Obtaining known concentrations of fluorescein and lissamine green from strips

Purpose: New compounding pharmacy laws in the United States have made it difficult to obtain 2% fluorescein (FL) or 1% lissamine green (LG) for clinical or research use. The purpose of this study was to determine whether repeatable and known concentrations of these dyes could be obtained from soaking commercially available strips.

Methods: A standard curve was generated using successive dilutions of compounded 2% FL and 1% LG solutions (O’Brien Pharmacy, Mission, KS) in a preservative free eye wash (Ocufresh®). Samples were pipetted into a 24 well plate (Costar® 3526) and measured with an ELIZA plate reader (Fluostar® Galaxy) at wavelength of 485 nm for excitation and 520nm for emission of FL and absorbance at 595 nm for LG. 1-3 FL (Glostrips®) and LG (GreenGlo™) strips were soaked in 200μl of the same eye wash for 0.5, 1, 2, 3, 4 and 5 minutes and the concentration of FL and LG determined using the calibration curves. Each strip and time period was repeated 3 times to assess the variability of the measure.

Results: Soaking one to three FL strips for increasing times (0.5 – 5 minutes) produced concentrations from 0.21% to 1.84% FL. One strip yielded concentrations ranging from 0.21% to 0.50% with a average standard deviation of ±0.07%; two strips ranged from 0.40% to 0.99% (±0.14%) and three strips ranged from 0.59% to 1.84% (±0.30%). The overall variability (standard deviation) among repeated FL measurements from all trials was ±0.17% FL. Soaking LG strips for increasing times produced concentrations from 0.25% to 2.28% LG. One strip yielded concentrations ranging from 0.25% to 0.82% with a average standard deviation of ±0.03%; 2 strips ranged from 0.51% to 1.7% (±0.04%) and 3 strips ranged from 1.14% to 2.28% (±0.05%). The overall variability (standard deviation) among repeated LG readings was ±0.04% LG. These results are shown in the attached figure.

Conclusions: Soaking FL and LG strips for controlled times in a measured amount of saline yielded repeatable results, especially for LG. Although 3 strips of FL soaked for 5 mins did not quite yield 2% FL, lesser amounts were easily obtainable. Concentrations close to 1% LG could be obtained using 2 or 3 strips and varying the soaking times, with very high repeatability. These results indicated that clinicians can overcome the lack of availability of FL and LG solutions by using this or similar methods.
FL and LG concentrations from soaked strips
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