CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology Review

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Submission Date(s)	December 6, 2016; SDN 2 May 5, 2017; SDN 13
Submission Type	New NDA 505(b)(2)
Brand Name	Gliolan®
Generic Name	5-aminolevulinic acid hydrochloride [5-ALA HCl] for oral solution
Dosage Form and Strength	Powder for oral solution, 1.5 g / vial
Route of Administration	Oral
Dosing Regimen	20 mg 5-ALA HCl per kg body weight (corresponding to 15.6 mg 5-ALA per kg bw), orally 3 hours (2 to 4 hours) before anesthesia.
Indication (s)	To facilitate the real time detection and visualization of malignant tissue during glioma surgery.
Applicant	NX Development Corp
Associated IND/NDA	IND 110655
OCP Review Team	Christy S John, Ph.D., Gene Williams, Ph.D.

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1. EXECUTIVE SUMMARY

Gliolan® (5-aminolevulinic acid hydrochloride for oral solution, 5-ALA HCl) is an imaging agent to facilitate the real time detection and visualization of tumor tissue during glioma surgery. Gliolan® is administered as an oral solution two to four hours prior to surgery.

Gliolan is a pro-drug, the active moiety is the metabolite protoporphyrin IX (PpIX), which like 5-ALA, is found endogenously. During neurosurgery, Gliolan is used with an operating microscope adapted with a blue emitting light source and filters for excitation light of wavelength 400 to 410 nm, and observation at wavelengths of 620 to 710 nm. This allows tumor tissue to be visualized as red fluorescence. Tissue lacking sufficient PpIX concentrations appears blue.

The diagnostic efficacy of 5-ALA HCl is demonstrated by a high positive predictive value (PPV) in identifying glioma tissue that may otherwise be overlooked during conventional (white light) resection. Three studies demonstrated the efficacy of 5-ALA HCl (Study MC-ALS.28/GLI, Study MC-ALS.30/GLI, and Study MC-ALS.3/GLI) all utilized the 20 mg/kg dosing regimen. Biopsy-based estimates of PPV across the three studies were high (98-100% in the strong fluorescent group and 92-97% in the weak fluorescent group). The PPV for strong (i.e., red) fluorescence was slightly higher in each study compared to the PPV for weak (i.e., pink) fluorescence.

The recommended dose is 20 mg/kg. The solution is swallowed by the patient three hours (range two to four hours) prior to induction of anesthesia. If the anesthesia/surgery is delayed by some hours, additional doses are not given. If the dose is not taken two to four hours prior to anesthesia, anesthesia and surgery are to be postponed for at least two hours. If the surgery is delayed by one or more days, another dose can be administered two to four hours prior to anesthesia.

In Study MC-ALS.8-I/GLI, the applicant studied the dose-efficacy relationship between three dose levels (0.2, 2 and 20 mg/kg). A dose-efficacy relationship between the dose levels and the extent and quality of fluorescence in the tumor core was present; the highest dose of 20 mg/kg was most effective.

Literature data shows that after oral administration of about 40 mg/kg, mild nausea, vomiting, hypotension, transient increases in liver enzymes and photo-toxicity occurred. Higher doses (30 to 60 mg/kg) appear to be associated with an increase in the frequency of adverse effects.

Considerable variability in fluorescence intensity was observed for the dose of 20 mg/kg. Doses between 2 and 20 mg/kg were not studied. Thus, the ability of doses from 2 to 20 mg/kg to provide less variability in visualization is unknown. Doses higher than 20 mg/kg were not studied. Thus, the ability of doses from 20 to 40 mg/kg to provide better visualization without causing adverse effects is unknown. An optimal dose has not been determined. Based upon the high PPV for detection of tumor, a dose of 20 mg/kg appears reasonable.

Drug development of 5-ALA did not include a mass balance study. Approximately 30% of an orally administered dose of 20 mg/kg 5-ALA HCl is excreted unchanged in urine within 12 hours. The ratio of the plasma AUC of PpIX to that of 5-ALA was < 6%. Additional data on the fate of administered 5-ALA and PpIX are not available.

There are no studies that examined the effect of renal impairment on the PK of 5-ALA. Literature data show that patients with renal impairment have endogenous 5-ALA plasma concentrations that increase linearly with creatinine clearance. This leads to an expectation that after exogenous 5-ALA administration, plasma concentrations in patient with impaired renal function will be increased relative to the concentrations seen in patients with normal renal function. The package insert (b) (4) (b) (4).

There are no studies that examined the effect of hepatic impairment on the PK of 5-ALA. The role of the liver in eliminating endogenous (as well as exogenous) 5-ALA is unclear. The package insert (b) (4)

1.1 Recommendations

From a clinical pharmacology perspective, the application is approvable provided agreement can be reached on package insert language.

1.2 Post-Marketing Requirements and Commitments

From the clinical pharmacology perspective, there are no recommendations for post-marketing requirements or commitments.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

5-aminolevulinic acid (5-ALA) is a natural biochemical precursor of hemoglobin. Exogenous administration of 5-ALA results in increased production and intracellular accumulation of the fluorescent 5-ALA metabolite, PpIX. PpIX preferentially accumulates in tumors of epithelial and mesenchymal origin. The mechanism responsible for preferential accumulation is not fully elucidated.

Pharmacokinetics
In 12 healthy subjects, the mean half-life of 5-ALA following the approved recommended dose of Gliolan solution was 0.9 $\pm$ 1.2 hours (mean $\pm$ std dev) with a range of 0.8 to 1.3 hours. Maximum concentrations of the PpIX metabolite (Tmax for PpIX) occurred with a median of four hours and a range of 1.2 to 7.8 hours. The elimination half-life of PpIX was 3.6 $\pm$ 1.8 hours (mean $\pm$ std dev) with a range of 1.2 to 7.8 hours.

Absorption

In 12 healthy subjects, the absolute bioavailability of 5-ALA following the approved recommended dose of Gliolan solution was 100% and 105% calculated from $AUC_{0\text{-}inf}$ (n=12) and Ae,ur (urine excretion data) (n=10) ratios, respectively. Maximum 5-ALA plasma concentrations were reached with a median of 0.8 hour (range 0.5 – 1.0 hour).

