# Pharmacokinetics and Safety of Intravenous Ferric Pyrophosphate Citrate: Equivalence to Administration via Dialysate

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**Abstract**

Ferric pyrophosphate citrate (FPC) is indicated to maintain hemoglobin in patients with stage 5 hemodialysis-dependent chronic kidney disease on chronic hemodialysis by addition to the dialysate. An intravenous (IV) FPC presentation containing 6.75 mg of iron in 4.5 mL was developed. The objective was to establish the equivalence of iron delivery via dialysate and IV infusion using a pharmacokinetic approach. An open-label, randomized, multiple-period, single-dose, crossover study was conducted in 27 patients with CKD-5HD. Each patient received (1) a basal iron profile over 12 hours, (2) FPC 6.75 mg Fe IV predialyzer, (3) FPC 6.75 mg Fe IV postdialyzer, and (4) FPC 2 $\mu$M (110 $\mu$g Fe/L of hemodialysate). Serum and plasma iron was analyzed for total Fe and transferrin bound iron (TBI). Equivalence was determined by comparing maximum observed concentration and area under the concentration-time curve from time 0 to the last observation of 110 $\mu$g Fe/L of hemodialysate (reference) and test treatments Fe predialyzer and postdialyzer iron profiles. The main outcome measure was the measurement of bioequivalence between the reference and test treatments. Bioequivalence parameters showed that infusion of FPC iron IV, predialyzer and postdialyzer delivered equivalent iron as via hemodialysate. The increment in serum total Fe from predialysis to postdialysis was the same as observed in the long-term clinical studies of FPC. FPC IV was well tolerated. IV infusion of 6.75 mg iron as FPC during 3 hours of HD delivers an equivalent amount of iron as when Triferic is delivered via hemodialysate. The IV presentation of FPC extends the ability to provide FPC iron to all patients receiving hemodialysis or hemodiafiltration.

**Keywords**

equivalence, hemodialysis, iron, IV, pharmacokinetics, transferrin

The goals of iron therapy in adult patients with stage 5 hemodialysis-dependent chronic kidney disease (CKD-5HD) are to avoid depletion of iron stores, prevent iron-restricted erythropoiesis and maintain hemoglobin levels while minimizing erythropoiesis-stimulating agent therapy and avoiding blood transfusions that may sensitize patients and limit chances for a kidney transplant.[1]

Iron supplementation with macromolecular intravenous iron (m-IVFe) is provided to patients who receive maintenance hemodialysis (HD). However, these intravenous (IV) iron products are ferric hydroxide cores within a carbohydrate shell that only donate free unbound iron directly to transferrin. Instead, the complexes must be taken up by reticuloendothelial macrophages that free the iron from the carbohydrate shell for subsequent export to the blood via ferroportin. In HD patients, a considerable portion of the iron derived from these iron-carbohydrate complexes is sequestered within macrophages and is not readily available for transport to the erythroid marrow for use in hemoglobin synthesis due to the high concentrations of hepcidin that block iron absorption from the duodenum and iron egress from stores in macrophages.[2] The use of m-IVFe, which is indicated for repletion of iron stores and correction of hemoglobin in iron deficiency anemia, is primarily used as a maintenance therapy in patients who receive HD in efforts to reduce the doses of erythropoiesis-stimulating agent.[3] Consequently, iron stores in adult patients, as measured by serum ferritin levels, have increased from 200 $\mu$g/L in the 1990s to over 800 $\mu$g/L in 2019.[4] This change in the management of anemia have led to concerns that increased use of m-IVFe may produce iron overload and contribute to inflammation, oxidative stress, endothelial dysfunction,

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cardiovascular disease, immune deficiency, and the risk of bacterial infections.[5,6]

Triferic (ferric pyrophosphate citrate, FPC), added to the liquid bicarbonate concentrate for HD, is approved in the United States as a maintenance iron therapy. FPC donates iron directly to transferrin for optimal utilization in erythropoiesis, avoiding sequestration within reticuloendothelial macrophages.[7] When administered to adults via the dialysate at each HD session, FPC maintains hemoglobin concentrations without increasing iron stores.[8] FPC provides 5 to 7 mg of elemental iron with each HD session, which is the amount of iron that is typically lost due to retained blood in the dialyzer circuit plus other HD- and uremia-associated blood losses.

