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Simplified assay of diethylene glycol and ethylene glycol in various raw materials by capillary gas chromatography

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Synopsis

The FDA has recently taken steps to reduce risks due to raw materials affected by economically motivated adulteration (EMA). One area of great interest is diethylene glycol (DEG) or ethylene glycol (EG) adulteration of glycerin, propylene glycol, and solutions of sorbitol, for which the USP monographs have recently been revised (1). Such adulterations have occurred many times and in many countries, including a tragic episode between November 2008 and January 2009 in which 84 children in Nigeria died after ingesting teething syrup contaminated with DEG (9,10). To eliminate this problem, the FDA has required manufacturers of finished products to assay and confirm that incoming glycerin, propylene glycol, and sorbitol solutions meet the USP limits, and the FDA/USP has incorporated such testing into the identity requirements of its updated monographs.

Unfortunately, even though USP test procedures detail a simultaneous DEG and EG assay for these materials, different standard solutions are specified depending upon whether the incoming sample is glycerin, propylene glycol, or a sorbitol solution; in addition, a certain gas chromatography (GC) capillary phase is detailed for sorbitol solutions, while the assays for glycerin and propylene glycol use a different capillary phase, requiring column changeovers, separate GC systems, or front/rear column configuration. In addition, NF monographs for polyethylene glycols (PEG) and polyethylene glycol monomethyl ethers (MPEG) used in pharmaceutical products also require DEG and EG testing (detailing their own specific tests); three separate test procedures for these types of raw materials (the larger PEG-type polymers are assayed differently than their smaller counterparts), making assay at QC unwieldy.

This paper describes a single, simple test procedure that is applicable to the simultaneous assay of DEG and EG in all types of the described raw materials, using one standard solution. The assay procedure involves straightforward isolation, trimethylsilylation, and simultaneous capillary gas chromatographic quantitation using capillary GC with flame ionization detection. Although the USP-NF limits are 0.10% DEG and 0.10% EG (and 0.25% total DEG plus EG for the PEG and MPEG products), in reality any EMA would be at levels significantly higher than that, as low-level illegal EMA would not be economically advantageous. The scope of this project was not to fully validate this technique for inclusion in USP-NF, but just to demonstrate its applicability for those wishing to utilize it or take it further.

INTRODUCTION

The FDA has become more concerned about raw materials affected by economically motivated adulteration (EMA)—willfully adulterating more expensive desired material by adding less expensive material. Many times, this adulteration is done using significant scientific understanding, to produce adulterated material that passes most quality checks.

This is problematic because such adulterated materials can present serious public health risks and consequences. Recent examples of EMA that resulted in deaths include the 2008 adulteration of milk and infant products with melamine (12), and the diethylene glycol adulteration of glycerin (Nigeria 2008–2009, Panama 2006, India 1998, and Haiti 1995) (9,10), which will be addressed here. In response to this, the FDA requested that the USP incorporate into the identity section of its glycerin monograph the requirement for finished product manufacturers to assay and confirm that DEG and EG meet the limit maximum of 0.10% (each); this updated glycerin monograph became official in May 2009. In addition, the FDA/USP has incorporated similar DEG and EG requirements into its updated monographs for propylene glycol and for sorbitol solutions, effective February 2010 (2,3). The monographs for these three materials detail a simultaneous DEG and EG assay, but require different standard solutions depending upon whether the incoming sample is glycerin, propylene glycol, or a sorbitol solution. In addition, monograph GC tests for the DEG and EG in glycerin and propylene glycol use a G43 stationary phase while the test for sorbitol solutions details a G46 stationary phase. Polyethylene glycols (PEG) and polyethylene glycol monomethyl ethers (MPEG) have a USP-NF maximum level of 0.25% for the combination of DEG and EG; their USP-NF test procedures utilize older technology, packed-column gas chromatography (GC). Higher-molecular-weight samples of PEG and MPEG require unwieldy, time-consuming vacuum distillation followed by separation and colorimetric quantitation of the total of DEG and EG (1).

