# HPLC ASSAY FOR 5 – AMINOLEVULINIC ACID AND ITS APPLICATION TO ASSESSMENT OF SKIN PENETRATION

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**Introduction:** 5 – Aminolevulinic acid (ALA) is used in topical photodynamic therapy (PDT) of superficial skin lesions such as basal cell carcinoma. Absorption of ALA through skin lesions is poor, therefore several attempts have been undertaken to increase its skin penetration [1]. Dermaportation is a modified inductive energy technology to enhance skin penetration of drugs. The proposed mechanism of action is bioinduction caused by the generation of an electric effect. This reduces the barrier effect of the bilayer lipids to increase the skin penetration of a concurrently applied solute.

**Objective:** The purpose of this study was to assess the effect of the novel skin penetration enhancement technology, Dermaportation on the transdermal delivery of ALA.

**Methods:** An accurate and efficient HPLC method was developed for the detection of ALA obtained after transdermal permeation. The hydrochloride salt of ALA shows poor UV absorbance. A derivatization step was therefore necessary for analytical purposes. ALA was derivatized by addition of 0.1% fluorescamine solution and 0.1M borate buffer pH 8.0 in the ratio 1:1:3 to form a fluorescent derivitive suitable for HPLC analysis. *In vitro* diffusion across excised human epidermis was determined using Franz type diffusion cells (Fig. 1) maintained at 37°C . The donor compartment consisted of 2% w/v ALA in 1mL phosphate buffered saline pH 7.4 (PBS). The receptor compartment was filled with PBS and stirred throughout. Aliquots (500 μL) were taken from the receptor at 0, 0.5, 1, 1.5, 2, 3 and 4 h and immediately replaced with an equal volume of PBS. A aliquot was also taken from donor phase at 4 h. Dermaportation was applied continuously over the 4 h period and compared with a passive control. ALA content in all samples was assayed by HPLC with fluorescence detection (excitation 395nm and emission 480) following derivitization. Four cells were conducted for both active Dermaportation and three for passive control.

**Results:** The HPLC method developed was accurate and ALA eluted as a single peak with good linearity, accuracy and repeatability. Dermaportation enhanced transdermal delivery of ALA as compared to passive diffusion. This was shown by the flux values for Dermaportation and passive diffusion through the human epidermis during the period when Dermaportation was applied.

Table 1:HPLC method validation for ALA

| Validation Parameters | |
|---|---|
| **Linearity** | **$R^2$ = 0.9995** |
| **Precision (100 μg/mL,0.3 μg/mL)** | **RSD = 0.2%, 1.4%** |
| **Interday repeatability (5 μg/mL,25 μg/mL)** | **RSD = 0.8%, 1.9%** |
| **Intraday repeatability (5 μg/mL,25 μg/mL)** | **RSD = 1.13%, 1.70%** |
| **Lowest limit of detection (LOD)** | **120 ng** |
| **Lowest limit of Quantitation (LOQ)** | **400 ng** |
| **Accuracy (25 μg/mL)** | **100.6%** |
| **Stability of the fluorescent derivative** | **99% stable for 4 days under refrigeration** |

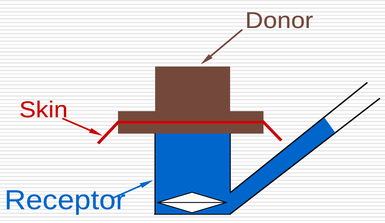


Fig 1: Franz cell

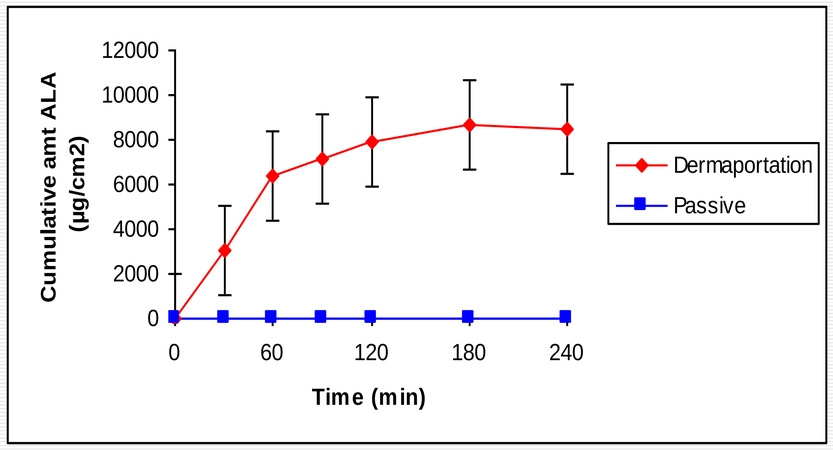


Fig 2: Cumulative amount of ALA penetrating human epidermis following application of 2% ALA solution with Dermaportation (0 - 4 h) or passive diffusion: mean ± sem, n = 4/3

**Conclusions:** Marked enhancement of transdermal delivery was obtained during Dermaportation with flux of 76.5 μg/cm$^2$/h as compared to passive flux of 0.12 μg/cm$^2$/h. The Dermaportation technology is being further evaluated to provide optimal skin penetration enhancement with a range of therapeutically relevant solutes.

## References

[1] Steluti R. et al. Topical glycerol monooleate/propylene glycol formulations enhance 5 – aminolevulinic acid in vitro skin delivery and in vivo protoporphyrin IX accumulation in hairless mouse skin. Eur J Pharm Biopharm 60: 439-444 (2005)