Informal Commentary
Limit of Diethylene Glycol (DEG) and Ethylene Glycol (EG) in Sorbitol Solution, Sorbitol Sorbitan Solution and Noncrystallizing Sorbitol Solution
December 2009

Monograph/Section(s): Sorbitol Solution, Sorbitol Sorbitan Solution and Noncrystallizing Sorbitol Solution/Identification C: Limit of EG and DEG
Expert Committee(s): Excipient Monographs 1
No. of Commenters: 10

Comment Summary #1: The commenter tested the proposed EG/DEG limit test for Sorbitol Solution and did not see peaks in their *Standard solution* for EG and DEG (EG and DEG, 0.08 mg/mL each).
Response: Through communications with USP, the commenter identified and resolved what turned out to be an instrument problem (column related).

Comment Summary #2: The commenter questioned why a 96:4 Acetone:DI Water diluent was being used. Sorbitol is very soluble in DI Water and the commenter could not get the sorbitol to go into solution with the acetone diluent.
Response: The Expert Committee recommended adding a clarification note to state, [NOTE—Acetone is used to precipitate sorbitol] in the *Sample solution* preparation section of the proposed method. This statement was incorporated because the Expert Committee determined that during a USP Web Meeting held on 16-17 March 2009, discussions on previous USP laboratory experiments conducted during the method development stage indicated that sorbitol solution samples prepared in water were unsuitable for the GC method, and as a result, requires a water- and sorbitol-free sample preparation. In order to prepare water- and sorbitol-free test solutions that would be suitable for the GC analysis, precipitation of sorbitol with water miscible organic solvents was explored. Sorbitol solution samples prepared by precipitation with Acetone:DI Water mixture (96:4, v/v) yielding adequate EG and DEG recoveries gave the desired results on the GC columns. It produced:

- No splitting or fronting of EG or DEG peaks was observed.
- Late-eluting sorbitol related peaks were greatly minimized
- No carry-over was observed.

Comment Summary #3: The commenter requested USP's detailed sample preparation for Sorbitol Solution and questioned why there is a need to dilute and vortex as described in the sample procedure.
Response: The Expert Committee determined that both EG and DEG are highly soluble in water and acetone, while sorbitol is only soluble in water. The detailed sample procedure involving dilution with diluent (Acetone:DI water 96:4, v/v) in three portions and vortexing after each addition was designed to achieve close to 100% recoveries of both analytes from the water-based Sorbitol Solution by keeping a balance between two processes occurring simultaneously, resulting in the precipitation of sorbitol and

extraction of EG and DEG from the matrix. However, the addition of all diluent at once resulted in poor recoveries.

Comment Summary #4: The commenter suggested that the *System suitability* requirement of NLT 30 between EG and DEG in the Sorbitol method seems excessive.
Response: The Expert Committee determined that the Propylene Glycol peak is quite often observed in the *Sample solution* after Ethylene Glycol peak and before Diethylene Glycol peak, therefore, the *Resolution* between Ethylene Glycol and Diethylene Glycol must ensure separation and agreed that this resolution is easily achieved under the stated conditions.

Comment Summary #5: The commenter questioned the resolutions the USP laboratory is routinely getting in sorbitol since the retention times are 1.2 and 3.1
Response: Based on the laboratory data, the Expert Committee determined that the Resolution is about 55. Also, the Expert Committee determined that this is the combined Resolution of Diluent peak (14) and DEG peak (41).

Comment Summary #6: The commenter indicated the requirement for holding a 25-mL volumetric flask on a vortexer for 12 minutes seemed excessive.
Response: Based on the laboratory data, the Expert Committee determined that due to volume expansion during an initial trial in the lab, sonication may not be a viable option as it is difficult to achieve accurate dilution volume by addition of diluents in three aliquots. Also, poor recoveries of EG and DEG were observed. The Expert Committee determined that another option could be by shaking and mixing well by available alternative or equivalent methods.

Comment Summary #7: The commenter suggested specifying the phase for the sample extract to avoid confusion because two phases are present and the analyst only should filter the top phase.
Response: Comment incorporated. The Expert Committee recommended replacing the phrase “Filter a portion of the test solution so obtained” with, “Pass a portion of the supernatant layer obtained.”

Comment Summary #8: The commenter observed the appearance of coating (sorbitol?) on the walls of a glass storage vial after filtration and questioned:

- Does this indicate timing is important? Is adequate precipitation of sorbitol time and temperature dependent?
- Requested that perhaps more detail on timing of extract preparation is warranted.