Current official USP-NF test procedures (1) for these polyol-type materials detail:

(1) Capillary GC using phase G43 for glycerin samples and propylene glycol samples
(2) Capillary GC using phase G46 for sorbitol solutions
(3) Packed-column GC using phase G13 for PEG samples with nominal molecular weight less than 450, with quantitation done by peak height
(4) Packed-column GC using support S2 for MPEG samples with nominal molecular weight less than 600, with quantitation done by peak height
(5) Complex vacuum distillation followed by colorimetric total assay for PEG samples with nominal molecular weight 450 or above, but not more than 1000, and MPEG samples with nominal molecular weight 600 or above but not more than 1500

The FDA states that "a manufacturer may use an equivalent identification procedure that includes a test to detect and quantify DEG provided it meets the relevant safety limit." (3). Advances in capillary gas chromatography have greatly enhanced capabilities for resolving complex mixtures; frequently, the resolving capacity of capillary columns can eliminate the need for extensive sample preparations or cleanups. In reality, any EMA with DEG or EG would occur at levels much higher than the USP-NF limits, and so any test procedure that could simply determine levels at the USP-NF limits or higher should be able to be utilized if documented. Since the author had expertise (including publications) for components similar to DEG and EG, and with matrices such as these, it was logical for this to be investigated using modern techniques. The sought-for analytes and the sample components themselves were reacted with BSTFA trimethylsilyl derivatizing agent, then taken for capillary GC analysis. The sample preparation is straightforward and requires approximately five minutes, and GC quantitation is completely automated, including calculations of the DEG and EG levels.

EXPERIMENTAL

INSTRUMENTS AND CONDITIONS

Analyses were performed on an Agilent Technologies 5890 gas chromatograph system that included a flame ionization detector, a model 7673 autosampler, and ChemStation software (Agilent Technologies, Palo Alto, CA). The column was a 30-m × 0.32-mm i.d. HP-5 fused silica capillary column coated with 5% diphenyl–95% dimethylsiloxane copolymer (crosslinked) at 0.25-μm film thickness (Agilent Technologies #19091J-413). The inlet split liner was an SGE FocusLiner. The column was installed into a split/splitless injection port held at 300°C and connected to a flame ionization detector held at 300°C; the carrier gas was helium held at 10 psi head pressure with a split ratio of about 30:1. The GC oven temperature was held at 90°C for four minutes, then programmed at a rate of 10.0°C/min. to reach a temperature of 280°C, where it was then held constant for five minutes. With these conditions, the retention times for silylated ethylene glycol, silylated propylene glycol, silylated diethylene glycol, silylated glycerin, and silylated sorbitol were approximately 2.8 minutes, 3.1 minutes, 7.7 minutes, 8.2 minutes, and 17 minutes, respectively.

REAGENTS AND SOLUTIONS

ACS reagent grade DMF (N,N-dimethylformamide) was obtained from Fisher Scientific (Pittsburgh, PA). BSTFA reagent (bis[trimethylsilyl]-trifluoroacetamide containing 1% trimethylchlorosilane) was obtained from Regis Technologies (Morton Grove, IL). Diethylene glycol, ethylene glycol, glycerin, and propylene glycol reference standards were purchased from either USP or Sigma Aldrich (St. Louis, MO). USP sorbitol solution was obtained from ADM (Archer Daniels Midland). Real-world USP-grade glycerin samples were produced by Dial Corporation's bar soap manufacturing facility (Montgomery, IL, now owned by VVF Corporation); PEG-6 methyl ether, PEG-8, and PEG-12 were provided by Lambent, Huntsman, and Dow. To prepare the mixed standard solution, about 0.15 g each of DEG and EG were accurately weighed (± 0.0001 g) into a single 100-ml volumetric flask, then mixed and diluted to volume with DMF; then 5.00 ml was pipetted into a 50-ml volumetric flask and mixed and diluted to volume with DMF. On each day of use, a 250-μl portion from the dilute mixed standard was transferred to an autosampler vial where it was mixed with 500 μl of BSTFA reagent.

ASSAY PROCEDURE AND CALCULATION

Two and one half grams of well-mixed sample was weighed (± 0.0001 g) into a 25-ml volumetric flask and mixed and diluted to volume with DMF. Two hundred fifty microliters was transferred to an autosampler vial where it was mixed with 500 μl of BSTFA reagent. Two microliters was then injected into the GC and compared to 2-μl injections of the silylated standard mixture. Routine external standard calculations were used to determine percent DEG and EG.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

