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**APPLICATION NUMBER: 20-965**

**CLINICAL PHARMACOLOGY AND  
BIOPHARMACEUTICS REVIEW(S)**

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NOV 9 1998

## Clinical Pharmacology/Biopharmaceutics Review

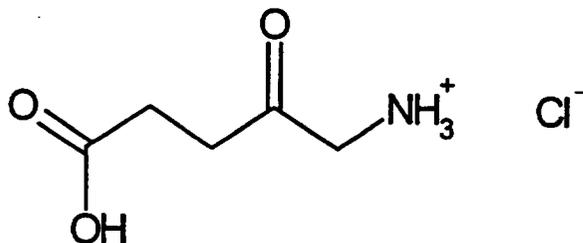
Aminolevulinic HCl, 20% Topical Solution  
Levulan® Kerastick™  
Reviewer: A. Noory  
NDA 20-965

DUSA Pharmaceuticals Inc.  
Valhalla, NY 10595  
Submission Date:  
June 29, 1998

### Review of an NDA

#### I. Background:

Levulan® Kerastick™ is a two component system applicator. The system (shown on Page 8 of the appendix), consists of two ampoules, one contains the vehicle solution and the other contains 354mg of Levulan® (aminolevulinic acid HCl). Levulan® is mixed with the vehicle solution to produce a 20% solution just before application by a health professional by crushing the ampule in the applicator. Levulan® photosensitizes actinic keratoses lesions (AK) by inducing the accumulation of protoporphyrin IX (PpIX) in cells and tissues for photodynamic therapy with the 4170 Blue Light Photodynamic Therapy Illuminator (BLU-U™). As part of this NDA, the applicant has submitted the results of a pharmacokinetic study using both intravenous and oral dosing, a pharmacokinetic study using topical administration, and an in vitro Percutaneous absorption study. Aminolevulinic acid HCl is a white to off-white crystalline powder, highly soluble in water, sparingly soluble in ethanol and methanol, and insoluble in mineral oil or hexane with a molecular weight of 167.59. The chemical name of Aminolevulinic acid HCl is 5-amino-4-oxopentanoic acid hydrochloride with the following structural formula.



#### II. Recommendation:

In support of the human pharmacokinetic and bioavailability portion of this NDA the applicant submitted the result of three studies. These studies adequately characterized the pharmacokinetic and bioavailability of aminolevulinic acid from the Levulan® Kerastick™. From the biopharmaceutics point of view the NDA 20-965 is approvable.

INDEX

I. Background	-	-	-	-	-	-	-	-	1
II. Recommendation	-	-	-	-	-	-	-	-	1
III. Overview of pharmacokinetic section	-	-	-	-	-	-	-	-	2
Analytical	-	-	-	-	-	-	-	-	2
Pharmacokinetic study (protocol # PK-01)	-	-	-	-	-	-	-	-	3
Pharmacokinetic study (protocol # Pharm-03)-	-	-	-	-	-	-	-	-	4
In Vitro Percutaneous study (Study # ALA-97-1)-	-	-	-	-	-	-	-	-	5
IV. Conclusion	-	-	-	-	-	-	-	-	6

III. Overview of pharmacokinetic section/disease:

The human pharmacokinetic and bioavailability section of this NDA consists of three study reports:

1. Pharmacokinetics of 5-Aminolevulinic Acid (ALA) and protoporphyrin IX (PpIX) in healthy Volunteers after Intravenous and Oral Dosing, (PK-01)
2. Pharmacokinetics of Levulan Induced Protoporphyrin IX in Actinic Keratosis and Adjacent Skin, (Pharm-03)
3. In Vitro Percutaneous Absorption of [<sup>14</sup>C]-Aminolevulinic Acid in Human Skin.

Chronic exposure to sunlight may increase the incidence of squamous and basal cell carcinoma of the skin in fair, white-skinned persons which is directly related to the amount of yearly sunlight to the exposed area. Precancerous keratotic lesions (actinic keratoses [AK] ) are frequent consequences of many years of over-exposure. The keratoses are usually hard and sharp on palpation, and gray to dark in color. They differ from warty brown seborrheic keratoses, which increase in number and size with age but occur on covered as well as uncovered areas of the body and are not premalignant.

The accumulation of protoporphyrin IX (PpIX) in actinic keratoses and photosensitization of the lesion by light of a certain wavelength will result in necrosis of the lesion. Aminolevulinic Acid (ALA), an endogenous substance, is an intermediate in the biosynthesis of porphyrins, it induces the accumulation of PpIX. Attachment 9 of the appendix shows the pathway for the synthesis of PpIX.

Levulan® Kerastick™ will be applied by a health professional to assure the proper amount and method of application. As mentioned previously the system consists of two ampoules, one contains the vehicle solution and the other contains 354mg of Levulan® (aminolevulinic acid HCl). The formulation of the vehicle solution is shown in page 10-11 of the appendix.

Analytical:

The analysis of PpIX in human plasma was carried out by [redacted] based on a method by Meyer and Vogt "Ion-Pair Reversed HPLC Determination of Porphyrins from Red Blood Cells" Chromatographia Vol. 16, P190. The assay was shown to be specific for PpIX and linear over a range of [redacted] ng/ml. The lower limit of quantitation [redacted] ng/ml. Representative chromatograms are included in the appendix, page 12. The analysis of ALA was based on a method by Okayama et al, Clin. Chem. 36:1494-1497, 1990. The

assay was shown to be specific for ALA and linear over a range of [redacted]  $\mu\text{g/ml}$ . The lower limit of quantitation [redacted]  $\mu\text{g/ml}$ . Representative chromatograms are included in the appendix, page 13.

