

The FDA is providing to the public a Thin Layer Chromatography (TLC) procedure that can be used as an initial screening procedure to detect the presence of Diethylene Glycol (DEG) in toothpaste. The purpose of the screening procedure is to enable testing of a wide range of products in a cost and time effective manner. This TLC procedure has the capability of detecting DEG in toothpaste at a level of 0.1 % DEG and can be considered as an alternate procedure. Once a positive result for the presence of DEG is found a second procedure such as GC-MS, which is also provided, can be performed for confirmation and a more accurate estimation. The GC-MS method is currently used by the FDA laboratories for the determination of DEG content in toothpaste samples that have been collected by FDA investigators.

### **Identification of Diethylene Glycol (DEG) in Toothpaste by Thin Layer Chromatography (TLC)**

The method of Kenyon *et al.*<sup>1</sup> has been used for determination of Diethylene glycol in toothpaste. This method is capable of detecting DEG in toothpaste at 0.1%. Below is a concise description of the modified sample preparation step and the procedure to be used.

#### Materials:

Any commercially available TLC developing tank

Corning 50 mL Polypropylene Rim Seal Cap Centrifuge Tubes - Catalog #25335

Bio-Rad 1.5 mL Polypropylene Micro Test Tubes with attached cap – Catalog # 223-9501

TLC Plates - Whatman Silica Gel G flexible plates with polyester backing, 20 x 20 cm with layer thickness of 250  $\mu$ m

Developing Solvent - Acetone: 5M Ammonium Hydroxide: Toluene (85:10:5) (prepare daily)

Spotting volume - 5 micro liters

Detection - Oxidation by Potassium Permanganate

(Potassium Permanganate staining solution concentration 6.7 mg/mL in developing solvent prepared immediately before use)

Sample Preparation – Weigh approximately 1.0 g of toothpaste (equivalent to approximately one application on a toothbrush) into a 50 mL polypropylene centrifuge tube. Add 5 mL water and vortex approximately 1 minute to disperse the toothpaste (some foaming may occur). Add 5mL acetonitrile to the mixture and vortex approximately 1 minute. Centrifuge the mixture until a clear solution is obtained. Collect approximately 1 mL of the supernatant. Transfer 500 micro liters of the supernatant to a 1.5 mL micro test tube and add 1.0 mL methanol.

Reference Standard Preparation – Dilute 0.2 mL of diethylene glycol to 5.0 mL methanol. Dilute 500 micro liters of this solution with 1.0 mL methanol. This solution is used only in determination of the  $R_f$  (retardation factor)<sup>2</sup> and not for any quantitative comparisons.

Chromatographic Procedure – A spotting line (origin) is drawn 2 cm from the bottom of the sheet without cutting into the coating by very lightly applying a pencil tip. A development stopping line is marked 10 cm from the spotting line on the TLC sheet by placing a straight edge across the sheet and removing approximately 1 mm of the silica coating by bearing down on a pencil tip. Spot 5 micro liters of the diluted test solution and of the reference standard

solution onto the sheet and allow to dry. Place the sheet into the developing tank which has been previously prepared by equilibrating with 100 mL of developing solvent for approximately 30 minutes. After the developing solvent has reached the stopping line remove the plate from the tank and allow to air dry.

Detection Procedure – Submerge the dried sheet in a freshly prepared (immediately before use) solution of potassium permanganate for approximately 5 seconds. Remove the sheet; as it begins to dry yellow spots will appear on the purple background, DEG is the last spot to appear. Circle the spots with a pencil since all spots will fade. Compare the  $R_f$  of the sample spots to the  $R_f$  of the diethylene glycol standard for determination of the presence of DEG in the toothpaste product.

Discussion: A common practice for increasing the sensitivity of TLC is to multiply spots by applying the first spot (5  $\mu$ L) allowing it to dry and spotting additional spots on top of the dried spot.

This method can also be used to estimate the concentration of DEG in toothpaste, glycerin and syrup samples by spotting standard solutions of DEG in different or bracketing concentrations and comparing spot intensities.

This method has also been used in the identification of DEG in children's cold preparations (elixirs and syrups).

1. Simple At-Site Detection of Diethylene Glycerol/Ethylene Glycol Contamination of Glycerin and Glycerin-Based Raw Materials by Thin-Layer Chromatography”

Alan S. Kenyon, Shi Xioye, Wang Yan, and Ng Wai Har: Journal of AOAC International Vol. 81, No. 1, 1998, pages 44-50

2. USP 23, <621> Chromatography, pg 1777