A project was initiated here to develop an assay to confirm that USP glycerin, USP propylene glycol, USP sorbitol solutions, and incoming polyethylene glycol and polyethylene glycol monomethyl ethers (Figure 1) did not contain DEG or EG adulteration. Since setting up five to six separate test procedures for these materials at QC would be difficult due to space and instrument requirements, the need for a single, straightforward assay existed. A review of the literature found a TLC procedure for DEG and EG suggested by the FDA as an alternative to the USP monograph test (4), but this required followup GCMS confirmation and quantitation (5), and thus would not be amenable to QC use. Two papers described an assay for trace ethylene glycol in used motor oil by GC (6,7). The author saw the opportunity to develop a single test method that would require less operator time, be amenable to automated analysis, and provide reliable quantitation. This laboratory has expertise in assaying similar materials, including DEG and glycerin, at low levels by capillary GC, and it was decided to evaluate that technique to see if it was amenable to all four of the raw materials stated above. Materials like these, with –OH (hydroxyl) functionalities, form strong internal hydrogen bonding, which makes volatilization and gas chromatography challenging, especially for materials with multiple hydroxyl groups such as those detailed above. Because of this, derivatization using reagents such as BSTFA (bis[trimethylsilyl]-trifluoroacetamide) can be used to improve volatilization

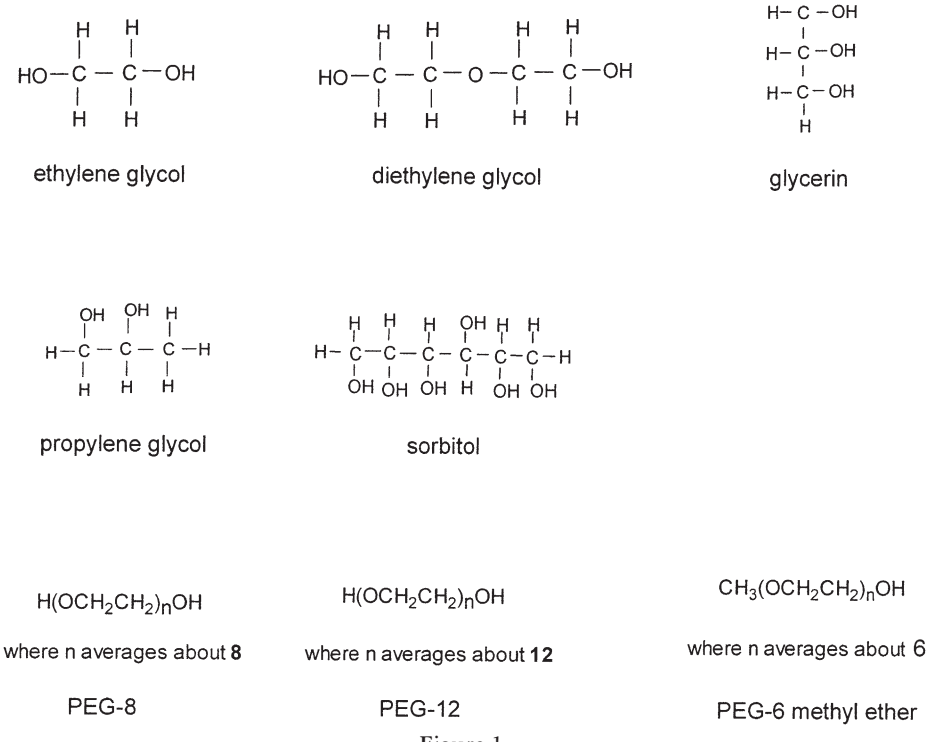

Figure 1.

and chromatography by reacting with active hydrogens of the hydroxyl groups; even though an active hydrogen is replaced by a heavier trimethylsilyl group, the resulting derivative is usually more volatile and delivers sharper peaks due to the polarity of the molecule being decreased. For example, for glycerin (Figure 2), the following technique was employed. This technique was published by this author in 1987 for quantitation of glycerin in consumer products at both use levels and trace levels (8) and was considered for application to the issue of EG and DEG quantitation at low levels.

Standards were prepared by dissolving DEG and EG in N,N-dimethylformamide solvent (DMF) and mixing with BSTFA reagent in autosampler vials; samples were prepared similarly, as was a reagent blank. Since DMF and BSTFA reagent are not much more volatile than derivatized EG, the GC oven program was initiated at 90°C, held there for 4.0 minutes, and then programmed to remove the derivatized polyol components off the GC column prior to the next injection.