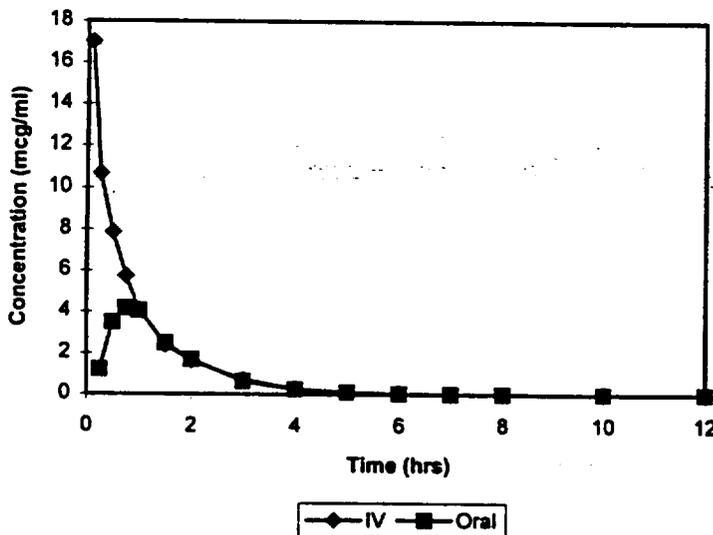
**Pharmacokinetic and Bioavailability: Study # PK-01**

The objective of this study was to determine the bioavailability of 5-aminolevulinic acid.(ALA). Also the plasma concentrations of protoporphyrin IX (PpIX) were measured. Six healthy male volunteers were enrolled in this crossover study. Each subject received an intravenous and an oral dose of 128mg of ALA HCl (equivalent to 100mg of ALA) with a washout period of 6-7 days. The solution for injection was also used for oral administration. The study design was a two-way crossover, and the trial is summarized in the appendix page 14-17. The table below shows the AUC and the  $C_{\text{max}}$  for ALA after Oral and the IV administration.

Pharmacokinetic Parameters for ALA; Mean $\pm$ SD; N=6		
PK-parameter	Intravenous	Oral
AUC (0- $\infty$ ) $\mu\text{g}\cdot\text{hr/ml}$	12.50 $\pm$ 2.89	7.30 $\pm$ 1.25
$C_{\text{max}}$ $\mu\text{g/ml}$	15.44 $\pm$ 6.60	4.65 $\pm$ 0.94
% Bioavailability		60.3 $\pm$ 13.4

The mean plasma concentration-time curves for ALA are shown below.

**ALA: Plasma Levels after the Administration of Levulan**

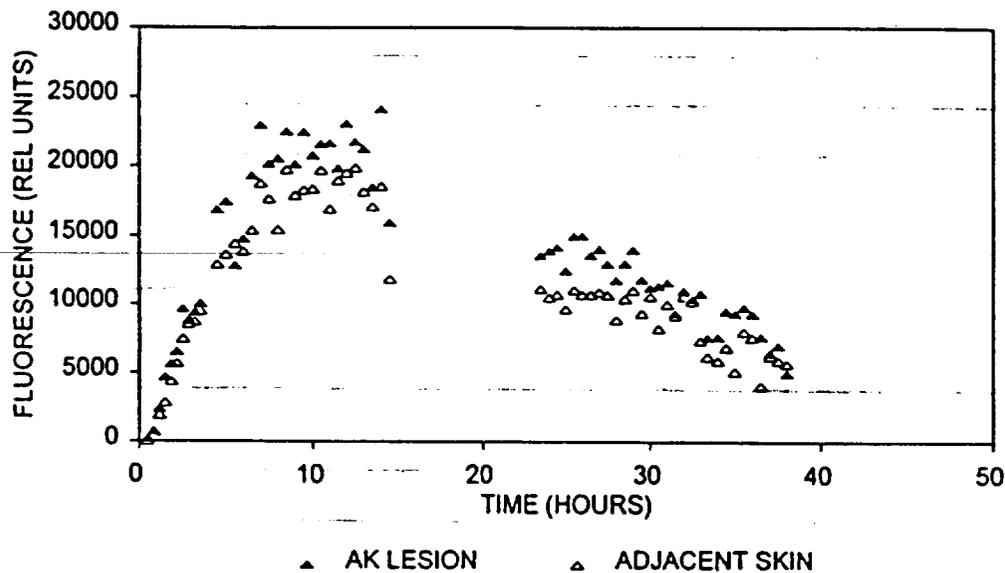


Although the plasma concentration profile for the ALA was determined easily, the plasma concentration profile for the PpIX could not be determined at all. The PpIX concentrations were low and erratic. Following the intravenous and oral administration of ALA, the levels of PpIX was only detectable in 48% and 38% of the plasma samples respectively. The levels are shown on page 18 of the appendix. The results of this study demonstrate that the level of PpIX does not correspond to ALA administered. In fact, in one subject (# 4) the concentration of PpIX was similar or higher

before the administration of ALA in both IV and oral dosing. This indicates that the production of PpIX could be dependent on the bodies need to maintain homeostasis.

**Pharmacokinetic in Actinic Keratosis: Study # Pharm-03**

The objective of this study was to evaluate the kinetics of PpIX in actinic keratoses and adjacent skin on the face and scalp following the application of Levulan using the Levulan® Kerastick™ for Topical Solution, 20%. PpIX fluorescence was used to follow relative changes in PpIX concentration as a function of time (fluorokinetics), following topical application of Levulan. Twelve patients were enrolled in this study. Two sequential topical applications of Levulan Topical 20% solution or Topical Solution Vehicle were made to each skin site using the Levulan Kerastick. A representative fluorokinetics curves for lesional and adjacent skin obtained is shown below.



The table below contains overall mean peak intensities for lesional and adjacent skin fluorescence, expressed in arbitrary relative units. The individual data are on page 19 of the appendix.

Pharm-03: Summary PpIX Fluorokinetics Parameters; (Mean ± SD); (N=12)				
Skin Site	Peak Intensity (relative units)	Peak Time (hours)	Plateau Period (hours)	T ½ (hrs)
Lesion	18000 ± 4000	11 ± 1	6 ± 1 to 19 ± 4	30 ± 10
Adjacent Skin	13000 ± 4000	12 ± 1	7 ± 1 to 20 ± 2	28 ± 6

These data indicate that there was little fluorescence selectivity following Levulan administration to AK lesion and adjacent skin sites on the face and scalp. The formation of PpIX in healthy skin is similar to that of AK lesion site. Therefore Levulan should only be applied to the effected skin.