Distribution

In in vitro experiments using 5-ALA concentrations up to approximately 25% of the maximal concentration that occurs in plasma following the approved recommended dose of Gliolan solution, the mean protein binding of 5-ALA was 12%.

Elimination

Metabolism

Exogenous 5-ALA is metabolized to PpIX, but the fraction of administered 5-ALA that is metabolized to PpIX is unknown. The average plasma AUC of PpIX is less than 6% of that of 5-ALA.

Excretion

In 12 healthy subjects, excretion of parent 5-ALA in urine in the 12 hours following administration of the approved recommended dose of Gliolan solution was 34 $\pm$ 8% (mean $\pm$ std dev) with a range of 27% to 57%.

Specific Populations

The effect of renal or hepatic impairment on the pharmacokinetics of 5-ALA following Gliolan administration is unknown.

Drug Interaction Studies

In in vitro studies using human microsomes studies, 5-ALA was found to not act as a substrate of cytochrome P450 (CYP) enzymes or P-glycoprotein (P-gp). The ability of PpIX to act as a substrate of cytochrome P450 (CYP) enzymes and P-glycoprotein (P-gp) was not studied.

5-ALA was tested for its inhibitory potential on seven human isoforms of CYP450 (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A). In human hepatic microsomes, 5-ALA was not a potent inhibitor of the major human CYP450 isoforms, with half maximal inhibitory concentrations (IC50s) > 300 μM. The ability of PpIX to act as an inhibitor of cytochrome P450 (CYP) enzymes was not studied.

In vitro studies suggest that phenytoin and other anti-convulsants may decrease cellular PpIX accumulation following Gliolan dosing. There are no clinical studies that examined the effect of concomitant drug administration on fluorescence intensity or image quality.

There are no clinical studies that examined the effect of concomitant drug administration on phototoxicity. Due to the risk of possible phototoxic interactions, phototoxic drugs are not to be used for up to 24 hours before and after administration of Gliolan.

Pharmacodynamics
The effect of the timing of Gliolan administration on fluorescence intensity or image quality in brain tissue is unknown. The relationship between systemic 5-ALA plasma concentrations at the time of visualization and fluorescence intensity in brain is also unknown. PpIX concentrations are higher in vital Grade IV tumors than Grade III tumors. There was heterogeneity within Grade IV tumors with PpIX displaying significantly lower levels in infiltration zones and necrotic regions as compared with "vital" tumor parts.

Photosensitization of the skin occurs post- 5-ALA dosing. The package insert advises patients to avoid sunlight for 24 hours post-surgery.

During drug development, a total of 452 patients were monitored with ECGs. QT was reported only for Study MC-ALS.20/BV (n=12). No QTc >470 msec were reported. The database of worldwide post marketing adverse drug reactions (ADR) was reviewed by the applicant; there are no reports of QT prolongation. Since 2007, more than 50,000 patients have been treated with Gliolan outside of clinical trials.

At concentrations almost 30-fold higher than Cmax following the clinical dose, 5-ALA altered hERG channel current less than 10%.

2.2 Dosing and Therapeutic Individualization

The recommended dose is 20 mg/kg. The solution is swallowed by the patient three hours (range two to four hours) prior to induction of anesthesia. If the anesthesia/surgery is delayed by some hours, additional doses are not given. If the dose is not taken two to four hours prior to anesthesia, anesthesia and surgery are to be postponed for at least two hours. If the surgery is delayed by one or more days, another dose can be administered two - four hours prior to anesthesia.

In study MC-ALS.8-I/GLI, the applicant studied the dose-efficacy relationship between three dose levels (0.2, 2 and 20 mg/kg) and the extent of fluorescence of the tumor core (compared to demarcation of the tumor core under white operation light) and the quality (strong, weak, none) of the tumor core. 5-ALA HCl was administered orally three hours (range 2.5 to 3.5 hours) preceding induction of anesthesia. A dose-efficacy relationship between the dose levels and the extent and quality of fluorescence in the tumor core was detected: higher doses of 5-ALA HCl enhanced the fluorescence quality and the fluorescence extent of the tumor core in a monotonic fashion. The highest dose of the investigative drug (20 mg/kg) was determined to be the most effective by the surgeons.

Literature data shows that after oral administration of about 40 mg/kg, mild nausea, vomiting, hypotension and a transient increase of liver enzymes in serum occurred. The increase in liver enzymes did not correlate with clinical symptoms. Phototoxic skin reactions were observed over a time period of 48 h. Higher doses (30 to 60 mg/kg) appear to be associated with an increase in the frequency of side effects, especially phototoxic skin reactions, gastrointestinal symptoms, hemodynamic changes and an increase in liver enzymes.

Considerable variability in fluorescence intensity was observed for the dose of 20 mg/kg. Doses between 2 and 20 mg/kg were not studied. Thus, the ability of doses from 2 to 20 mg/kg to provide less variability in visualization is unknown. Doses higher than 20 mg/kg were not studied. Thus, the ability of doses from 20 to 40 mg/kg to provide better visualization without causing adverse effects is unknown. An optimal dose has not been determined. Based upon the high PPV for detection of tumor, a dose of 20 mg/kg appears reasonable.

2.3 Outstanding Issues

There are no outstanding issues.

2.4 Summary of Labeling Recommendations

The clinical pharmacology related portions of the applicant's proposed package insert, together with our recommended edits (as tracked changes), are shown on next page. The labeling is not final, as it is yet to be negotiated with the applicant.

7 Page(s) of Draft Labeling have been Withheld in Full as B4 (CCI/TS) immediately following this page

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

Gliolan is an imaging agent to facilitate the real time detection and visualization of malignant tissue during glioma surgery. The pre-Investigational New Drug (pre-IND) application number forthis product is 110655. No studies were conducted under an IND. A pre-IND meeting with FDA was held on September 22, 2014 to discuss the suitability of the existing nonclinical and clinical data to support the submission of an NDA.

The applicant has requested a waiver for conducting pediatric studies on the basis that Gliolan for the visualization of tissue during surgery for malignant glioma has been designated as an orphan drug.

Gliolan is approved for marketing in the European Union (EU), Japan, Korea and many other countries for use in adult patients for visualization of malignant tissue during surgery for malignant glioma (WHO grade III and IV).