Not all HD machines use liquid bicarbonate as part of the 3-steam manufacture of dialysate. Solid bicarbonate cartridges or bags and online generation of ultrapure dialysate for pre- or postdilutional hemodiafiltration are increasingly available.[9,10] FPC cannot be added to the solid bicarbonate due to differential solubility of FPC compared to sodium bicarbonate and uniformity of dispersal. An IV presentation of FPC, for administration directly into the pre- or postdialyzer blood lines, makes FPC available to all HD patients regardless of machine configuration (liquid bicarbonate, solid bicarbonate, or hemodiafiltration).

This study was conducted to establish the equivalence of the IV FPC presentation to the approved FPC delivery via dialysate in patients receiving conventional HD treatments.

## Methods

The protocol was reviewed and approved by an independent central institutional review board (IntegReview, Austin, Texas) before enrolling patients. The study adhered to the Declaration of Helsinki, and informed consent was obtained from all patients. The study was listed on Clinicaltrials.gov under NCT03303144 (October 5, 2017).

### Study Design

An open-label, randomized, multiple-period, single-dose, crossover study was conducted in 27 patients with CKD-5HD (Figure S1). Each patient received (1) a basal iron profile over 12 hours, followed by a randomized sequence; (2) FPC 6.75 mg Fe IV predialyzer; (3) FPC 6.75 mg Fe IV postdialyzer; and (4) FPC 2 $\mu$M (110 $\mu$g Fe/L of hemodialysate).

### Test Products

FPC solution for hemodialysis was provided as 5-mL ampules containing 27.2 mg Fe (5.44 mg/mL). The contents of one ampule was added to 2.5 gallon (9.5 L) of liquid bicarbonate concentrate. Acid concentrate, liquid bicarbonate concentrate, and reverse osmosis water are proportioned in the HD machine (mixing ratio, 1:1.72:4.28) to produce hemodialysate (with or without FPC). The final dialysate iron concentration is 2 $\mu$M Fe (110 $\mu$g Fe/L). Patients were dialyzed for 4 hours at a blood flow rate of 350 mL/min and a dialysate flow rate of 500 mL/min.

FPC for IV use contains 6.75 mg Fe/4.5 mL in low-density polyethylene blowfill sealed luer lock ampules. The ampule is designed for easy, needle-free withdrawal of the dosing solution. The contents of the vial are drawn up into a syringe, attached to the HD machine syringe pump and administered IV over 3 hours predialyzer or postdialyzer using existing connections to the blood lines.

### Pharmacokinetic Methods

Blood samples for the pharmacokinetic (PK) analysis were collected at the following times: 0 (before dosing), 1, 2, 3, 3.5, 4, 4.5, 5, 6, 8, 10, and 12 hours. The 0-hour sample for all visits was obtained as close to 8 am as possible, just before the start of HD. Total serum iron ($sFe_{total}$), transferrin saturation (TSAT), total iron-binding capacity (TIBC), and ferritin values were determined by the clinical laboratory using established methods on the COBAS (Roche, Basel, Switzerland) platform. The clinical assay for iron was a Gen2 Fe assay. Under acidic conditions, iron is liberated from transferrin. Ascorbate reduces the released $Fe3^+$ ions to $Fe2^+$ ions, which then react with ferrozine to form a colored complex. The color intensity is directly proportional to the iron concentration and can be measured photometrically. The quantification range for the assay is 0 to 1000 $\mu$g Fe/dL.

To establish equivalence between the dialysate administration of FPC and IV administration, plasma iron (pFe) and transferrin-bound iron (TBI) were analyzed according to the US Food and Drug Administration (FDA) product-specific guidance.[11] Plasma iron was determined by inductively coupled plasma mass spectrometry (ICP-MS) and TBI was quantified using a liquid chromatography–ICP-MS technique (Supplemental Information). All methods were validated according to the 2015 recommendations for bioanalytical methods.[12]

PK parameters were calculated with Phoenix WinNonlin 6.3 (Certara, Princeton, New Jersey) using actual sampling times. All iron end points were summarized using actual and baseline-corrected values by treatment using nominal PK sampling times. Estimated PK parameters included maximum observed concentration ($C_{max}$), time to $C_{max}$, time of last observation, area under the concentration-time curve from time 0 to the last observation ($AUC_{0\text{-last}}$), and calculated half-life. The nominal dose of FPC iron is 6.75 mg; however,

due to the residual volume in the delivery line upon completion of the IV infusion, a dose of 6.5 mg iron was used for the calculation of dose-dependent parameters.

Equivalence to the reference treatment (FPC via Dialysate) was formally assessed on the basis of $AUC_{0\text{-last}}$ and $C_{max}$ for pFe and TBI, using a two 1-sided hypothesis (alpha = 0.05) test approach. The primary analyses were performed on natural log-transformed data. Upon back transformation, standard bioequivalence boundaries for the geometric mean for $AUC_{0\text{-last}}$ and $C_{max}$ between 0.80 and 1.25 were assessed for both absolute values and baseline corrected values.