Either the DMF-BSTFA reagent blank or a concentrated standard EG-DEG-BSTFA mix could be used to positively identify which peaks were EG and DEG. Routine external standard quantitation was used; the assay was straightforward.

RESULTS

A sample of real-world USP grade glycerin, a sample of propylene glycol, a sample of USP 70% sorbitol, two suppliers' samples of PEG-8, two suppliers' samples of PEG-12, and three suppliers' samples of PEG-6 methyl ether were assayed (Figs 3–7). All the samples assayed above would meet current USP-NF DEG and EG level requirements. The glycerin, propylene glycol, and sorbitol solution were below detection level (BDL). The results are detailed in Table I.

Small artifact peaks in reagent blanks (and in standards and samples) when using BSTFA trimethylsilyl derivatization have been well-documented in the literature (13,14) but do not elute near the retention times of DEG or EG, and so these do not interfere with the assay; one such artifact typically found is $C_5H_7N_2OF_3$.

ACCURACY AND PRECISION

System suitability calculations for the above had %RSDs of 0.10 and 1.24 for the EG retention times and peak areas, respectively, and a tailing factor of 0.99, all within cGMP

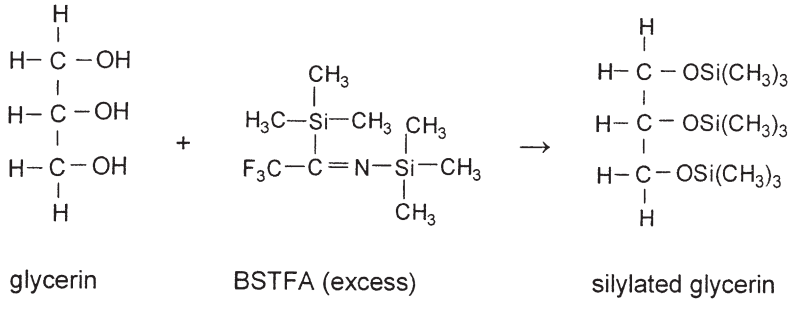

Figure 2.

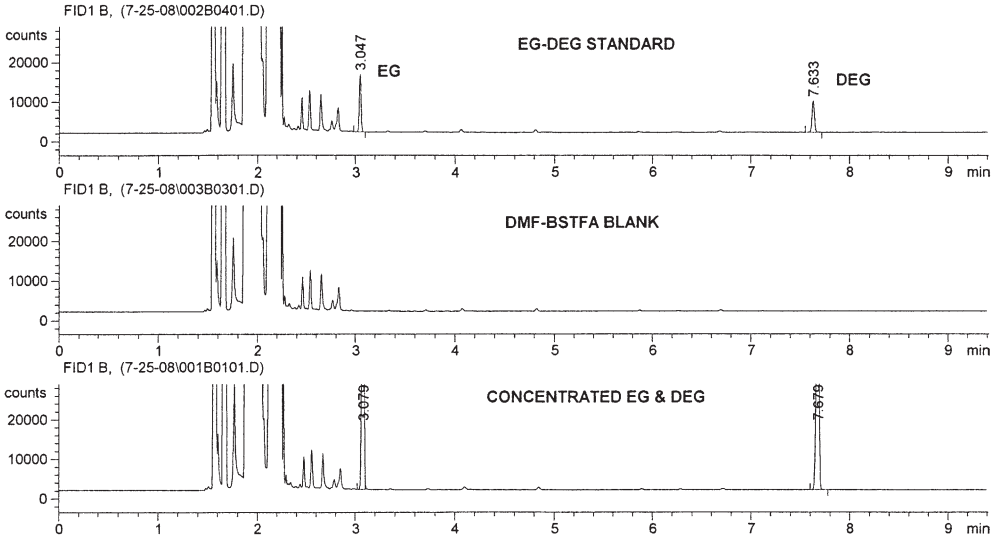

Figure 3. Chromatograms detailing injection of EG-DEG mixed standard (top), reagent blank (middle), and concentrated EG-DEG (bottom) to positively identify EG and DEG peaks.