Gliolan contains 5-aminolevulinic acid (5-ALA) in the form of a powder as the hydrochloride salt of 5-ALA (5-ALA HCl), for reconstitution in 50 mL tap water to form a solution. The product is a lyophilized powder in glass vials, containing 1.17 g 5-ALA/vial (1.5 g 5-ALA HCl). The oral solution is prepared by dissolving the amount of one vial in 50 mL tap water, which leads to a concentration of 23.4 mg 5-ALA/mL.

Gliolan oral solution is swallowed by the patient 2 to 4 hours prior to induction of anesthesia. During glioma surgery, the excitation light of the appropriate wavelength (λ = 400 to 410 nm) is coupled with the operating microscope. Protoporphyrin IX (PpIX, the active metabolite of 5-ALA) causes red fluorescence. Tissue lacking fluorescence appears blue.

3.2 General Pharmacology and Pharmacokinetic Characteristics

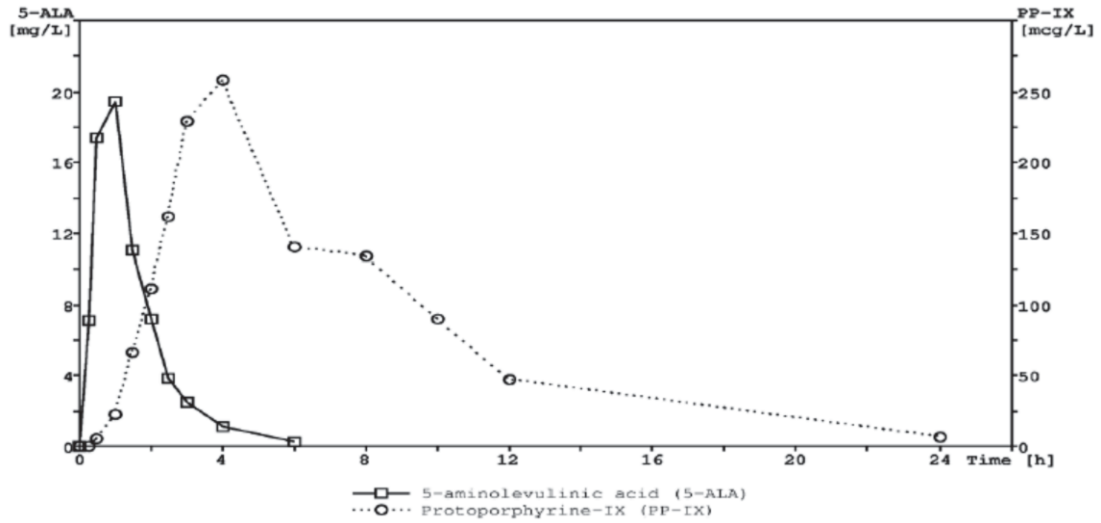
5-aminolevulinic acid (5-ALA) is a natural biochemical precursor of hemoglobin. Exogenous administration of 5-ALA results in increased production and accumulation of intracellular PpIX. PpIX preferentially accumulates in tumors of epithelial and mesenchymal origin. This preferential accumulation holds for malignant glioma. The mechanism responsible for preferential accumulation is not fully elucidated. The penetration of 5-ALA into brain tumor tissue compared to normal brain cells might be due to a disturbance of the BBB of the tumor and/or increased brain tumor vascularization as gliomas are characterized by very high levels of neovascularization. Changes in enzymes involved in the heme synthesis, uptake of 5-ALA and transport of PpIX may be involved.

Pharmacokinetics

5-ALA is an endogenous substance, average 5-ALA concentrations in healthy subjects are below 0.08 ug/L (pre-dosing samples from Study MC ALS.20/BV). After p.o. administration of 20 mg/kg 5-ALA HCL to 12 healthy subjects, PpIX plasma concentrations upto 562 µg/L were reached, which is more than 7000 times higher than endogenous PpIX plasma concentrations (pre-dosing samples from Study MC ALS.20/BV).

The highest concentrations of 5-ALA and PpIX are reached after 0.8 and 4.0 hours, respectively (**Figure 1.**). For clinical use in brain tumor surgery, 5-ALA HCl is administered orally 3 ± 1 hour before induction of anesthesia. Since PpIX is responsible for the tissue fluorescence, the 3 ± 1 hour administration schedule should result in plasma concentrations of PpIX being adequate during tumor surgery.

Figure 1. Geometric Mean Concentrations of 5-ALA [mg/L] and PpIX [µg/L] versus Time [h] in 12 Healthy Volunteers (Study MC-ALS.20/BV)



Dose Linearity

The dose linearity of 5-ALA was evaluated in Study MC-ALS.8-I/GLI. Healthy subjects received 0.2, 2.0, or 20 mg/kg 5-ALA HCl in solution. Results are shown in **Tables 2. - 4**. Ten-fold increases in doses of 5-ALA HCl resulted in less than ten-fold increases (2.7 fold increase) in PpIX exposure in plasma. The relationship between plasma and tumor concentrations of PpIX is not known.

Table 2. Dose Linearity (values are ratios of means)			
	5-ALA Dose		
	0.2 mg/kg	2 mg/kg	20 mg/kg
5-ALA AUC relative to that of 0.2 mg/kg	1	6.2	49.9
5-ALA half-life relative to that of 0.2 mg/kg	1	1.3	3.6
5-ALA Cmax relative to that of 0.2 mg/kg	1	8.2	32.2
5-ALA Tmax relative to that of 0.2 mg/kg	1	1.2	1.8
PpIX AUC relative to that of 2 mg/kg	NC[1]	1	3
PpIX half-life relative to that of 2 mg/kg	NC	1	2.7
PpIX Cmax relative to that of 2 mg/kg	NC	1	2.7
PpIX Tmax relative to that of 2 mg/kg	NC	1	1.2
NC=Not calculated [1]The values for PpIX are were not calculated for 0.2 mg/kg doses as the values were below LLOQ			

Table 3. Geometric means (dispersion factors) and in italics medians (ranges) (n=7) of the model independent target parameters of 5-ALA following single dose administration of three different doses

