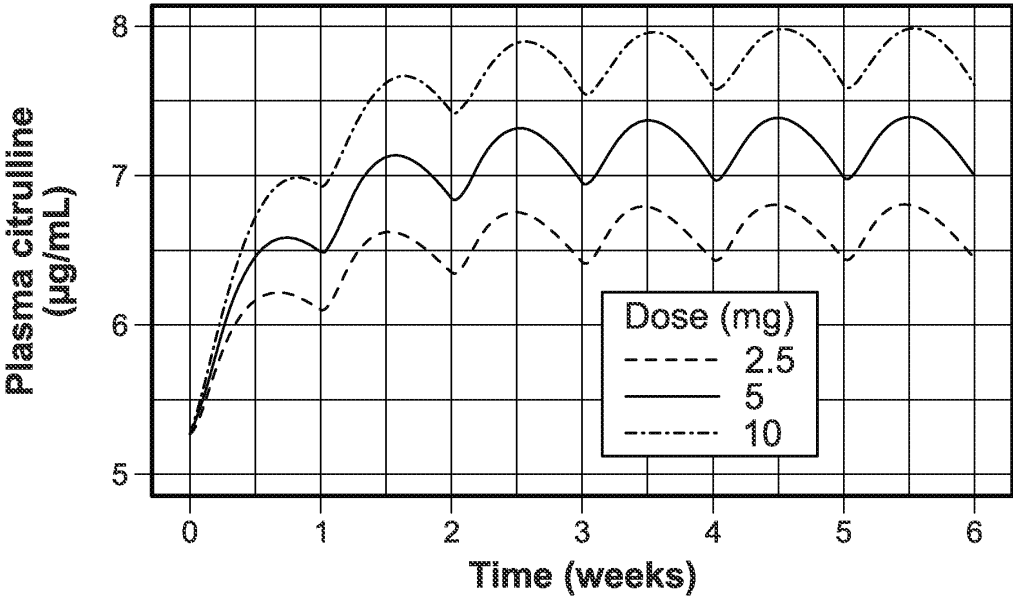
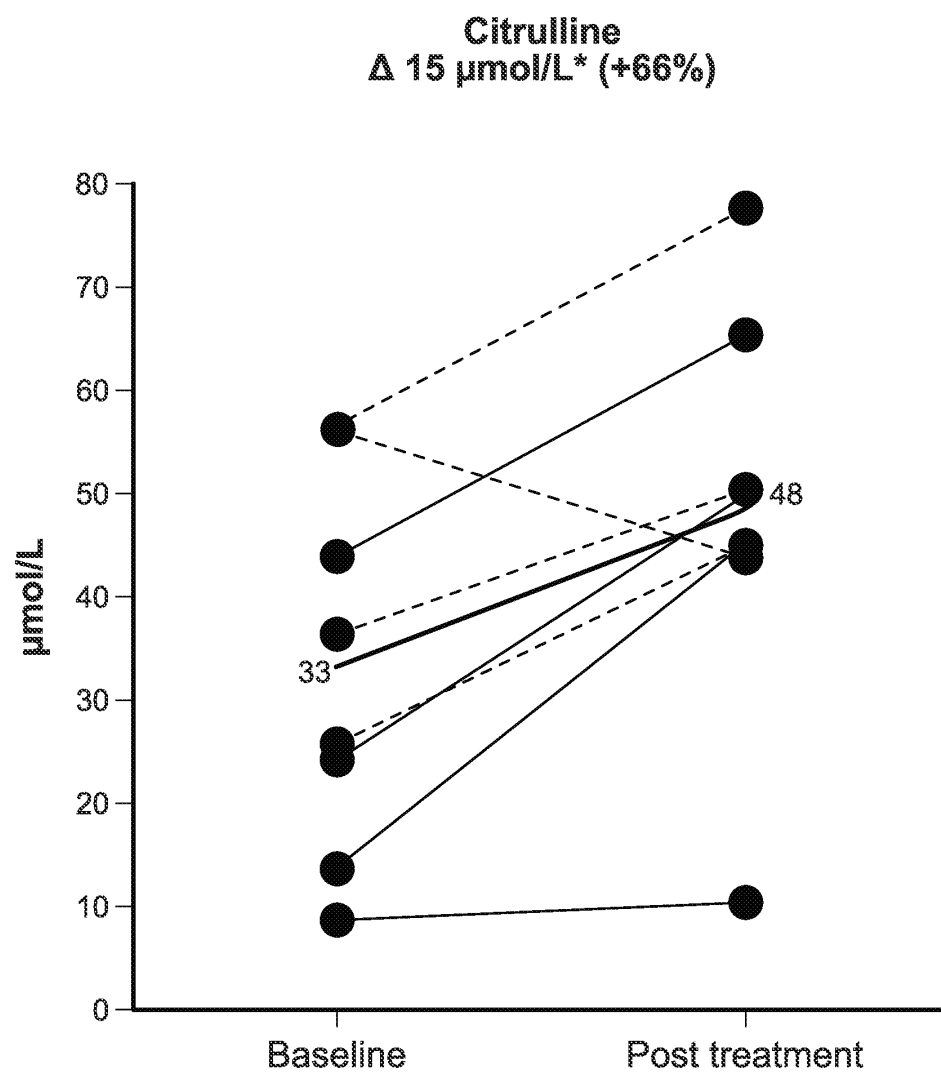