### Subjects

Healthy male and female HD patients aged 18 to 55 years with a body mass index between 20.0 and 32.0 kg/m$^2$ and no clinically relevant abnormalities based on medical history, physical examination, vital sign measurements, electrocardiograms, or safety laboratory tests were enrolled in the study. Female subjects were not pregnant or breastfeeding and were at least 9 months postpartum. Subjects agreed not to use iron preparations during the 14 days before administration of the test material and to use acceptable birth control measures for the duration of the study.

Subjects were excluded if they had a hemoglobin concentration of <11 g/dL, a hematocrit of <30%, or a serum iron concentration of <70 $\mu$g/dL (male or female) at screening or had received IV iron within the previous 14 days.

Subjects were dialyzed for 4 hours each period using 1.7m$^2$ dialyzers (Polysulfone; Fresenius F180nr [Fresenius Renal Care, Waltham, Massachusetts], n = 25; or Cellulose Triacetate; Baxter Exceltra 190 [Baxter International, Deerfield, Illinois], n = 2). The dialysate composition was acetate based with Ca = 2.5 mEq/L and K = 3.0 mEq/L. All subjects received intermittent bolus heparin to maintain dialyzer patency.

**Table 1.** Demographics

| Characteristics | N = 27 |
|---|---|
| Age | |
| Mean (SD) | 53.9 (8.11) |
| Min-max | 36-68 |
| Weight, kg | |
| Mean (SD) | 84.5 (14.26) |
| Min-max | 51.1-121.5 |
| Race | |
| White, n(%) | 4 (14.8) |
| Black n(%) | 23 (85.2) |
| Baseline laboratory values | |
| CRP, ng/mL | |
| Mean (SD) | 0.91 (0.71) |
| Min-max | 0.01-2.44 |
| Normal | 0.9-27 |
| sFe, $\mu$g/dL | |
| Mean (SD) | 65.1 (24.90) |
| Min-max | 28-129 |
| Normal | 25-156 |
| TSAT (%) | |
| Mean (SD) | 29.9 (12.92) |
| Min-max | 11-63 |
| Normal | 16 - 45 |
| Ferritin, $\mu$g/L | |
| Mean (SD) | 1152 (575.00) |
| Min-max | 281-2679 |
| Normal | 10-322 |
| TIBC, $\mu$g/dL | |
| Mean (SD) | 222.9 (26.68) |
| Min-max | 177-280 |
| Normal | 200-450 |
| Transferrin, $\mu$g/dL | |
| Mean (SD) | 170.9 (34.63) |
| Min-max | 108-240 |
| Normal | 215-380 |
| Hgb (g/dL) | |
| Mean (SD) | 11.9 (1.4) |
| Min-max | 9.2-15.2 |
| Normal | 12-16 |

CRP, C-reactive protein; Hgb, hemoglobin; Min, minimum value; Max, maximum value; SD, standard deviation; sFe, serum iron; TIBC, total iron-binding capacity; TSAT, transferrin saturation.

## Results

A total of 27 patients were enrolled in the study. The patient demographics are summarized in Table 1. All patients were iron replete as measured by baseline iron parameters. Due to the variability in basal serum iron concentration, each patient served as their own control to assure accurate baseline corrected PK parameters.

To establish equivalence between dialysate delivered iron and iron delivered IV it was necessary to develop validated assays for total plasma iron ($pFe_{total}$) and TBI. The TBI assay showed a high correlation with the clinical sFe assay (Figures S8 and Figure S9). Hemolysis had an impact on the ICP-MS assay for $pFe_{total}$ due to the presence of free hemoglobin and haptoglobin released from lysed red blood cells. A priori rules for excluding nonphysiologic $pFe_{total}$ values were in place before the analysis of plasma iron and TBI were started.