Pharmacokinetic characteristics [Unit]	**5-ALA HCl 0.2 mg/kg p.o.**	**5-ALA HCl 2 mg/kg p.o.**	**5-ALA HCl 20 mg/kg p.o.**
$AUC_{0\text{-inf.}}$ [mcg*h/L]	539.9 (1.98) *424.2* *(246.8-1779.7)*	3326.0 (1.60) *2901.3* *(1602.2-5880.1)*	26914.9 (1.19) *27143.5* *(20413.0-34625.8)*
$t_{1/2}$ [h]	0.85 (1.71) *0.75* *(0.51-2.38)*	1.12 (2.00) *0.84* *(0.45-2.44)*	3.05 (2.09) *1.94* *(1.60-10.04)*
C_{max} [mcg/L]	256.9 (1.20) *274.7* *(196.2-310.8)*	2103.6 (1.57) *1862.6* *(987.3-3757.9)*	8272.2 (1.11) *8239.1* *(7416.8-9700.6)*
t_{max} [h]	0.50 (1.75) *0.50* *(0.23-1.00)*	0.61 (1.77) *0.50* *(0.25-1.47)*	0.94 (1.51) *1.00* *(0.52-2.00)*

Table 4. Geometric means (dispersion factors) and in italics medians (ranges) (n=7) of the model independent target parameters of PPIX following single dose administration of three different doses of MC 506/1; *nc*: not calculated[1]

Pharmacokinetic characteristics [Unit]	PP-IX 0.2 mg/kg p.o.	PP-IX 2 mg/kg p.o.	PP-IX 20 mg/kg p.o.
$AUC_{0\text{-inf.}}$ [mcg*h/L]	*nc*	*255.80 (2.46)* *318.94* *(54.97-572.60)*	*779.90 (2.73)* *862.04* *(247.96-2655.06)*
$t_{1/2}$ [h]	*nc*	*2.90 (1.36)* *3.19* *(1.62-3.83)*	*2.61 (1.63)* *3.38* *(1.52-4.08)*
C_{max} [mcg/L]	*nc*	*32.28 (2.28)* *27.44* *(9.87-83.20)*	*nc* *101.71* *(0.00-258.83)*
t_{max} [h]	*nc*	*4.81 (1.37)* *4.92* *(2.90-6.92)*	*5.73 (1.58)* *5.48* *(2.97-11.92)*

[1]**Note:** The applicant did not calculate Cmax for the 20 mg/kg dose. However, the geometric Cmax (std dev) as calculated by the reviewer from raw data was 81.01(106.35).

Absorption

The absolute bioavailability of the orally administered dose of 20.0 mg/kg of 5-ALA HCl is 100% ± 1% calculated from $AUC_{0\text{-inf}}$ (n=12).

Distribution

Across the concentration range of 0.05 to 5 µg/L, the mean protein binding of 5-ALA in human plasma was 12% (for comparison, the average Cmax is approximately 20 ug/L – see **Figure 1.**).

Elimination

Metabolism

Endogenous 5-ALA is metabolized by a series of enzymatic reactions to protoporphyrin IX (PpIX) and subsequently to heme. The sites of metabolism of exogenously administered 5-ALA are unknown.

Drug development of 5-ALA did not include a mass balance study or metabolic profiling. Following oral administration of the clinical dose of 20 mg/kg, the ratio of the plasma AUC of PpIX to that of 5-ALA was 6% (Study MC-ALS.20/BV). A relative mass of PpIX cannot be derived from this AUC ratio because the volume of distribution of PpIX is unknown.

Excretion

Drug development of 5-ALA did not include a mass balance study or metabolic profiling. Approximately 30% of an orally administered dose of 20 mg/kg 5-ALA HCl is excreted unchanged in urine within 12 hours (Study MC-ALS.20/BV).

Endogenous porphyrins and porphyrin precursors are excreted in feces, but in limited amounts, as only limited amounts are not transformed into heme. Heme is excreted in bile.

Pharmacodynamics

Johansson et al. (5-Aminolevulinic acid-induced protoporphyrin IX levels in tissue of human malignant brain tumors. Photochem. Photobiol. 86(6), 1373-1378; 2010) measured PpIX concentrations in human brain tissue following p.o. administration of 20 mg/kg 5-ALA. The mean ± standard deviation (SD) concentration of PpIX was significantly higher in vital grade IV tumors (5.8 ± 4.8 μM; n = 8) than grade III tumors (0.2 ± 0.4 μM; n = 4). There was heterogeneity within grade IV tumors with PpIX displaying significantly lower levels in infiltration zones and necrotic regions as compared with "vital" tumor parts.

Photosensitization

Photosensitization of the skin was studied by observing the minimal erythema dose (MED) and corresponding PpIX plasma concentrations after p.o. treatment in 21 healthy volunteers Small skin areas on the dorsum and the gluteal region (which were not usually exposed to sun light) were exposed to a progressively graded series of defined ultraviolet A (UVA)-light doses 1 day before (baseline) as well as 12, 24, and 48 hours ± 30 minutes after p.o. administration of 20 mg/kg 5-ALA HCl (**Table 5.**). The MED was defined as the dose of irradiation after which a minimal skin reaction was visible in the respective area.

Table 5. Photosensitization of the skin, immediate and late reaction (MED) in healthy volunteers after oral administration of 20 mg/kg bw 5-ALA HCl together with PpIX concentration
PpIX values are geometric, arithmetic mean; Immediate and Late reaction values are mean (std dev) minimum/maximum.

Sub-jects	PP-IX [μg/L]	Time-point of irradiation	Immediate reaction (16 min after irradiation) [J/cm^2]	Late reaction (24 h after irradiation) [J/cm^2]
(b) (6)	BLLQ	day –1 (baseline)	18.19 (4.38) 14/28	23.81 (7.59) 14/40
(n=21)	72.08,104.44	12 h a. a.	7.38 (3.41) 5/14	6.05 (2.22) 5/14
	8.20, 10.12	24 h a. a.	8.52 (3.39) 5/14	21.71 (7.16) 7/28
	BLLQ	48 h a. a.	17.33 (5.49) 10/28	28.00 (12.87) 14/56
(b) (6)	BLLQ	day –1 (baseline)	20.33 (4.25) 14/28	26.17 (7.98)14/40
(n=12)	46.85, 59.53	12 h a. a.	8.67 (4.03) 5/14	6.08 (2.61) 5/14
	6.58, 8.52	24 h a. a.	7.83 (3.41) 5/14	18.83 (7.30) 7/28
	BLLQ	48 h a. a.	15.83 (4.71) 10/28	24.00 (12.71) 14/56
(b) (6)	BLLQ	day –1 (baseline)	15.33 (2.65) 14/20	20.67 (6.08) 14/28
(n=9)	128.02,164.32	12 h a. a.	5.67 (1.00) 5/7	6.00 (1.73) 5/10
	11.01, 12.24	24 h a. a.	9.44 (3.32) 5/14	25.56 (5.08) 14/28
	BLLQ	48 h a. a.	19.33 (6.08) 10/28	33.33 (11.66) 20/56