### In Vitro Percutaneous Absorption OF [<sup>14</sup>C]-ALA in Human Skin:

The study was conducted to evaluate the *in vitro* percutaneous absorption of ALA 20% Levulan Topical Solution in human cadaver skin. A comparison of penetration in intact skin versus the "stripped" skin was conducted. The ALA solution was radiolabeled with ALA (aminolevulinic acid hydrochloride,  $\delta$ -[4-<sup>14</sup>C]). The skin was cut into 54 specimens approximately 9 cm<sup>2</sup> each. Half of the skin specimens were tape-stripped to remove the stratum corneum, using up to 22 sequential strips of [redacted] transparent tape until a "glistening" of the skin surface was obtained or until epidermal separation started to occur. This skin was placed on the chambers of 54 [redacted] Glass Chambers. The exposed surface area was 1.77 cm<sup>2</sup>. Two successive applications of 8.5  $\mu$ L (8.5 mg) of the test formulation were applied to the skin surface (1.7 cm<sup>2</sup>) and allowed to air dry for 5 minutes before reapplying. This represented a dose of 3.4 mg ALA/1.7 cm<sup>2</sup> or 2 mg ALA/cm<sup>2</sup> applied to each of the chambers. Nine groups were designated according to the following schedule:

Group # (n-9)	Skin	ALA Concentration	Cumulative Penetration (Hrs)	Tissue Recovery (Hrs)
1	Intact	20%	1, 4, 8	8
2	Intact	20%	1, 4, 8, 16	16
3	Intact	20%	1, 4, 8, 16, 24	24
4	Stripped	20%	1, 4, 8	8
5	Stripped	20%	1, 4, 8, 16	16
6	Stripped	20%	1, 4, 8, 16, 24	24

Data were analyzed to include all of the DPM data to calculate the percent and micrograms recoveries (Analysis I), and after discarding statistical "outlier" values for each compartment (Analysis II). The following table provides the results of the assays.

In Vitro cumulative Penetration of [ <sup>14</sup> C]-ALA						
Time (hrs)	Intact Skin (Mean $\pm$ SD)			STRIPPED Skin (Mean $\pm$ SD)		
	N	Percent	$\mu$ g/1.77 cm <sup>2</sup>	N	Percent	$\mu$ g/1.77 cm <sup>2</sup>
0	23	0.0 $\pm$ 0.0	0 $\pm$ 0	26	0.0 $\pm$ 0.0	0 $\pm$ 0
1	23	0.1 $\pm$ 0.2	3 $\pm$ 5	26	7.7 $\pm$ 10.1	262 $\pm$ 343
4	23	0.2 $\pm$ 0.3	7 $\pm$ 9	26	18.9 $\pm$ 19.8	643 $\pm$ 674
8	23	0.5 $\pm$ 0.5	15 $\pm$ 17	26	22.3 $\pm$ 17.8	757 $\pm$ 6.5
16	15	0.7 $\pm$ 0.6	25 $\pm$ 19	17	29.2 $\pm$ 19.8	991 $\pm$ 675
24	7	1.4 $\pm$ 1.0	47 $\pm$ 35	9	43.3 $\pm$ 17.2	1472 $\pm$ 585

The results also show that the intact stratum corneum presents a significant barrier to ALA absorption and skin uptake. Many skin diseases, including actinic keratosis, have been reported to have a defective skin barrier. Therefore, one can view the results of the present study as approximating the range of skin absorption of ALA in intact skin relative to skin in which the barrier has been abrogated by disease.

**IV. Conclusion:**

The results of these studies demonstrate that the systemic exposure to PpIX following the topical application of Levulan® Kereastick™ will most likely be undetectable as the body produces about 350mg of ALA to maintain the homeostasis. Additionally the topical absorption of ALA is about 2% of the applied dose, furthermore, for the synthesis of one mole of PpIX eight moles of ALA is needed. The resulting PpIX could practically be indistinguishable from the endogenous production of PpIX ✓

**/S/** 11/5/98  
Assadollah Noory  
Pharmacokineticist  
Division of Pharmaceutical Evaluation III

Team Leader: E. Dennis Bashaw, Pharm.D. **/S/** 11/7/98

CC: NDA 20-965 (ORIG),  
HFD-540/DIV. File  
HFD-540/Prj. Mjr./Cintron  
HFD-880 (Noory)  
HFD-880 (Bashaw)  
HFD-880 (Lazor) ✓  
(CDR. Attn. B. Murphy)  
HFD-344 (Viswanathan)