Baseline iron concentrations demonstrated no diurnal variation in sFe, TBI, TSAT, or TIBC (Figure 1A and B). With administration of FPC via dialysate, there is a linear increase in serum iron, TBI, and TSAT with the peak concentration observed at the end of dialysis (t = 4 hours). Infusion of the IV FPC iron over 3 hours showed a similar linear increase in absolute and baseline corrected $pFe_{total}$, TSAT, and TBI concentrations, with $C_{max}$ observed at the end of the 3-hour infusion (Figure 1A and B). Changes in absolute TSAT followed the changes in $pFe_{total}$ and TBI concentrations (Figure 2). There was no impact of ultrafiltration as evidenced by

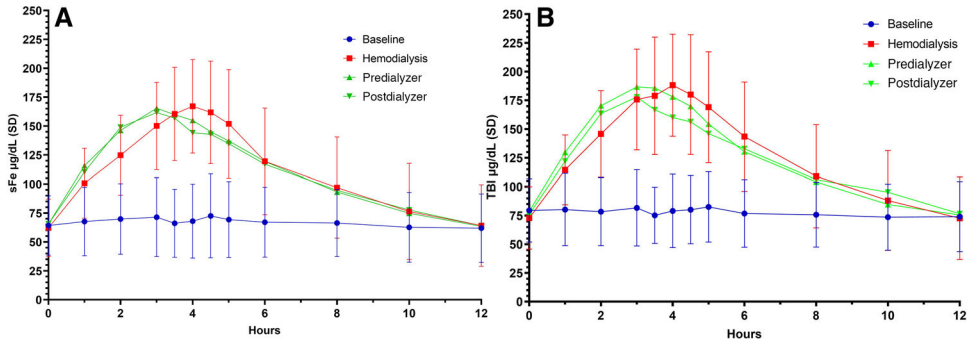


**Figure 1.** Serum iron and transferrin bound iron for ferric pyrophosphate citrate (FPC) administration by dialysate and intravenously. Treatment: ● Baseline (no iron); ■ FPC dialysate; ▲ FPC predialyzer; ▽ FPC postdialyzer. (A) Serum iron concentration (sFe) by time and treatment. (B) Transferrin-bound iron (TBI) concentration by time and treatment (mean ± standard deviation [SD]). FPC iron was administered at a dialysate concentration of 110 $\mu$g/L at a dialysate flow rate of 650 mL/min for 4 hours. Intravenous FPC was administered into the predialyzer or postdialyzer blood lines over 3 hours by the on-machine syringe pump. The iron concentration was 1.5 mg Fe/mL, the total amount of iron administered was 6.5 mg to accommodate the small quantity of solution remaining in the tubing between the syringe head and the blood line.

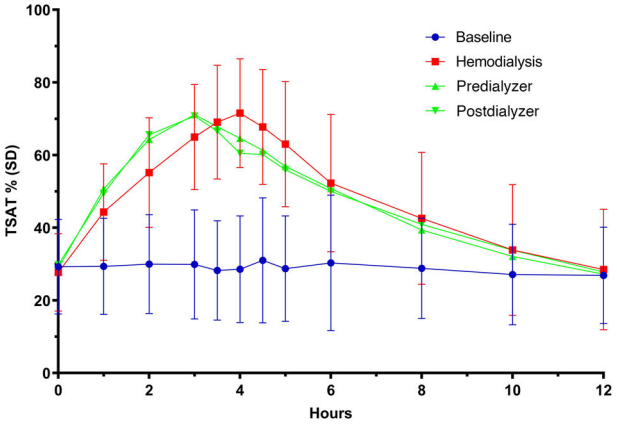


**Figure 2.** Transferrin saturation for administration of ferric pyrophosphate citrate (FPC) by dialysate and intravenously. Treatment: ● Baseline (no iron); ■ FPC dialysate; ▲ FPC predialyzer; ▽ FPC postdialyzer. The transferrin saturation (TSAT) (mean ± standard deviation [SD]) paralleled the serum iron concentration. The maximum TSAT at time of maximum concentration was 68.5% (95%CI, 64.9-72.0) for hemodialysis administration and 67.8% (95%CI, 64.5-71.6) for intravenous administration predialyzer and 65.9% (95%CI, 62.0-69.8) for intravenous administration postdialyzer.

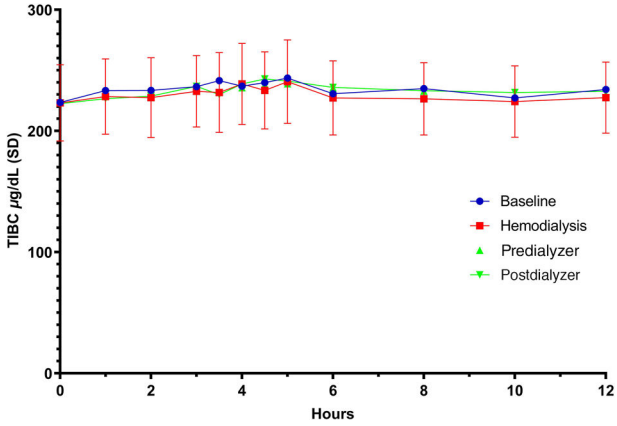


**Figure 3.** Iron binding capacity for administration of ferric pyrophosphate citrate (FPC) by dialysate and intravenously. Treatment: ● Baseline (no iron); ■ FPC dialysate; ▲ FPC predialyzer; ▽ FPC postdialyzer. Total iron-binding capacity (TIBC) (mean ± standard deviation [SD]) was unchanged during the 4 hours of hemodialysis indicating that volume removal during hemodialysis did not affect the measurements of iron.

the lack of change in TIBC, a surrogate measure of transferrin concentrations (Figure 3).