BLLQ: Below the lower limit of quantification a. a.: after administration

Cardiac Electrophysiology

Across four studies [MC-ALS.20/BV (bioavailability study), MC-ALS .8-I/GLI, MC-ALS.28/GLI and MC-ALS.3/GLI (three efficacy studies)], a total of 452 patients were monitored with ECGs. QT was reported only for Study MC-ALS.20/BV (n=12). No QTc >470 msec were reported. The database of worldwide post marketing adverse drug reactions (ADR) was reviewed by the applicant; there are no reports of QT prolongation. Since 2007, more than 50,000 patients have been treated with Gliolan outside of clinical trials.

The potential effect of 5-ALA HCl on hERG potassium channels was tested in hERG channels in HEK293 cells exposed to 6.0 mM 5-ALA (close to the limit of solubility, this concentration is almost 30-fold higher than the maximal 5-ALA plasma concentrations observed after oral administration of 20 mg/kg 5-ALA HCl). The hERG current changed less than 10%.

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

Although not the primary clinical endpoint in the single controlled Phase 3 trial, the primary effectiveness measure by which Gliolan will likely be judged is the positive predictive value of fluorescence in tumor biopsies. An effectiveness measure likely to provide supportive evidence of effectiveness, and the primary clinical endpoint in the Phase 3 trial, is percentage of patients without residual tumor on early postoperative MRI. Both of these are indirect, i.e., pharmacodynamic, measures.

Biopsy-based Estimates of PPV

Biopsy-based estimates for Study MC-ALS.3/GLI, combined with the results for two other studies, are shown in **Table 6**. Estimates of PPV were consistently very high across the three studies: 96.2% in Study MC-ALS.28/GLI (N=33), 96.6% in Study MC-ALS.30 (N=36), and 97.8% in Study MC-ALS.3/GLI (N=170). The PPV for strong (i.e., red) fluorescence was slightly higher in each study compared to the PPV for weak (i.e., pink) fluorescence, with the lowest PPV reported for weak fluorescence in Study MC-ALS.28/GLI 92.2%.

Table 6. Biopsy-based estimates of positive predictive value stratified by fluorescence quality

Fluorescence Quality of Biopsies	MC-ALS.3/GLI	MC-ALS.28/GLI	MC-ALS.30/GLI
Strong:			
n1/N1 (PPV)	151/153 (98.7%)	95/95 (100%)	161/164 (98.2%)
95% CI of PPV	95.4-99.8	96.2-100	94.7-99.6
Weak:			
n1/N1 (PPV)	161/166 (97.0%)	83/90 (92.2%)	181/190 (95.3%)
95% CI of PPV	93.1-99.0	84.6-96.8	91.2-97.8
Any Fluorescence:			
n1/N1 (PPV)	312/319 (97.8%)	178/185 (96.2%)	342/354 (96.6%)
95% CI of PPV	95.5-99.1	92.4-98.5	94.2-98.2

N = total number of patients in Full Analysis Set; n1 = number of fluorescent biopsies having tumor cells >0%; N1 = number of fluorescent biopsies; n2 = number non-florescent biopsies having 0 tumor cells; N2 = number of non-florescent biopsies; PPV = positive predictive value (n1/N1*100); NPV = negative predictive value (n2/N2*100); CI = confidence interval.

Exact 95% CIs are presented using the Clopper-Pearson method.

Study MC-ALS.3/GLI

The study population was males or females aged 18 to 72 years with cranial MRI justifying diagnosis of malignant glioma (WHO grades III and IV) for whom primary surgical treatment was indicated. Of the three studies, only Study MC-ALS.3/GLI was a Phase 3 study with a

conventional surgery (i.e., white-light = "WL"group, patients receiving 5-ALA are in the fluorescent light = "FL"-group). Gliolan was administered at 20 mg/kg two to four hours prior to anesthesia. Tumor resection was performed continuously under both conventional white light and blue excitation light – the surgeon alternated freely between the two types of illumination. The pre-specified primary endpoint was percentage of patients without definite residual contrast-enhancing tumor in the early post-operative MRI (within 72 hours after surgery). Additional (non-primary) endpoints included positive predictive value of fluorescence and progression-free survival, defined via radiographic assessment, at the six-month visit, and overall survival.

For the full analysis set (FAS, n=349), 63.6% of all patients in the FL-group and 37.6% of all patients in the WL-group (Table VII) did not show residual tumor on early postoperative MRI (Chi-square test, $p < 0.0001$). 20.5% of patients in the FL-group and 11.0% of patients in WL-group were alive at the six-month visit without progression (**Figure 2**). The difference was statistically significant using the chi-square test (p=0.0152). Overall survival results for Study MC-ALS.3/GLI are shown in **Figure 3**.