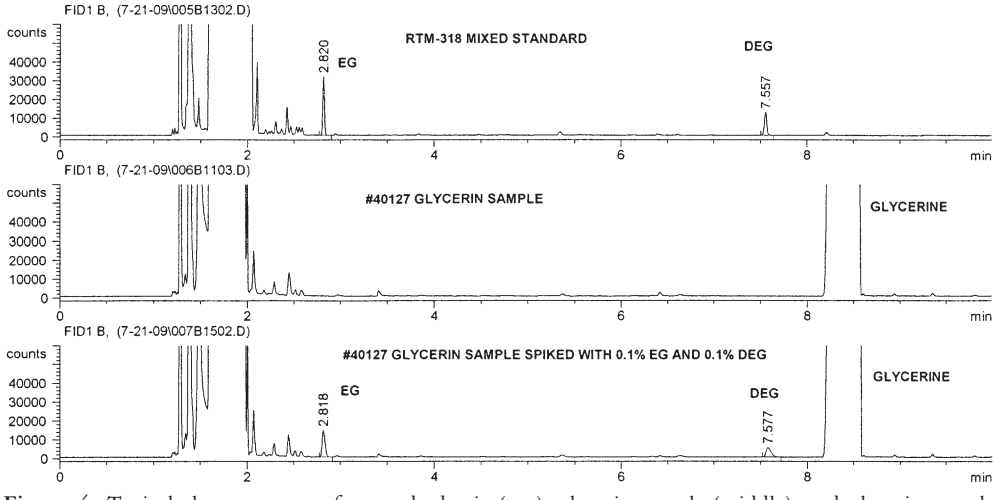

Figure 4. Typical chromatograms for standard mix (top), glycerin sample (middle), and glycerin sample spiked at USP limits of 0.10% EG and 0.10% DEG to demonstrate how sample at compliance threshold would appear (bottom).

guidelines. For DEG, the %RSD was 0.06 for retention times and 1.09 for peak areas, with a tailing factor of 1.00, also meeting cGMP guidelines. Full cGMP validation of this test procedure was not performed, as any values measured above 0.10% would render the material out of compliance, and so determining how far a sample might be out of compliance is not the goal of either this test or the USP-NF tests. Figure 8 demonstrates reproducibility.

Since sample #39191-2 was assayed at below detection limits for EG and DEG, it was used for spiking/recovery studies (Figure 9). Sample #39191-2 was spiked at two known levels with EG and DEG, then assayed. Recoveries of the sample spiked with 0.0792%

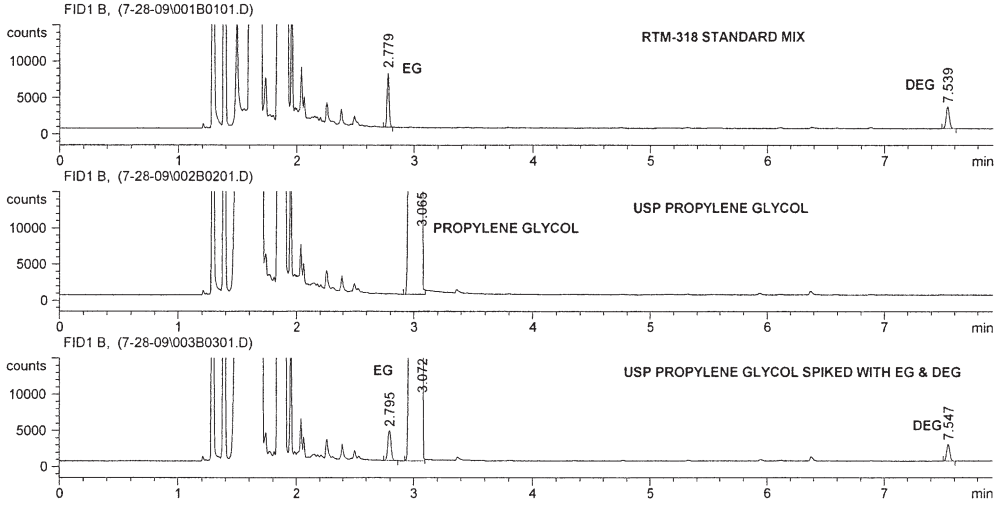

Figure 5. Typical chromatograms for standard mix (top), propylene glycol sample (middle), and propylene glycol sample spiked at USP limits of 0.10% EG and 0.10% DEG to demonstrate how sample at compliance threshold would appear (bottom).