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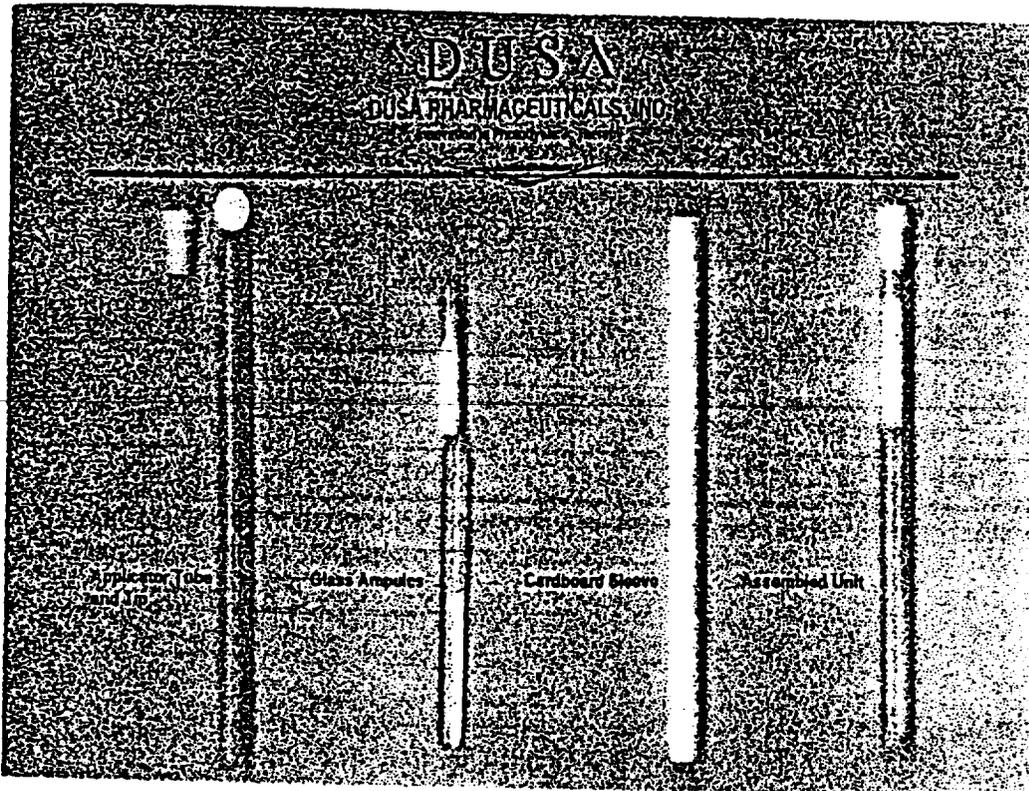
# Appendix

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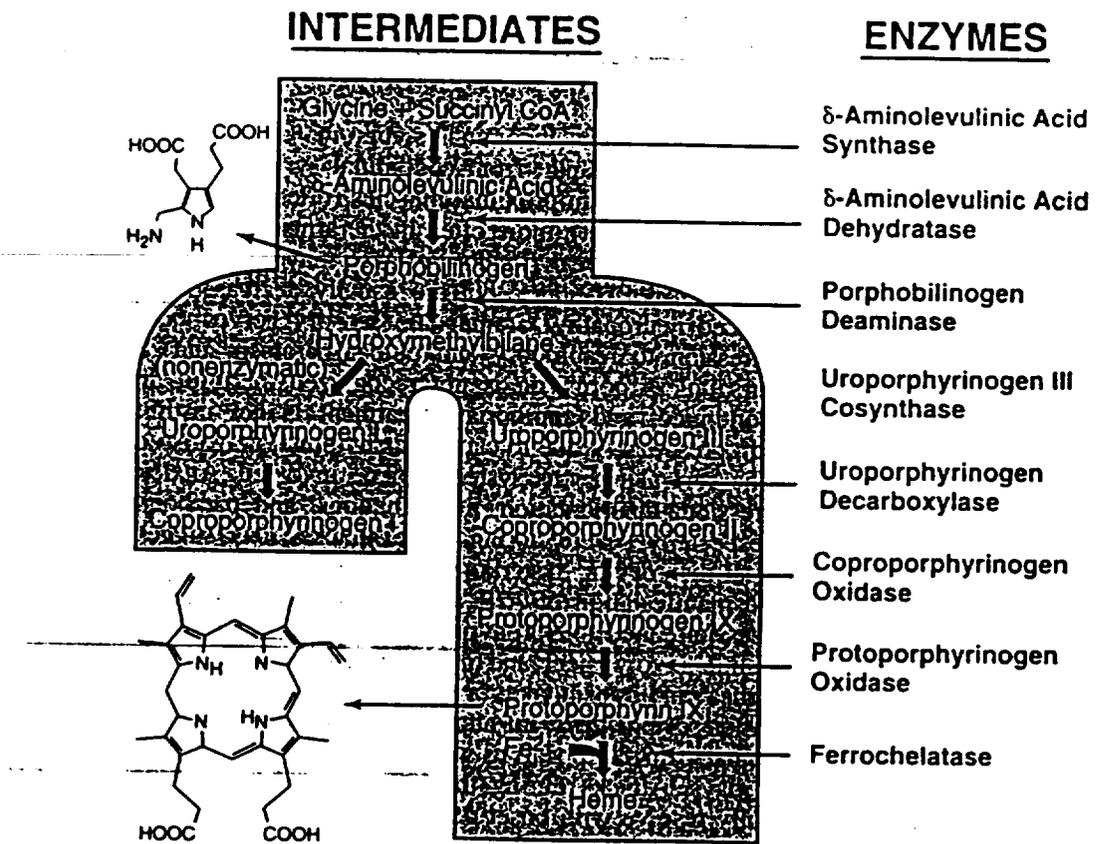
Figure D.2

Levulan® Kerastick™ and Component Parts



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Table D.3

STUDY: PHARM-03				
ACTIVATED LEVULAN® KERASTICK™ FOR TOPICAL SOLUTION, 20%				
Ingredient	Function	Theoretical Quantity (mg/applicator)	Theoretical Quantity (mg/mL)	Theoretical Percent (w/v)
Levulan	Active	354.0		
Alcohol USP	Vehicle			
Purified Water USP				
Laureth-4				
Isopropyl Alcohol USP	Vehicle			
Polyethylene Glycol				
Total				NA