Response: Based on the laboratory data, the Expert Committee determined that the sample preparation including extraction has been optimized to achieve spike recovery greater than 90%. Most importantly, any timing for extract preparation or storage was not identified in the method development and validation studies because sorbitol was not the analyte of interest. The USP laboratory did observe some precipitation of sorbitol in vials stored in a refrigerator.

Comment Summary #9: The commenter indicated that working with such small sample volumes (i.e., a 1 μL injection) a 10:1 split magnifies any mechanical difficulty with the equipment. A lesser split (perhaps 5:1) would allow greater repeatability, while retaining the sensitivity the assay is intended to achieve.
Response: Comment not incorporated. The Expert Committee determined that a 10:1 split is commonly used in GC separations. During the course of USP's laboratory method development and validation experiments, no issues were encountered with regard to repeatability or sensitivity warranting the use of a lesser split.

Comment Summary #10: The commenter indicated that the column has a maximum programmable temperature of 280°, while the method calls for a final oven temperature of 300°. The temperature caused no problem in this initial study, it may however, reduce the life of the column with continuous use.
Response: The Expert Committee determined that according to the manufacturer's specification, the column is programmable up to 300° for a gradient method. Addition of a temperature hold for 5 minutes at 300° did not lead to any deterioration in the column performance during the course of extensive method development and validation studies.

Comment Summary #11: The commenter indicated that the USP procedure describes the *Standard solution* as containing 0.08 mg/mL EG and 0.08 mg/mL of DEG in diluent but it does not include a protocol for preparing the *Standard solution*. The commenter asked that USP provide instructions for the GC *Standard Solution* preparation
Response: The Expert Committee determined that according to the redesigned USP monograph format, if there is no special preparation involved in *Standard solution* and in *Sample solution*, the detailed procedures are not specified. USP *General Notices* states *"When a specified concentration is called for in a procedure, a solution of other normality or molarity may be used provided that allowance is made for the difference in concentration and that the change does not increase the error of measurement. Unless otherwise indicated, analyte concentrations shall be prepared to within ten percent (10%) of the indicated value. In the special case in which a procedure is adapted to the working range of an instrument, solution concentrations may differ from the indicated value by more than ten percent (10%), with appropriate changes in associated calculations. Any changes shall fall within the validated range of the instrument."*

Comment Summary #12: The commenter indicated they had questions on the stability of the GC *Standard solution* and whether or not the *Standard solution* needs to be prepared daily or weekly and whether, if prepared weekly, it should be refrigerated.
Response: Based on the laboratory data, the Expert Committee determined that during the method validation standard solutions were prepared fresh and used immediately.

Comment Summary #13: The commenter indicated they had questions on the stability of the prepared samples and whether or not they can be stored overnight or should be analyzed within a certain time period.
Response: Based on the laboratory data, the Expert Committee determined that during the method validation sample solutions were prepared fresh and used immediately.

Comment Summary #14: The commenter suggested a GC method similar to the GC method proposed in the USP drafts. The commenter stated that two major differences were observed between the USP proposed method and their method:

1. The USP-proposed GC method does not include a derivatization step. As polyols are not volatile compounds, the derivatization step helps to obtain volatile compounds for the GC analysis. This also helps avoid clogging of the column.
2. The method developed by the commenter applies a derivatization step. Also, the GC method proposed by USP uses external standards. The GC method submitted by the commenter uses internal standard. Usually, external standards require work with accurate volumes and weights. This leads frequently to human and systematic errors. The use of internal standards decrease the risk of errors during the preparation of samples and solutions and the risk of deviation linked to the sampling.

Response: Comment not incorporated. The Expert Committee determined that the method submitted by the commenter is only validated for Ethylene Glycol. There is no data supporting its use for Diethylene Glycol.

Comment Summary #15: The commenter indicated that in the draft monographs, the relative retention times are listed for Ethylene Glycol and Diethylene Glycol as 1.0 and 2.6 respectively, but one laboratory obtained slightly different relative retention times of 1.0 and 1.8 respectively. The commenter noticed that General Chapter <621> *Chromatography* states:

> *"Relative retention times may be provided in monographs for information purposes only, to aid in peak identification. There are no acceptance criteria applied to relative retention times."*

The commenter mentioned that although the above statement indicates that relative retention times are informational, they believe that relative retention times may be interpreted as a requirement in this particular case since there is such high visibility for this particular identification test. Therefore, the commenter requested either that relative retention times be adjusted according to data provided to USP, or that relative retention times be removed from the monograph since they are only informational.

Response: Comment incorporated. The Expert Committee recommended to revise the Note to read as [NOTE—Diethylene Glycol elutes after Ethylene Glycol in the chromatogram.]