FIG. 26B

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**FIG. 27**

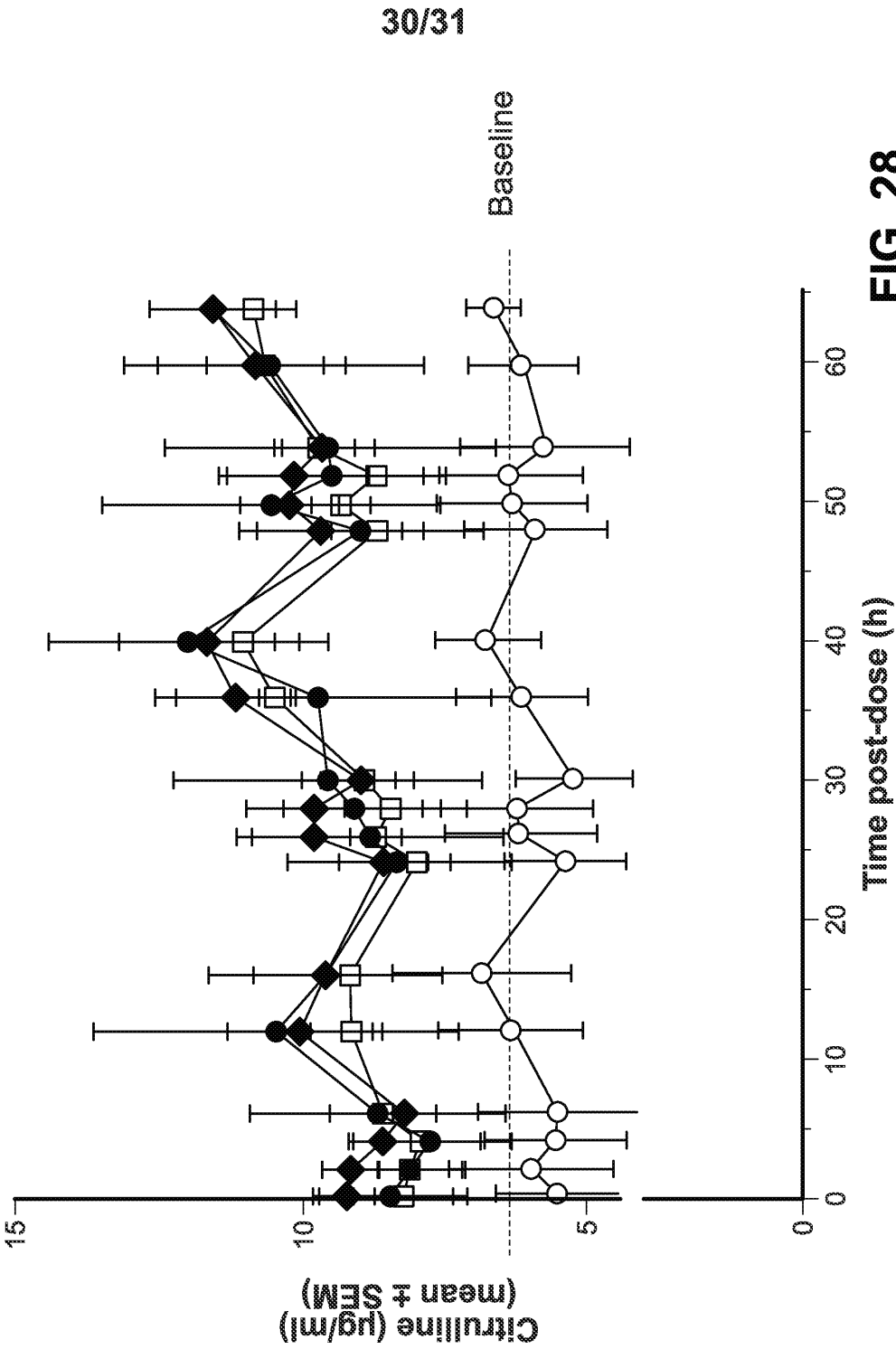