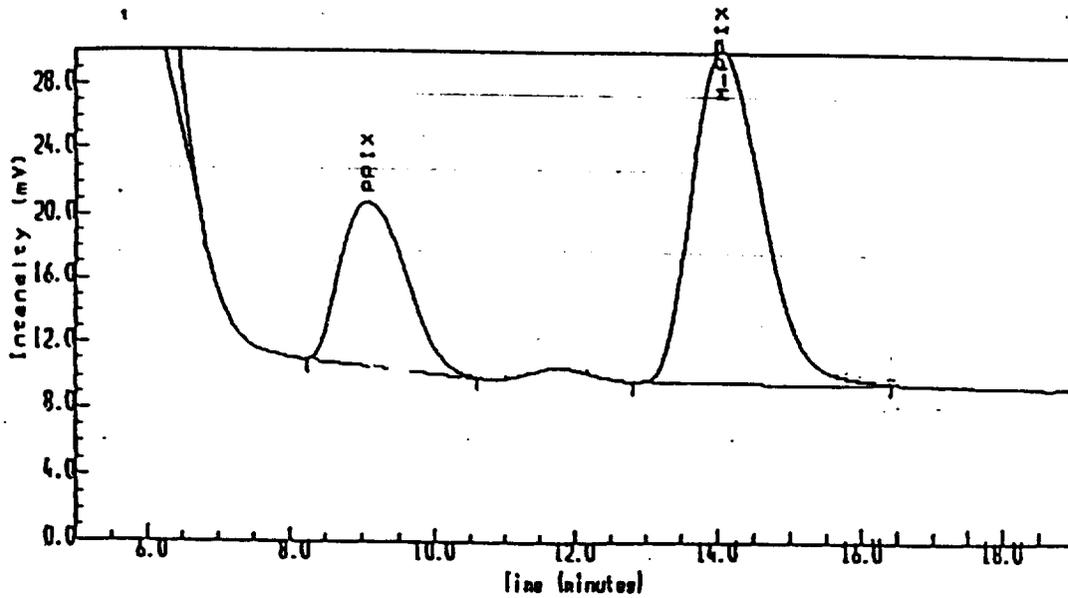
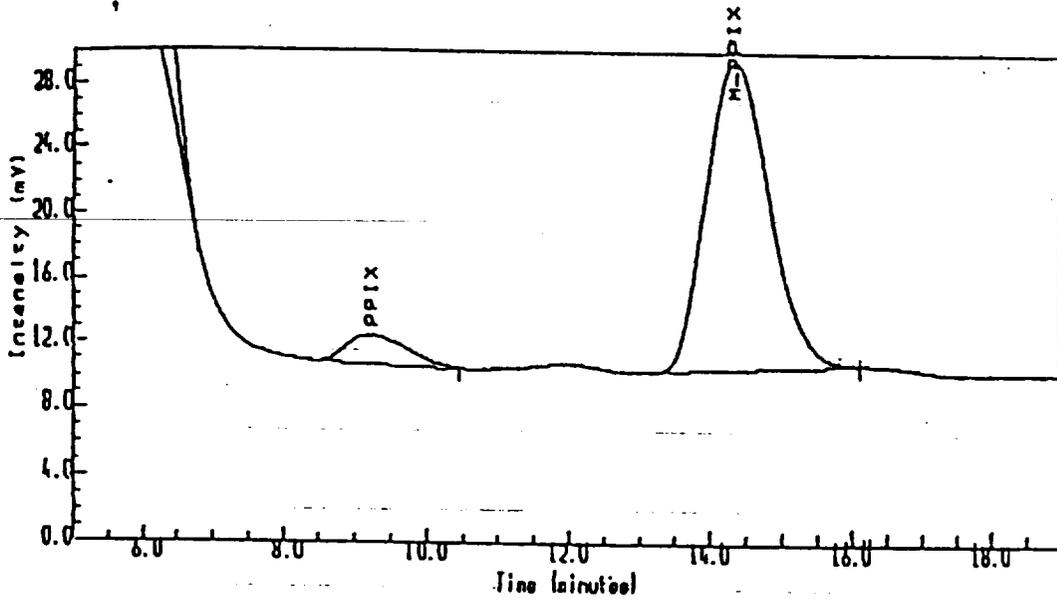
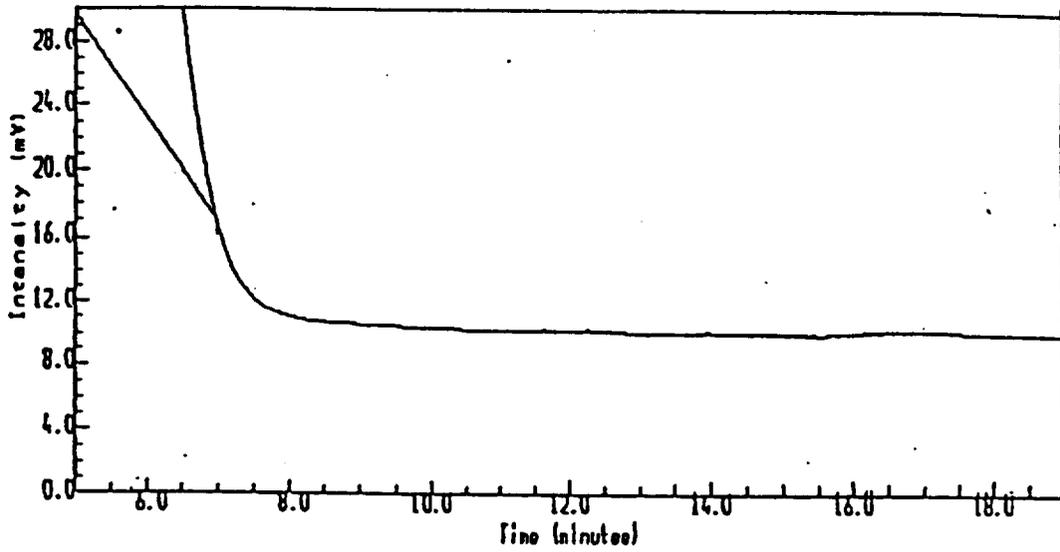
Table D.4

STUDY: PHARM-03				
ACTIVATED LEVULAN® KERASTICK™ FOR TOPICAL SOLUTION, VEHICLE				
Ingredient	Function	Theoretical Quantity (mg/applicator)	Theoretical Quantity (mg/mL)	Theoretical Percent (w/v)
Alcohol USP	Vehicle			
Purified Water USP				
Laureth-4				
Isopropyl Alcohol USP	Vehicle			
Polyethylene Glycol				
Total				NA