Comment Summary #16: The commenter suggested the following changes to paragraph 1. Bold text reflects commenter recommendations.

- First sentence should read: "Because of the serious hazards associated with the use of Diethylene Glycol **and Ethylene Glycol**-contaminated materials…
- With regards to last sentence: "the testing of USP Sorbitol Solution should demonstrate the absence at NMT 0.10%
    - There should be some consideration of daily intake

Response: The Expert Committee determined that the previously posted **BRIEFING** will not appear as part of the Revision Bulletin. The spec limits for Diethylene Glycol and Ethylene Glycol were recommended by the Food and Drug Administration (FDA), please refer to FDA letter received by USP on 12-Jan-2009.

http://www.usp.org/pdf/EN/hottopics/2009-01-12-FDALetterPropyleneGlycolSorbitalSolution.pdf

Comment Summary #17: The commenter suggested the following changes to paragraph 2.). .

- Second sentence should read: "The Gas Chromatography procedure for the limit of Diethylene Glycol and Ethylene Glycol is based on analysis performed with the J & W Scientific DB-1701 brand of G46 column"…

Response: The Expert Committee determined that the previously posted **BRIEFING** will not appear as part of the Revision Bulletin.

Comment Summary #18: The commenter provided the following comments and suggested the following two changes to paragraph 3.Bold text reflects commenter recommendations.

1. *Standard solution*: The concentration of Standard solutions may be too low for detection with older equipment
2. Sample Solution should read:
    a) Transfer 2.0 g of Sorbitol Solution to a 25ml volumetric flask. Add 1.0ml of diluent to the flask and vortex the flask for 3 min. Add the remaining Diluent to the flask to volume in 3 equal portions **and bring up solution to a final volume of 25ml**." Vortex the flask for **about** 3 minutes after each addition of Diluent. Filter a "portion" of the **extract** so obtained through a 0.45-um nylon filter."
    b) Commenter questioned if a volume should be assigned to the "portion" and asks if 10mL would be appropriate.

Response: The Expert Committee determined for comment 1, that validation studies and public reports indicated that the concentration of *Standard solution* in current proposed methods for Sorbitol Solutions is appropriate for detection with any basic GC instrument via direct injection. However, USP *General Notices* also states that "When a specified concentration is called for in a procedure, a solution of other normality or molarity may be used, provided that allowance is made for the difference in concentration and that the change does not increase the error of measurement."
USP hosted an open microphone Web Meeting on 10 August 2009 to collect public comments on USP proposed methods. After that meeting, USP posted all the presentation slides made during the web meeting on USP website under Hot Topics for Propylene Glycol and Sorbitol Solutions. Laboratory validation data and important chromatograms are all included in these presentation slides.
For comment 2a), the Expert Committee recommended the following additional changes to the *Sample solution* for clarification purposes by using the phrase "Pass a portion of the supernatant layer obtained" to replace "Filter a portion of the test solution so obtained," and by adding "about" as suggested by the commenter.
The committee determined that the suggestion of using "**and bring up solution to a final volume of 25 ml"** is not necessary because in the USP monograph format, a 25-mL volumetric flask is used in the experiment, "Add the remaining *Diluent* to the flask to volume…" should be equivalent to "Add the remaining *Diluent* to the flask to 25 mL…"

For comment 2b), the Expert Committee determined that it is unnecessary to provide a defined volume for the "portion" as long as there is an adequate quantity of filtrate to be used in the GC analysis.

Comment Summary #19: The commenter suggested the following changes on Chromatographic System. Bold text reflects commenter recommendations.

- **Injection Type: Split injection; Split Ratio is about 10:1**

Response: Comment incorporated. The Expert Committee changed the current text: "Injection mode: Split 10:1" to "Injection type: Split injection; Split ratio is about 10:1"

Comment Summary #20: The commenter suggested the following changes on *System suitability*. Bold text reflects commenter recommendations.

- Note above chart at top of the page should read:
  - "NOTE-See the relative retention times **in** table below
  - Title for second column in chart should read Relative Retention Time (Approximately)

Response: Comment incorporated. The Expert committee changed the Note to read as "[NOTE—Diethylene glycol elutes after ethylene glycol in the chromatogram.]"

Comment Summary #21: The commenter indicated they had questions on the *Resolution* under *Suitability Requirements* and whether or not the *Resolution* of "NLT 30" is too large or should it be 3.0 or smaller?
Response: Comment not incorporated. Please refer to response to *Comment Summary #4.*

Comment Summary #22: The commenter suggested the following changes on *Acceptance criteria*. Bold text reflects commenter recommendations.