Iron was rapidly cleared after IV administration with an apparent half-life of ≈2.5 hours. Serum iron and TBI concentrations returned to baseline by 8 hours after the cessation of infusion or dialysis (Figure 1A and B) for all FPC administration methods (dialysate and IV). PK parameters for absolute sFe and baseline corrected sFe (BLC sFe) are presented in Table 2. Baseline corrected values reflect the addition of FPC iron to the basal iron concentrations. There is no difference in $C_{max}$ and clearance of FPC iron between dialysate and IV administration.

Equivalence calculations followed the method recommended by the US FDA for $pFe_{total}$ and TBI. The 90%CI of the geometric mean of the ratio between the reference treatment (FPC via dialysate) and the test treatment (FPC IV pre- or postdialyzer) were contained within the bounds of 80% to 125% indicative of equivalence between the two methods of delivery for absolute pFe and TBI (Table 3) as well as baseline-corrected pFe and TBI (Table 4).

## Discussion

FPC administered via dialysate is the only iron replacement product indicated to maintain iron balance and hemoglobin concentrations in HD patients. The clinical trials that supported approval used a final dialysate iron concentration of 2 $\mu$M (110 $\mu$g Fe/L) in iron-replete

**Table 2.** Noncompartmental Pharmacokinetic Parameters for Absolute and Baseline Corrected Serum Fe

| | | $C_{max}$, $\mu$g/dL | $AUC_{0\text{-last}}$, $\mu$g • h/dL | $t_{\frac{1}{2}}$, h |
|---|---|---|---|---|
| FPC-dialysate mean (SD), N = 26 | Absolute | 216 (60.4) | 1570 (492) | 7.32 (3.70) |
| | BLC | 141 (65.7) | 541 (346) | 3.04 (1.94) |
| Predialyzer mean (SD), N = 25 | Absolute | 219 (43.9) | 1600 (430) | 7.86 (2.87) |
| | BLC | 138 (49.6) | 535 (180) | 2.03 (0.798) |
| Postdialyzer mean (SD), N = 25 | Absolute | 201 (48.6) | 1520 (465) | 7.04 (3.70) |
| | BLC | 121 (54.7) | 485 (262) | 2.62 (1.19) |

$AUC_{0\text{-last}}$, area under the concentration-time curve from time 0 to the last observation; BLC, baseline corrected; $C_{max}$, maximum observed concentration; SD, standard deviation; $t_{\frac{1}{2}}$, half-life.

**Table 3.** Absolute pFe and TBI Equivalence Parameters FPC-IV vs FPC-Dialysate

| Plasma Analyte | Treatment | Parameter | Geometric LS Mean (FPC-IV) | Geometric LS Mean (FPC-Dialysate) | Test/Reference (%) | 90%CI |
|---|---|---|---|---|---|---|
| Total plasma iron, N = 16 | Predialyzer | $C_{max}$ | 211.39 | 207.52 | 101.9 | 93.3-111.2 |
| | | $AUC_{0\text{-last}}$ | 1524.50 | 1489.88 | 102.3 | 94.7-110.6 |
| | Postdialyzer | $C_{max}$ | 194.41 | 207.52 | 93.7 | 85.8-102.2 |
| | | $AUC_{0\text{-last}}$ | 1441.95 | 1489.88 | 96.8 | 89.6-104.6 |
| TBI, N = 15 | Predialyzer | $C_{max}$ | 193.71 | 186.73 | 103.7 | 97.5-110.4 |
| | | $AUC_{0\text{-last}}$ | 1419.92 | 1411.27 | 100.6 | 91.4-110.8 |
| | Postdialyzer | $C_{max}$ | 178.25 | 186.73 | 95.5 | 89.7-101.6 |
| | | $AUC_{0\text{-last}}$ | 1288.90 | 1411.27 | 91.3 | 82.9-100.6 |

$AUC_{0\text{-last}}$, area under the concentration-time curve from time 0 to the last observation; $C_{max}$, maximum observed concentration; FPC, ferric pyrophosphate citrate; IV, intravenous; LS, least squares; pFe, plasma total iron; SD, standard deviation; TBI, transferrin-bound iron.