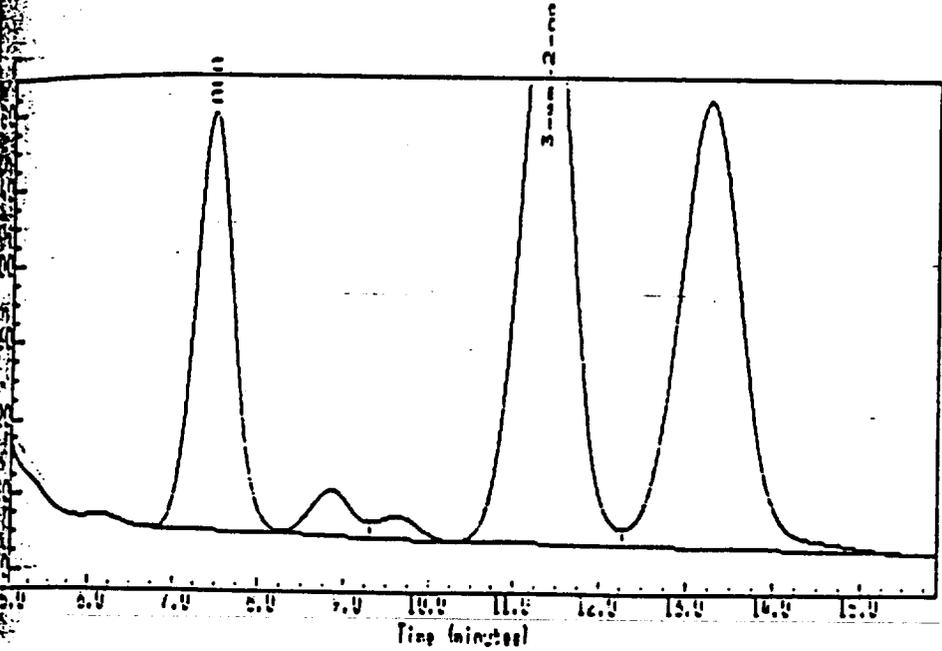
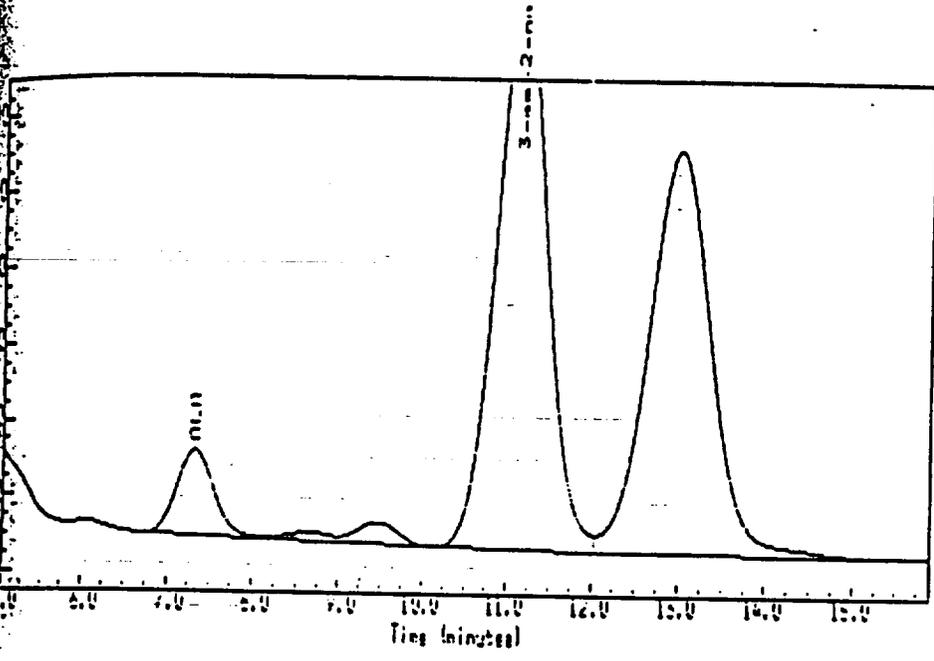
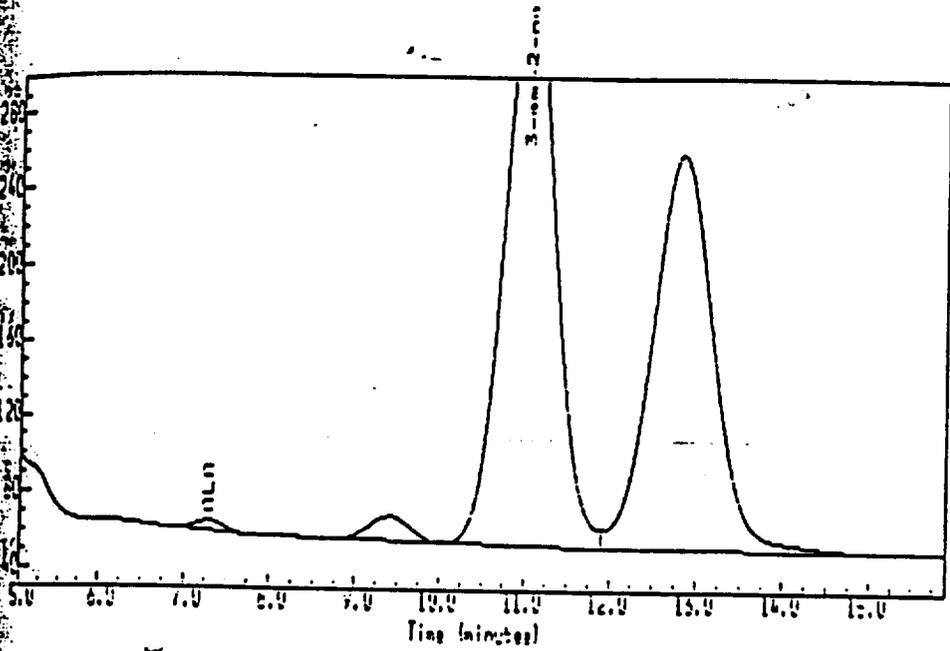
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NDA # 20965