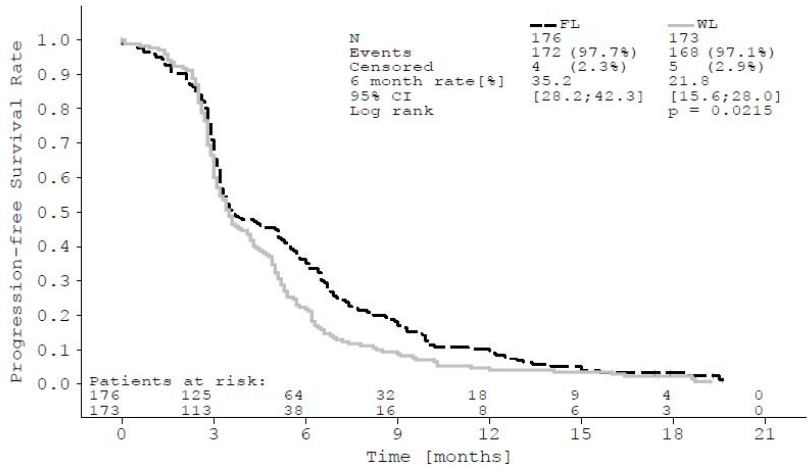


Figure 2. Progression free survival, Study MC-ALS.3/GLI

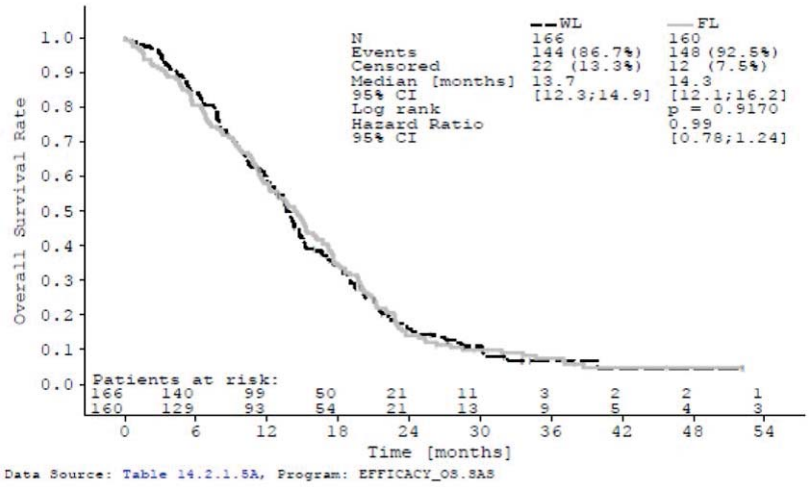


Figure 3. Overall survival, Study MC-ALS.3/GLI

Study MC-ALS.28/GLI
The study population was males or females aged 18 to 75 years with cranial MRI justifying a diagnosis of malignant glioma (WHO grades III and IV) for whom primary surgical treatment was indicated. Gliolan was administered at 20 mg/kg 2.5 – 3.5 hours prior to anesthesia. Tumor resection was performed continuously under both conventional white light and blue excitation light – the surgeon alternated freely between the two types of illumination.

The primary objective of the study was to determine the positive predictive value (PPV) of tissue fluorescence at the patient level. A secondary objective was to determine the positive predictive value of tissue fluorescence at the biopsy level. To evaluate PPV, three test regions remote from one another and as distant as possible from tumor necrosis were selected using spectrometric evaluation: : one region with no fluorescence, one with weak fluorescence and one with strong fluorescence. Three biopsies were taken from each of the three test regions. The positive predictive value of tissue fluorescence at the patient level was 84.8%. The positive predictive value of strong fluorescence was higher (100.0%) than that of weak fluorescence (83.3%). The positive predictive value of fluorescence at the biopsy level was 96%. The positive predictive value of strong fluorescence was 100%. Among 185 evaluated biopsies, seven samples failed to reveal any tumor cells (false-positive specimens). In all false-positive specimens, the quality of fluorescence was weak.

Study MC-ALS.30/GLI
The study population was males or females aged 18 to 75 years with cranial MRI justifying diagnosis of recurrent malignant glioma (WHO grades III and IV), for whom repeat-surgery was indicated. Patients must have undergone previous surgery including open craniotomy for newly diagnosed glioma plus standard radiotherapy. Gliolan was administered at 20 mg/kg 2.5 – 3.5 hours prior to anesthesia. Tumor resection was performed continuously under both conventional white light and blue excitation light – the surgeon alternated freely between the two types of illumination. The primary objective of the study was to determine the positive predictive value (PPV) of tissue fluorescence at the patient level. A secondary objective was to determine the positive predictive value of tissue fluorescence at the biopsy level.

To evaluate PPV, the fluorescence quality was evaluated in two different tissue areas distinguishable under normal white light illumination: abnormal, pathologically changed tissue and "regular brain parenchyma" (tumor margins) adjoining the pathological tissue. For each tissue type, three separate areas for biopsy as distant as possible from any tumor necrosis were to be selected under white light. Then, after switching to the fluorescence mode, Spectrometric evaluation was used to select spots showing strong fluorescence, and spots showing weak fluorescence, from each tissue area. Three biopsies were taken from each of the four selected test sites (maximum of 12 per patient).

The positive predictive value of fluorescence at the patient level was 78%. The positive predictive value of strong fluorescent was higher (98.2%) than that of weak fluorescence (95.3%). The positive predictive value of fluorescence at the biopsy level was 97%. The positive predictive value of strong fluorescence was 98%. Among 355 biopsies, 12 sections failed to reveal any tumor cells (false-positive specimens). Nine of the 12 were of weak fluorescence, and three of the 12 were strongly fluorescing.

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

The recommended dose is 20 mg/kg. The solution is swallowed by the patient three hours (range two to four 4 hours) prior to induction of anesthesia. If the anesthesia/surgery is delayed by some hours, additional doses are not given. If the dose is not taken two to four hours prior to anesthesia, anesthesia and surgery are to be postponed for at least two hours. If the surgery is delayed by one or more days, another dose can be administered two to four hours prior to anesthesia.

In study MC-ALS.8-I/GLI, the applicant studied the dose-efficacy relationship between the dose levels (0.2, 2, and 20 mg/kg of study drug) and the extent of fluorescence of the tumor core (compared to demarcation of the tumor core under white operation light) and the quality (strong, weak, none) of the tumor core. The secondary objectives included the measurement of the fluorescence in the tumor core and tumor margin in areas selected by subjective assessment with strong, weak and no fluorescence using spectrometry as an objective control. A histological examination of biopsies taken intraoperatively for verification of the MRI diagnosis and comparison with the spectrometric fluorescence measurements were conducted.

Three hours (range 2.5 to 3.5 hours) preceding induction of anesthesia 1.5 grams of 5-aminolevulinc hydrochloride were dissolved in 50 ml of water as ready-for-use solution and administered orally immediately thereafter.

During the study, it was possible to alternate freely between the two types of illumination. After completion of resection, the global fluorescence extent of the tumor core compared to its demarcation under standard white operation light was assessed post-hoc by the principal investigator and by the second surgeon.