**Table 4.** Baseline Corrected Equivalence Parameters FPC-IV vs FPC-Dialysate

| Plasma Analyte | Treatment | Parameter | Geometric LS Mean (FPC-IV) | Geometric LS Mean (FPC-Dialysate) | Test/Reference (%) | 90%CI |
|---|---|---|---|---|---|---|
| Total plasma iron, N = 26 | Predialyzer | $C_{max}$ | 210.73 | 205.78 | 102.4 | 93.5-112.1 |
| | | $AUC_{0\text{-last}}$ | 1523.03 | 1472.13 | 103.5 | 95.8-111.7 |
| | Postdialyzer | $C_{max}$ | 198.15 | 205.78 | 96.3 | 87.9-105.5 |
| | | $AUC_{0\text{-last}}$ | 1479.86 | 1472.13 | 100.5 | 93.0-108.6 |
| TBI, N = 25 | Predialyzer | $C_{max}$ | 191.32 | 190.68 | 100.3 | 95.0-106.0 |
| | | $AUC_{0\text{-last}}$ | 1391.50 | 1467.93 | 94.8 | 87.4-102.9 |
| | Postdialyzer | $C_{max}$ | 179.60 | 190.68 | 94.2 | 89.2-99.4 |
| | | $AUC_{0\text{-last}}$ | 1299.41 | 1467.93 | 88.5 | 81.7-96.0 |

$AUC_{0\text{-last}}$, area under the concentration-time curve from time 0 to the last observation; $C_{max}$, maximum observed concentration; FPC, ferric pyrophosphate citrate; IV, intravenous; LS, least squares; SD, standard deviation; TBI, transferrin-bound iron.

patients. Hemoglobin concentrations were maintained at baseline values for up to 48 weeks without requiring supplemental traditional IV iron.[8] It was postulated that FPC delivered between 5 and 7 mg of iron at each treatment, but determining the exact dose was difficult. Measuring iron in expended dialysate was not accurate due to the low concentration and interference by uremic solutes in the dialysate. Therefore, a PK approach to the determination of net iron delivery was used during the development of an IV presentation that could be administered to patients that dialyzed on machines that used solid bicarbonate rather than liquid bicarbonate as part of the 3-stream dialysate production.

The PK of FPC are dose proportional up to a TSAT of 100%.[13] Therefore, a comparison between a known quantity of FPC iron and measuring the $AUC_{0\text{-t}}$ indicates the dose of iron administered.

Analyses of iron end points were based on absolute and time-matched baseline-corrected data. There was no clear circadian pattern in the standard basal HD iron profile as was seen in the study of FPC administered to healthy volunteers.[13] $pFe_{total}$ was determined by the bioanalytical laboratory using ICP-MS, and $sFe_{total}$ concentrations were determined by the clinical laboratory using the autoanalyzer-based Ferrozine assay. Both assays, while accurate, have different limitations.

The ICP-MS assay measures all iron in the sample, including iron bound to proteins (hemoglobin, haptoglobin, ferritin, and transferrin). Therefore, if any degree of hemolysis is present in the sample, total iron concentrations will be higher and will not accurately reflect the amount of iron that was administered via HD or IV. Samples were visually graded as to the presence of hemolysis to avoid inaccuracies in $pFe_{total}$ data analyzed by ICP-MS. The doses of FPC that were used in this study were selected to not exceed the unsaturated iron binding capacity of the subjects; therefore, concentrations of total Fe >1.25-fold of the time-matched TIBC value were excluded from the PK and equivalence analyses.

Clinical laboratory samples were collected at the same time points as the samples that were analyzed by the bioanalytical laboratory. The clinical assay has high precision due to the automated nature of the analyzer and does not measure iron covalently bound to heme proteins or ferritin. Lithium heparin in plasma is known to reduce the recovery of iron spiked to plasma. In this study, clinical laboratory samples were collected as serum in clot-activator serum separator tubes. However, due to the need to administer unfractionated heparin (UFH) to HD patients to prevent clotting within the dialyzer circuit, some inaccuracy in $sFe_{total}$ levels is possible even though UFH is not known to interfere with the autoanalyzer method for sFe.[14] Although the clinical assay is not validated to the same extent as the bioanalytical laboratory assay, it is highly relevant and accurate, as it is the assay used for patient care.