Submission Date: June 29, 1998

Volume: 1.23

Study Type:

Bioavailability

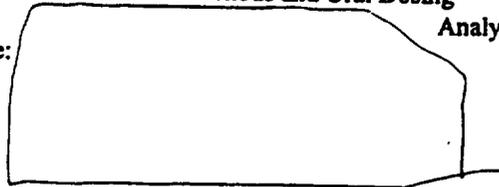
Study # PK-01

Study Title:

Pharmacokinetics of 5-aminolevulinic Acid (ALA) and protoporphyrin IX in Healthy Human Volunteers After Intravenous and Oral Dosing

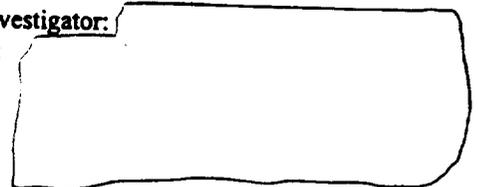
Clinical Investigator:

Site:



Analytical Investigator:

Site:



Study Objective: To describe the bioavailability of Levulan and the pharmacokinetics of Levulan and PpIX in the plasma of normal volunteers.

Study Design:

Single Dose:

Multiple Dose:

Randomized:

Washout Period: One Week

Cross-Over:

Parallel:

Other Design:

Fasted:

Post Dosing:

Food Study:

Food Type:

Study Subjects: Six healthy male subjects (5 Caucasian, 1 Black) were enrolled in this study. All subjects completed the study.

Subject Breakdown

Number of Subjects	Age (range); years	Height (range); inches	Weight (range); lb
6	28.3 (26.4 - 30.2)	70.2 (65.0 - 73.0)	164.8 (155.0 - 175.0)

Drug Products

Drug Product	Dose	Dosage Form	Strength	Packing Lot #
ALA HCl	128 mg, (eq. To 100 mg ALA)	Injection & Oral	100 mg	5141885
	Diluent for 5-ALA HCl injection; 10ml/vial			5111665

Sampling Times

Plasma:

Blood samples were collected at 0, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 hours after dose administration. The 0.1 hour was omitted for oral administration.

Assay Method:

[Redacted] was used for determination of protoporphyrin IX.

Assay Sensitivity:

10 ng/ml

Assay Accuracy:

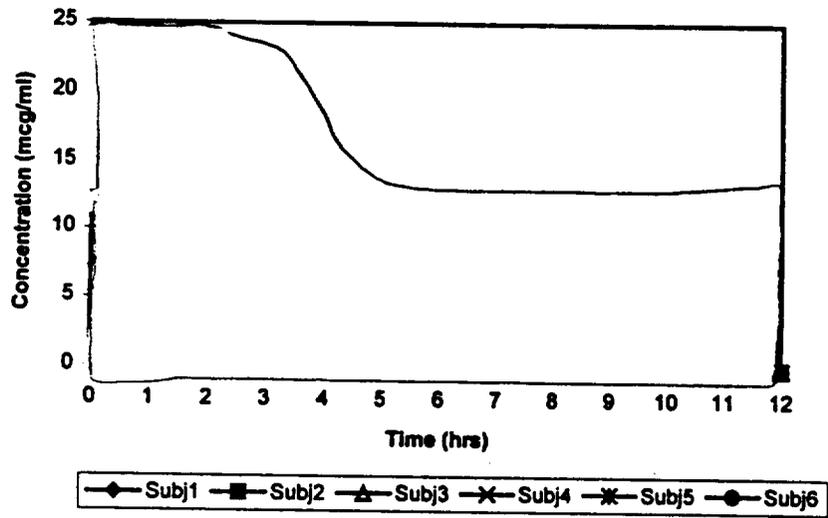
% Theor. range from [Redacted]

Assay Precision:

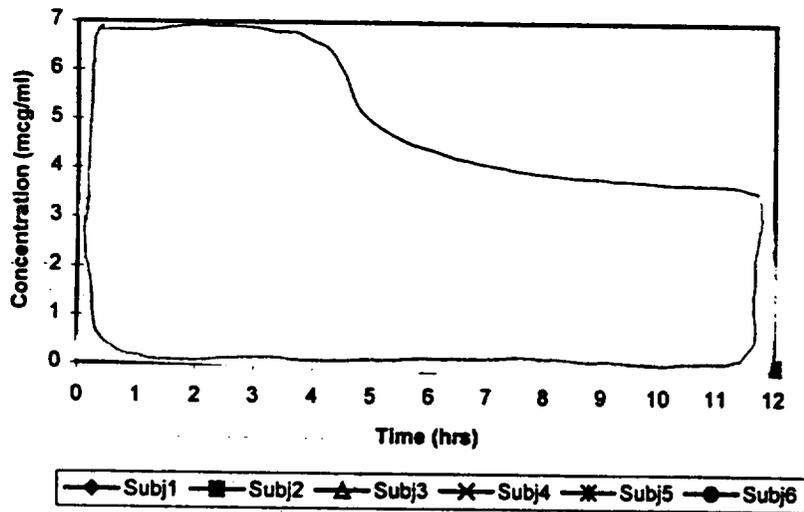
%CV range from [Redacted]

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### ALA: Plasma levels after Intravenous Administration