A dose-efficacy relationship between the dose levels and the extent and quality of fluorescence

in the tumor core was detected: higher doses of 5-ALA HCl enhanced the fluorescence quality and the fluorescence extent of the tumor core in a monotonic, fashion. The highest dose of the investigative drug (20 mg/kg) was determined to be the most efficient by the surgeons. The applicant considered the highest dose of the investigative drug (20 mg/kg) was the most efficient with respect to fluorescent quality and extent of the tumor core (**Figure 4.**).

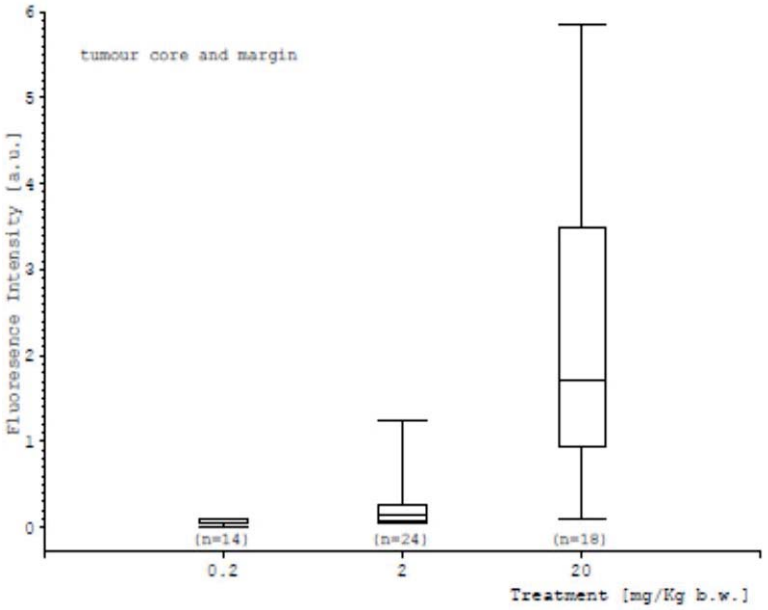


Figure 4. Mean fluorescence intensities as measured spectrometrically versus treatment group.

Literature data shows that after oral administration of about 40 mg/kg, mild nausea, vomiting, hypotension and a transient increase of liver enzymes in serum occurred. The increase in liver enzymes did not correlate with clinical symptoms. Phototoxic skin reactions were observed over a time period of 48 h. In the current study the oral dose was 20 mg 5-ALA HCl/kg. Higher doses (30-60 mg/kg) appear to be associated with an increase in the frequency of side effects, especially phototoxic skin reactions, gastrointestinal symptoms, hemodynamic changes and an increase in liver enzymes.)

Reviewer's comments
Considerable variability in fluorescence intensity was observed for the dose of 20 mg/kg. Doses between 2 and 20 mg/kg were not studied. Thus, the ability of doses from 2 to 20 mg/kg to provide less variability in visualization is unknown. Doses higher than 20 mg/kg were not studied. Thus, the ability of doses from 20 to 40 mg/kg to provide better visualization without causing adverse effects is unknown. An optimal dose has not been determined. Based upon the high PPV for detection of tumor, a dose of 20 mg/kg appears reasonable.

3.3.3 Is an alternative dosing regimen and/or management strategy required for sub populations based on intrinsic factors?

There are no studies that examined the effect of renal or hepatic impairment on the PK of 5-ALA. (b) (4).

It is possible that the PK of 5-ALA will be altered in patients with renal impairment. Literature data *(*A. Gorshein and R. Webber 5-Aminolaevulinic acid in plasma, cerebrospinal fluid, saliva and erythrocytes: studies in normal, uraemic and porphyric subjects. Clinical Sciences, 72, 103-112, 1987**)** showed that in patients with renal impairment, endogenous 5-ALA plasma concentrations are increased compared to healthy volunteers and that 5-ALA clearance is correlated with creatinine clearance (**Figure 5.**) This leads to an expectation that after exogenous 5-ALA administration, plasma concentrations in patient with impaired renal function will be increased relative to the concentrations seen in patients with normal renal function.

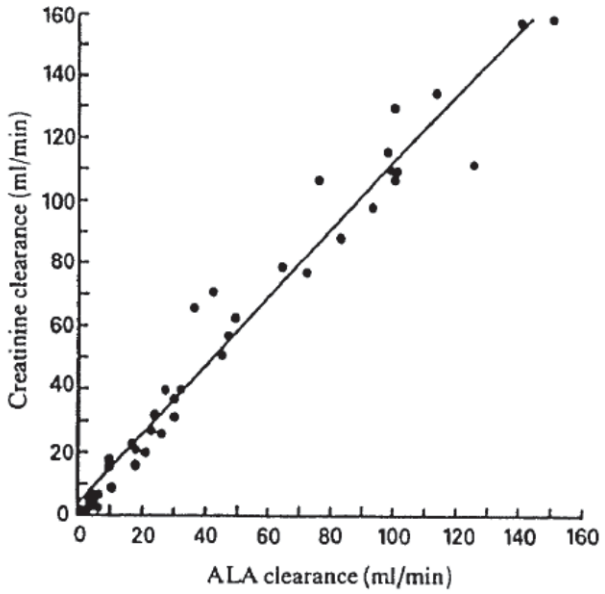


Figure 5. Correlation between clearance of ALA and creatinine clearance

The role of the liver in eliminating endogenous (as well as exogenous) 5-ALA is unclear. A literature report (Gibson, P.R., Grant, J., Cronin, V., Blake, D., and Ratnaike, S. (2000). Effect of hepatobiliary disease, chronic hepatitis C and hepatitis B virus infections and interferon-α on porphyrin profiles in plasma urine and faeces. *J. Gastroenterol. Hepatol. 15*, 192-201) states, "…the elevation of total porphyrins in urine and, to a lesser extent, in plasma in some patients with hepatobiliary disease is due an elevation of coproporphyrin I, as exemplified by an increase in the urinary coproporphyrin I:III ratio, which is consistent with reduced biliary excretion of coproporphyrin I. The degree of elevation of total plasma or urinary porphyrins correlates with the degree of hepatic dysfunction and cholestasis." The paper does not provide data to support the statement.

An IR was sent to the applicant requesting a summary of the use of Gliolan in patients with hepatic and renal impairment. The applicant's response is reproduced (indented).