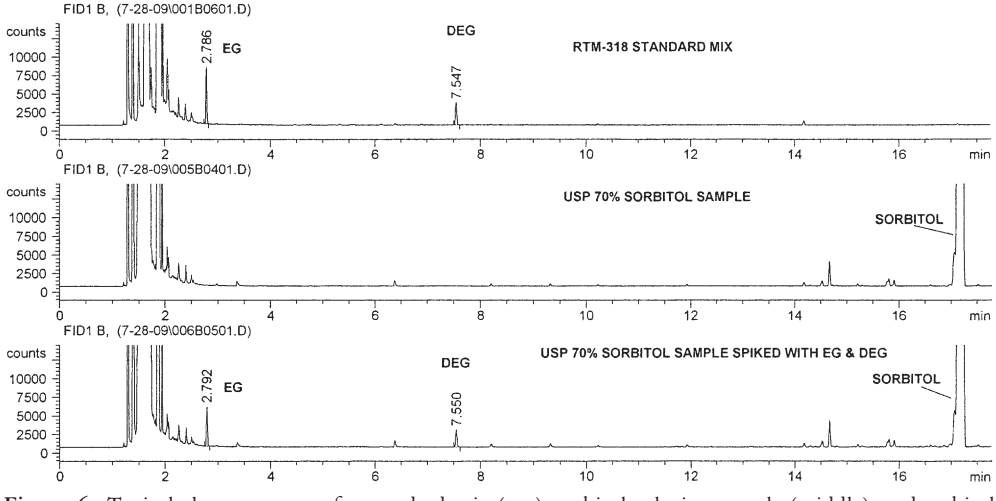

Figure 6. Typical chromatograms for standard mix (top), sorbitol solution sample (middle), and sorbitol solution sample spiked at USP limits of 0.10% EG and 0.10% DEG to demonstrate how sample at compliance threshold would appear (bottom).

and 0.1584% EG were 93.8% and 93.2%, respectively. Recoveries of the sample spiked with 0.0831% and 0.1662% DEG were 102.9% and 103.4%, respectively.

DISCUSSION

This test procedure is offered as a single simplified alternative to six separate DEG and EG test procedures specified in the USP-NF. This test procedure enables a single mixed standard and one set of GC conditions to be used that would replace five separate USP GC

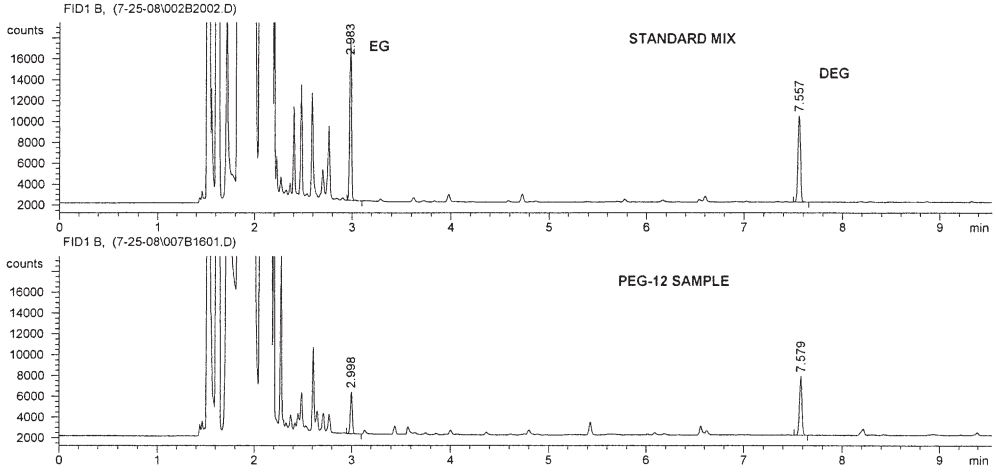

Figure 7. Typical chromatograms for standard mix (top) and PEG-12 sample (bottom).

Table I
Experimental Results

Identification	Sample type	Suppler code	% DEG	% EG
	Glycerin	B	BDL	BDL
	Proplene glycol	E	BDL	BDL
	70% Sorbitol solution	F	BDL	BDL
39157-1	PEG-8	A	0.0268	0.0118
			0.0291	0.0124
39157-2	PEG-8	C	0.0482	0.034
			0.0495	0.0349
39158-1	PEG-12	A	0.0477	0.0216
			0.0493	0.0223
39158-2	PEG-12	D	0.1138	0.0417
			0.1174	0.0428
39191-1	PEG-6 ME	A	0.0087	0.0087
			0.0083	0.0086
39191-2	PEG-6 ME	C	BDL	BDL
39191-3	PEG-6 ME	D	0.0218	0.0096

PEG-6 ME = PEG-6 methyl ether.
BDL = Below detection levels.

tests with four different sets of standards and four different column types (two capillaries and two packed columns). This test also replaces the vacuum distillation-colorimetric test used for larger PEG and MPEG homologs. Utilizing this technology could greatly simplify and improve efficiencies in the quality control laboratories of facilities receiving these USP-NF materials.