FIG. 28

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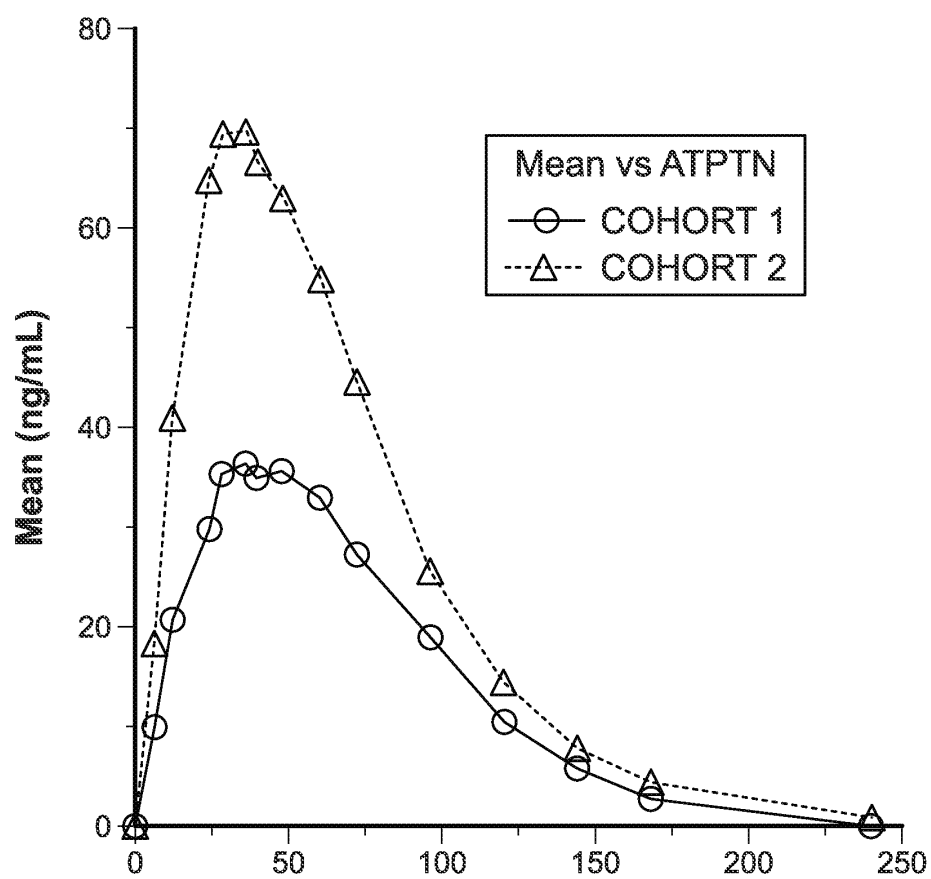


FIG. 29