### ALA: Plasma Levels after Oral Administration



11 MEAN ALA HUMAN PLASMA

TABLE II - MEAN ALA PLASMA CONCENTRATIONS (Blank Corrected)																
INTRAVENOUS ADMINISTRATION (µg/ml)																
SUBJECT	0.1 hr	0.25 hr	0.50 hr	0.75 hr	1.0 hr	1.5 hr	2.0 hr	3.0 hr	4.0 hr	5.0 hr	6.0 hr	7.0 hr	8.0 hr	10.0 hr	12.0 hr	24.0 hr
1																
2																
3																
4																
5																
6																
Mean	16.67	10.67	7.67	5.73	4.10	2.39	1.62	0.72	0.27	0.12	0.06	0.02	0.00	0.00	0.00	0.00
Std. Dev.	6.23	3.77	1.67	1.69	1.16	0.73	0.58	0.37	0.11	0.05	0.04	0.02	0.00	0.00	0.00	0.00
CV%	36.94	35.33	21.24	29.48	28.19	30.38	35.94	50.64	40.17	42.74	78.07	129.11				
ORAL ADMINISTRATION (µg/ml)																
SUBJECT	0.25 hr	0.50 hr	0.75 hr	1.0 hr	1.5 hr	2.0 hr	3.0 hr	4.0 hr	5.0 hr	6.0 hr	7.0 hr	8.0 hr	10.0 hr	12.0 hr	24.0 hr	
1																
2																
3																
4																
5																
6																
Mean	1.22	3.47	4.15	4.05	2.48	1.69	0.64	0.24	0.11	0.04	0.01	0.01	0	0	0	
Std. Dev.	1.29	1.91	0.88	0.47	0.53	0.39	0.15	0.13	0.08	0.04	0.02	0.02	0	0	0	
CV%	105.73	55.07	21.26	11.57	21.18	23.14	23.31	56.28	74.36	108.49	244.95	244.95				

"III" PKIN TABLE

TABLE III - SUMMARY OF ALA PLASMA PHARMACOKINETICS									
	PO	IV	PO AUC	IV AUC	% REL. BA				
SUBJ.	AUC(0-t)	AUC (0-t)	(0-∞)	(0-∞)	PO/IV	IV MRT	PO MRT	Ke(1/hr)	Ke(1/hr)
	µgHr/ml	µgHr/ml	µgHr/ml	µgHr/ml	AUC (0-∞)	Hr	Hr	PO	IV
1									
2									
3									
4									
5									
6									
MEAN	7.31	12.47	7.30	12.50	60.32	1.05	1.49	1.03	0.84
SD	1.24	2.88	1.25	2.89	13.42	0.24	0.15	0.24	0.05
CV%	16.9	23.1	17.2	23.1	22.3	22.6	10.3	23.6	6.3
SUBJ.	T1/2	T1/2	IV	IV	IV Vdss	IV Vdss	PO Cmax	IV Cmax	PO Tmax
	PO (hr)	IV (hr)	Tot. Cl.	Tot. Cl.	Liters	ml/kg	µg/ml	µg/ml	Hours
			(ml/min)	ml/min/kg					
1									
2									
3									
4									
5									
6									
MEAN	0.70	0.83	141.42	1.88	8.75	116.14	4.65	15.44	0.83
SD	0.18	0.05	42.42	0.51	2.58	30.27	0.94	6.60	0.20
CV%	25.0	6.6	30.0	27.1	29.5	26.1	20.3	42.7	24.5

VII SUMMARY OF PPIX CONCS

TABLE VII - PROTOPORPHYRIN IX PLASMA CONCENTRATIONS (µg/ml)																	
IV																	
SUBJECT	0 HR	0.1 hr	0.25 hr	0.5 hr	0.75 hr	1 hr	1.5 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr	10 hr	12 hr	24 hr
1																	
2																	
3																	
4																	
5																	
6																	
MEAN	0.0082	0.008	0.042	0.018	0.002	0.018	0.022	0.018	0.0435	0.0312	0.0258	0.0182	0.0105	0.008	0.0125	0	0.0237
ORAL																	
SUBJECT	0 HR	0.25 hr	0.5 hr	0.75 hr	1 hr	1.5 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr	10 hr	12 hr	24 hr	
1																	
2																	
3																	
4																	
5																	
6																	
MEAN	0.0085	0.0082	0	0.010	0.008	0.003	0.011	0.030	0.022	0.017	0.026	0.009	0.009	0.020	0.000	0	

Table 2. Summary of PpIX fluorokinetics results. When the peak or half-life occurred during a rest period, ranges or estimates are reported in brackets. The error on the peak and plateau time estimates is at least  $\pm 2$  hr, and  $\pm 2000$  rel. units on peak intensity estimates. Values derived from curves where peak times are not during the rest period have a smaller error.

patient	Site	lesion grade and assessment of adjacent skin	peak intensity (rel. units)	peak time (hr)	plateau period (hr)	$t_{1/2}$ (hr)
3-001	lesion	2	22000	11		32
	adjacent	not quite normal	19000	10		30
3-002	lesion	1	13000	11		30
	adjacent	not quite normal	6000	11		26
3-003	lesion	2	(22000)	(12)		(30)
	adjacent	reddened not rough	(11000)	(14)		(29)
3-004	lesion	2	(15000)	(13)		(30)
	adjacent	normal	(12000)	(14)		(28)
3-005	lesion	2	18000	9		(est.47-65)
	adjacent	normal	22000	11		35
3-006	lesion	1	(14000)	(10)		(22)
	adjacent	sun-damaged	(12000)	(14)		(25)
3-007	lesion	2	(20000)	(11)	(29)	
	adjacent	weathered	(13000)	(11)	(22)	
3-008	lesion	2	(21000)	(12)	(36)	
	adjacent	sun-damaged	(18000)	(14)	(23)	
3-009	lesion	2	(19000)	(10)	(23)	
	adjacent	sun-damaged	(10000)	(13)	(29)	
3-010	lesion	2	(13000)	(10)	(27)	
	adjacent	not quite normal	(16000)	(11)	(43)	
3-011	lesion	2	(14000)	(11)	(19)	
	adjacent	abnormal	(12000)	(10)	(18)	
3-012	lesion	2	(22000)	(10)	(25)	
	adjacent	not quite normal	(13000)	(11)	(26)	
overall lesion		average $\pm$ std.dev., or range	(18000 $\pm$ 4000)	(11 $\pm$ 1)	(6 $\pm$ 1 to 19 $\pm$ 4)	(30 $\pm$ 10)
overall adjacent		average $\pm$ std.dev., or range	(13000 $\pm$ 4000)	(12 $\pm$ 1)	(7 $\pm$ 1 to 20 $\pm$ 2)	(28 $\pm$ 6)