1. "The areas of Ethylene Glycol **or** Diethylene Glycol peaks in the *Sample solution* is NMT the peak areas of Ethylene Glycol **or** Diethylene Glycol in the *Standard solution*, respectively, corresponding to NMT 0.10% ethylene glycol and NMT 0.10% diethylene glycol in Sorbitol Solution.
2. The commenter also commented that Standard solution is low, extract concentration is very low, possibly needs to be higher. The commenter suggested that it may need an internal standard to demonstrate the formula for calculations.

Response: For comment 1, the Expert Committee recommended the creation of separate acceptance criteria for Ethylene Glycol and Diethylene Glycol in order to clarify the *Acceptance criteria* as follows:

Acceptance criteria:

Diethylene Glycol: The peak area of Diethylene Glycol in the *Sample Solution* is not more than the peak area of Diethylene Glycol in the *Standard solution*, corresponding to not more than 0.10% of Diethylene Glycol in Sorbitol Solution.

Ethylene Glycol: The peak area of Ethylene Glycol in the *Sample Solution* is not more than the peak area of Ethylene Glycol in the *Standard solution*, corresponding to not more than 0.10% of Ethylene Glycol in Sorbitol Solution.

For comment 2, the Expert Committee will consider revising *Standard solution* in the future if the necessary supporting data is submitted by the commenter. The Expert

Committee determined that extensive method development and validation studies as well as public reports demonstrated that *Standard solution* and extract concentration in current proposed methods for Sorbitol Solution are appropriate for detection with any basic GC instrument via direct injection. However, USP *General Notices* also states that "When a specified concentration is called for in a procedure, a solution of other normality or molarity may be used, provided that allowance is made for the difference in concentration and that the change does not increase the error of measurement." Extensive method development and validation studies also clearly exhibited that the method works well using external standards thus an internal standard is not necessary for this limit test. The Expert Committee suggests that the commenter refer to 10-Aug meeting presentation slides showing data for *Specificity, Accuracy*, *Precision, LOD* of the methods etc posted on USP website.
http://www.usp.org/pdf/EN/hottopics/ppgWebMeetingAug2009.pdf

Expert Committee-initiated Change #1: The Expert Committee emphasized the use of USP Reference Standards to revise the standard preparation In the *Standard solution* section, to read as below.

> **Standard solution:** 0.08 mg/mL of USP Diethylene Glycol RS and 0.08 mg/mL of USP Ethylene Glycol RS in *Diluent*.

Expert Committee-initiated Change #2: The Expert Committee checked an on-line catalog and found that that the liner used for the limit test for EG and DEG in Sorbitol containing solutions did not have "low pressure" as a part of its description. The Expert Committee removed "low pressure" from the description of the liner in the proposed monographs.

POSITIVE FEEDBACK

Monograph/Section(s): Sorbitol Solution, Sorbitol Sorbitan Solution and Noncrystallizing Sorbitol Solution/Identification C: Limit of EG and DEG
Expert Committee(s): Excipient Monographs 1
No. of Commenters: 4

Comment Summary #1: Pleased to find that the method works. The precipitation sample preparation approach was a clever addition to the GC measurement system. Nice job!

Comment Summary #2: Procedure seems acceptable as EG and DEG limit test (NMT 0.1%).

Comment Summary #3: No modifications to the procedure seem necessary. It is a very simple and straightforward procedure.

Comment Summary #4: EG and DEG are easily separated by gas chromatography. This is likely due to the significant difference in their boiling points (EG at 197°, and DEG at 245°)

Comment Summary #5: The GC parameters and temperature profile are very simple and the run time is short. The time between injections is approximately 20 minutes. Any basic GC instrument with direct injection should work fine for this type of analysis.

Comment Summary #6: Sample preparation is quick and easy. A polyol syrup (2.0 g) is vortexed with increasing amounts of Diluent (96:4 acetone/water) in a 25 mL volumetric flask. The sample is syringe-filtered into a GC vial prior to injection. It takes about 10 minutes to prepare a GC sample for analysis.

Comment Summary #7: During the open microphone web meeting, USP stated it will be adding clarification to the Sorbitol Solution *NF* monograph to indicate that the Sorbitol is supposed to precipitate out after addition of the organic dilution (acetone). Our laboratory also noted this during their testing. We fully endorse USP adding this clarification to the monograph.

Comment Summary #8: One large polyol manufacturer reported to USP that based on comments they received from several companies with which they interacted, they confirmed with USP that the method proposed by USP seems to be satisfactory and easy to use.