The existing chromogenic assays for TBI, which use separation of loosely bound iron via solid phase extraction and spectrophotometric assay, are not suitable for analysis of TBI in HD patients due to the presence of low concentrations of UFH and the slow development of fibrin strands on freezing of samples for transport.[15] The validated plasma TBI assay was developed using liquid chromatography followed by ICP-MS assay for iron. The column effluent was continuously injected into an ICP-MS analysis system. The concentration of TBI was quantitated by the area under the TBI peak. This method clearly separates the analyte of interest (TBI) from other iron-containing proteins, non–transferrin-bound iron (NTBI), and free unbound FPC. The speciation of iron from IV iron supplements has been an interest of regulatory authorities involved in the approval of nanoparticulate iron products. Because FPC is not a nanoparticle, measurement of total iron and TBI is sufficient to establish the lack of NTBI upon FPC administration. There are similarities between the method of Neu et al[16] and our assay in that both use liquid chromatography–ICP-MS in a low-ionic-strength buffer to separate iron species as well as to show any low-molecular-weight iron species. Because FPC is administered as small doses (≈6.5 mg/treatment), the generation of toxic NTBI is unlikely. This analysis also demonstrates that FPC is not present in high-molecular-weight aggregates, which require processing in macrophages to release the iron and may contribute to some of the adverse drug reactions noted with m-IVFe.[17–20]

Analysis of $C_{max}$ and $AUC_{0\text{-last}}$ for $pFe_{total}$ and TBI were the co–primary end points in this study to demonstrate the equivalence of dialysate-delivered FPC iron to IV-administered FPC iron. When FPC was administered via HD, $pFe_{total}$ concentrations peaked at 4 hours after the start of HD and returned to baseline levels by 12 hours. FPC iron (6.75 mg) administered IV via the predialyzer or postdialyzer line met the criteria for bioequivalence to FPC iron administered via the hemodialysate as measured by $C_{max}$ and $AUC_{0\text{-last}}$ for $pFe_{total}$, TBI, and sFe. The equivalence of predialyzer and postdialyzer iron delivery, confirms that the delivery of FPC iron immediately binds to transferrin and there is no free FPC, albumin-bound iron or NTBI to diffuse from blood to dialysate.

The secondary end points, baseline-corrected $C_{max}$ and $AUC_{0\text{-last}}$ for FPC iron (6.75 mg) administered IV predialyzer or postdialyzer met the criteria for bioequivalence to FPC iron administered via the hemodialysate.

The goal of this study was to bridge the delivery of FPC via the dialysate with IV administration of FPC. FPC shows dose-proportional PK up to a TSAT of 100%. The demonstration that the new IV presentation for administration into the blood lines delivers ≈6.5 mg of iron with each treatment corroborates the observations of the phase 2 and 3 clinical trials. In the pivotal clinical trials, the pre- to post-HD increment in serum total iron concentration demonstrated an increase of ≈100 $\mu$g Fe/dL. This increment corresponded to a maximum TSAT of 70% to 80%. The results of the clinical studies that examined the equivalence of dialysate-delivered FPC to IV-delivered FPC are shown in Figure 4. There was no difference between the observed pre- to postdialysis sFe in long-term FPC studies (CRUISE-1 and -2) and the current study. The delivery of 6.5 mg Fe/treatment for 1 year (156 treatments) would yield a total iron dose of 1014 mg. This is in contrast with current regimens of m-IV Fe, which deliver between 1200 and 3000+ mg/year depending on individual protocols. The rising ferritins in the United States, currently averaging 835 $\mu$g/L (95%CI, 181-1552)[21] can lead to iron overloading to maintain hemoglobin.[22,23] FPC can bypass the hepcidin block to iron exit from the reticuloendothelial macrophages, allowing administration of small amounts of immediately bioavailable iron to accommodate for ongoing