> A search of the global database, conducted on May 2, 2017, revealed no data on the use of 5-ALA in patients with renal impairment.
>
> In two cases, hepatic impairment might have existed at the time of administration.
> In the first case, a male patient with a history of cholecystectomy received 5-ALA and an increased alanine aminotransferase level was reported, which was considered related to the study drug. Outcome was reported as resolved within 1 week.
> In the second case, a male patient with a history of hepatitis B received 5-ALA and suffered from hypotension and severe metabolic acidosis during the use of 5-ALA for resection of a glioblastoma. The causality of metabolic acidosis and 5-ALA was reported as suspected; no causal relationship was reported for hypotension. The outcome of metabolic acidosis was reported as recovered; no outcome was provided for hypotension. There are no data available on liver function in these patients at time of administration, so degree of hepatic impairment cannot be judged.

The approved European package insert for 5-ALA HCl states, "No studies have been performed in patients with clinically relevant hepatic or renal impairment. Therefore, this medicinal product should be used with caution in such patients."

Reviewer's Comments
The effect of renal and hepatic impairment on 5-ALA and PpIX pharmacokinetics, and visualization, are unknown. We do not recommend a post-marketing requirement for acquiring data. Plasma concentrations of PpIX may not accurately reflect the amount of PpIX in tumor tissue. Thus, the results of a study with pharmacokinetic endpoints could not be used for dose adjustment based on "exposure-matching", as exposure matching would not assure that visualization was not compromised.

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

Food
Although 5-ALA is administered orally, the effect of food on the pharmacokinetics of 5-ALA and PpIX was not studied.

Reviewer's Comments
Patients will be fasting eight hours prior to the surgery and drug will be administered in absence of any food. The reviewer finds the lack of data on the effect of food on pharmacokinetics acceptable.

Drugs
In in vitro studies using human microsomes, 5-ALA was found to not act as a substrate of cytochrome P450 (CYP) enzymes or P-glycoprotein (P-gp). The ability of PpIX to act as a substrate of cytochrome P450 (CYP) enzymes or transporters was not studied.

5-ALA was tested for its inhibitory potential on seven human isoforms of CYP450 (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A). In human hepatic microsomes, 5-ALA was not a potent inhibitor of the major human CYP450 isoforms, with half maximal inhibitory concentrations (IC50s) > 300 μM.

There are no clinical studies that examined the effect of concomitant drug administration on fluorescence intensity or image quality. However, there is some evidence from in vitro studies that phenytoin may affect the 5-ALA-induced PpIX accumulations. Hefti et al. investigated the effect of the antiepileptic drugs phenytoin and levetiracetam on 5-ALA-induced PpIX accumulations in two glioma cells lines and primary glioma cells isolated from a human biopsy. The cells were treated with phenytoin and levetiracetam for three days then incubated with 5-ALA for four hours. The investigators observed a decrease in PpIX synthesis as measured by fluorescence of 55 ± 12% in primary glioblastoma cells after incubation with phenytoin. When irradiated with light, although less PpIX accumulated in phenytoin-treated cells, "the efficacy of photodynamic therapy was not affected." Similarly, literature describes accumulation of PpIX in a glioma cell line after pre-incubation of cells with dexamethasone and different anticonvulsants. Accumulation of PpIX was shown to be decreased by dexamethasone alone or in combination with the anticonvulsants to different extents.

There are no clinical studies that examined the effect of concomitant drug administration on phototoxicity. Due to the risk of possible phototoxic interactions, the applicant proposes that [redacted] (b) (4)

Reviewer's Comments
Plasma Cmax is of 5-ALA is approximately a thousand fold lower than the IC50 values estimated in the in vitro studies, therefore the likelihood of 5-ALA acting as a perpetrator of CYP-based drug interactions is remote. There is no data on in vitro metabolism of PpIX. This is acceptable as it is found very small amounts and the drug is administered only once.

The clinical relevance of the anticonvulsant data is unclear. A brief statement of the interaction is being included in section 12.3 of the package insert.

In all clinical studies for the use of Gliolan in glioma surgery, dexamethasone was administered before and after surgery, and thus visualization was studied under these conditions. Thus, if drug interactions are occurring, they resulted in, rather than interfered with, the observed clinical outcomes. [redacted] (b) (4)
.

The proposal to limit exposure to phototoxic drugs is acceptable.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

Analytical methods for Study MC-ALS.20/BV

Method validation
HPLC methods with UV detection were used for the analyses of 5-aminolevulinic acid (5-ALA) in plasma and urine and PPIX in plasma and urine.

Identity: Identity was assumed based upon equality of retention time between samples and reference standards. The chemical composition of peaks was not assessed.

Selectivity: The selectivity of the analytical method for 5-aminolevulinic acid could not be evaluated by the analysis of blank samples, as 5-aminolevulinic acid is an endogenous compound and therefore present even in blank plasma. The comparison between water and plasma processed by the described analytical method gives no evidence for interfering peaks caused by the method itself but interfering compounds caused by the biological matrix cannot be excluded.

Accuracy and precision: Standards did not deviate by more than 15% from nominal concentrations throughout the studied ranges above the LLOQ, nor did they differ by more than 20% from nominal at the LLOQ. Similarly, for all analytes (5-ALA in plasma and urine, and PP-IX in plasma), FDA requirements for precision were met.

Interference: The method validation report did not include testing of potential interfering substances (e.g., drugs).

Stability: Stability was evaluated in both matrices (plasma and urine) under relevant storage conditions (-20°C). Additional stability tests were performed on extract stability in the auto sampler of the HPLC. Freeze/thaw testing was also performed.

Reviewer's Comments
Analytical methods validation was acceptable.

Analysis Runs
Across 14 analytical runs for 5-ALA in plasma, four runs for 5-ALA in urine and five runs for Pp-IX in plasma, only minor deviations from FDA 15%/20 % standards regarding accuracy and precision of standard curves, and of quality control samples, occurred. The analytes were stable under sample processing and storage conditions (-20°C for 3.5 months).

Reviewer's Comments
The performance of the analytical methods was acceptable.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

CHRISTY S JOHN
05/12/2017

GENE M WILLIAMS
05/12/2017
I concur with the recommendations