CONCLUSION

This test procedure has not been validated, but has been detailed here to be amenable for the assay to determine DEG and EG compliance in various polyol raw materials. With

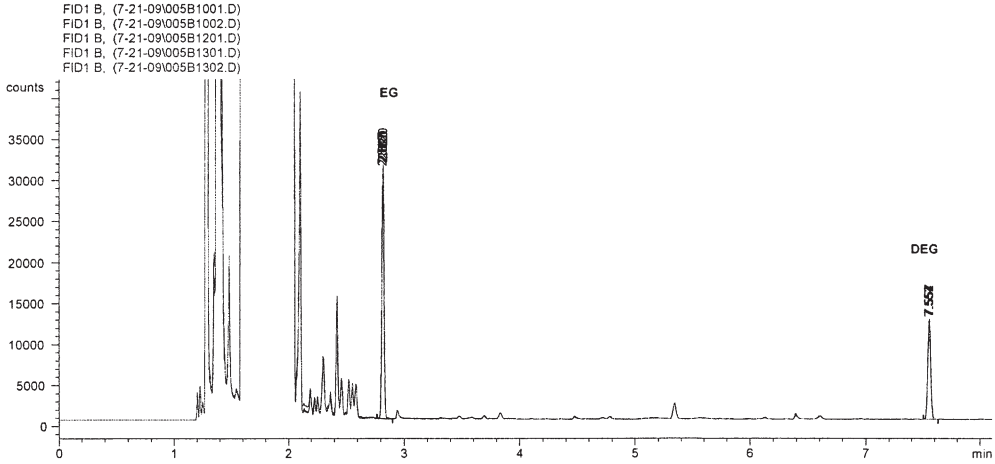

Figure 8. Five standard chromatograms overlaid, demonstrating reproducibility.

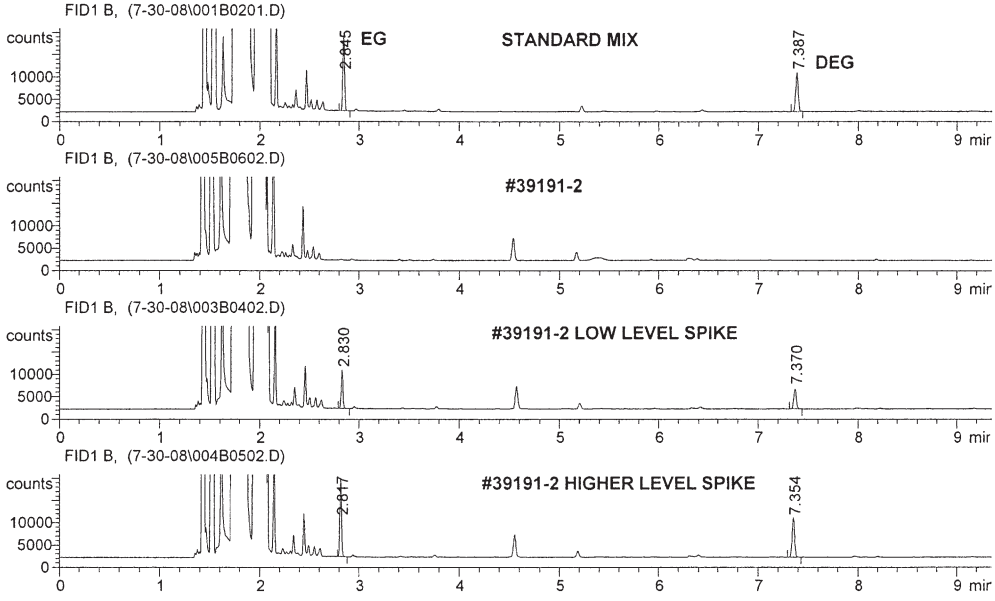

Figure 9. Chromatograms from spiking study.

appropriate standards, this basic test procedure could also be useful as an assay procedure for incoming glycerin, propylene glycol, and sorbitol.

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