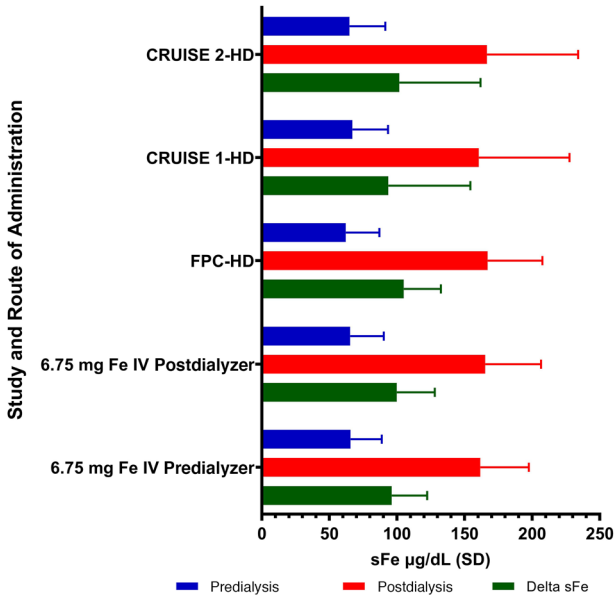


**Figure 4.** Pre- to post-hemodialysis change in serum iron by route of administration. Serum iron (sFe) (mean ± standard deviation [SD]) was measured at the start of hemodialysis (HD) and at the end of HD when ferric pyrophosphate citrate (FPC) was added to the dialysate at a concentration of 110 $\mu$g Fe/L of dialysate during the 2 large placebo controlled clinical studies (CRUISE-1 and CRUISE-2).[8] Similarly, sFe was measured at the start of hemodialysis and at the end of the intravenous (IV) infusion (t = 3 h) in the current equivalence study. The difference between pre- and post-hemodialysis serum iron and the change in serum iron (Delta sFe) was similar across HD and IV administration. Administration of FPC IV (6.75 mg Fe/4.5 mL) delivers the same quantity of iron as observed in the clinical studies of dialysate administered FPC.

iron losses and for incorporation into hemoglobin. FPC may also improve the efficiency of erythropoiesis.[24]

Hemodialysate can be used as a vehicle for administration of various compounds. Phosphate-enriched dialysate has been used for short-term phosphate supplementation in certain patients.[25] L-carnitine has also been administered via dialysate to maintain tissue carnitine levels.[26] FPC (Triferic) is the first drug approved in the United States as an iron replacement product to be added to the dialysate for the maintenance of hemoglobin in patients receiving chronic hemodialysis. The advantages of FPC added to dialysate include improving erythropoietic efficiency and reducing the total amount of iron administered to HD patients.[27] Macromolecular IV iron products cannot be added to the dialysate because they are nanoparticles that cannot diffuse across the dialyzer membrane. The IV formulation (Triferic AVNU) was approved by the FDA based on this equivalence study demonstrating that the amount of iron delivered via dialysate is the same as delivered in the approved IV presentation (6.75 mg Fe[III]/4.5 mL). The approved product labeling for FPC is not intended for use in peritoneal dialysis and has not been studies in patients receiving home hemodialysis.

FPC was well tolerated when administered as a continuous IV infusion or via dialysate. No unexpected serious adverse events were reported. One patient completed the study but reported an unrelated adverse event (exacerbation of chronic obstructive pulmonary disease) that occurred 3 days after the final on-study HD. Another patient required emergency surgery for vascular access complications 3 days after the second treatment (FPC IV predialyzer) leading to withdrawal from the study due to a prolonged hospitalization. This patient's sFe and TBI had returned to baseline values by 8 hours after IV infusion of FPC, and the event was considered unrelated to FPC.

## Conclusion

The results of this study demonstrate that FPC iron (6.5 mg) administered IV via the predialyzer (arterial) or postdialyzer (venous drip chamber) blood lines delivered an equivalent quantity of iron as FPC administered via the hemodialysate. FPC was well tolerated, with no unexpected adverse events. IV administration of FPC at each HD is a suitable therapeutic option for hemoglobin maintenance in patients who dialyze on machines using solid bicarbonate delivery or hemodiafiltration.

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Nuventra Inc. (Durham, North Carolina) performed the PK and bioequivalence analysis. QPS Netherlands BV (Groningen, The Netherlands) optimized, validated, and performed the bioanalytical methods for $pFe_{total}$ and TBI. Innovative Analytics (Kalamazoo, Michigan) provided database management and medical writing. The clinical studies were conducted at Orlando Clinical Research Center (Orlando, Florida). The authors thank the staff of Orlando Clinical Research Center and the patient volunteers who participated in this study.

## Conflicts of Interest

R.D.P. is a full-time employee of Rockwell Medical Inc. and receives equity in the form of salary, stock grants, and stock options. F.H. and E.H. are full-time employees of QPS Netherlands BV. T.M. received payment for conducting the clinical trial.

## Author Contributions

R.D.P. conceived the experiments; designed the research; supervised analysis, study report, and figure generation; and wrote and reviewed the manuscript. F.H. and E.H. developed and validated the assay for TBI, performed the bioanalytical assays, and reviewed the manuscript. T.M. performed the research, oversaw patient safety and protocol execution, and reviewed the manuscript.

## Data Sharing

Please contact the corresponding author at rpratt@rockwellmed.com.

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