U.S. FOOD & DRUG ADMINISTRATION

Gas Chromatography-Mass Spectrometry (GC-MS) Method for the Determination of Ethylene Glycol (EG) and Diethylene Glycol (DEG) in Cough, Cold, and Allergy Products containing Glycerin

Method

FDA developed and validated a GC-MS method following ICH Q2 (R2) guidelines for the detection and quantitation of DEG and EG in cough, cold, and allergy products containing glycerin[1]. The developed method met all ICH Q2 (R2) validation guidelines. The limit of quantitation (LOQ), limit of detection (LOD), and range of the method are summarized below:

	Diethylene Glycol	Ethylene Glycol
Limit of Quantitation (LOQ)	0.3 ppm (0.00003%)	1 ppm (0.0001%)
Limit of Detection (LOD)	0.1 ppm (0.000010%)	0.3 ppm (0.00003%)
Range	10 ppm – 1000 ppm (0.001% to 0.1%.)	

*1000 ppm = 0.1%

Purpose

This method can be used to detect and quantitate DEG and EG in glycerin when it is an ingredient in cough, cold, and allergy products.

Principle

DEG and EG are separated using gas chromatography (GC) and detected by mass spectrometry (MS). The GC-MS analysis utilizes single ion monitoring (SIM) mode for the detection of analytes, followed by extracted ion chromatograms for quantitation. The method was validated for use on representative children's cough medicine matrices (solutions, syrups, and suspensions). The method uses external standard calibration with internal standard (IS) normalization.

Reagents

Methanol, LC/MS grade
Ethylene Glycol standard
Diethylene Glycol standard
2,2,2-Trichloroethanol

Equipment/Instrument

Gas chromatograph, Agilent 7890A or equivalent
Mass spectrometry detector, Agilent 5975C or equivalent
GC Column: DB-WAX, 30-m x 0.25-mm I.D. x 0.25- µm film or equivalent
Analytical Balance
Vortex mixer
GC vials

[1] Research funded by FDA and supported through the contract 75F40123P00405.

Blanks
Blanks consist of methanol with and without internal standard. These solutions are filtered with 0.20 µm PTFE syringe filter membranes for comparison to samples that required filtration.

Stock DEG and EG Standard Preparation (1 mg/mL)
Accurately weigh 100 mg of each DEG and EG standard and transfer into a 100 mL volumetric flask. Dilute to volume with methanol and mix.

Internal Standard Solution (10 mg/mL)
Accurately weigh 0.1 g of 2,2,2-Trichloroethanol and transfer into a 100 mL volumetric flask. Dilute to volume with methanol and mix.

Working QC Standard preparation (50 µg/mL)
Transfer 0.5 mL aliquot volume of the stock DEG and EG standard into a 10 volumetric flask. In addition, transfer 0.10 mL of the internal standard solution to the 10 mL volumetric flask, dilute to volume with diluent, and mix with a vortexer.

Drug product sample preparation
Prepare un-spiked and spiked samples of each product matrix (solution, syrup, and suspension) in 10-mL volumetric flasks as described in the table below.

Sample Type	Conc. (µg/mL)	Spike Source	Source (mL)	Sample (g)	Stock IS (mL)
Un-spiked	NA	NA	NA	1.0	0.10

Mix samples thoroughly to homogenize prior to sampling. Dilute sample solutions to volume with MeOH, and vortex mix. Solutions were filtered (0.20 µm PTFE syringe filter) if solids or suspensions were observed after preparation.

Chromatographic Conditions:

GC Column	DB-WAX, 30-m x 0.25-mm I.D. x 0.25- µm film or equivalent
Oven Program	130 ºC (4 min hold) increase 17 ºC/min to 185 ºC increase 35 ºC/min to 250 ºC (5 min hold)
Column Flow Rate (He)	1.0 mL/min
Split Ratio	10:1
Injection Volume	1.0 µL
Inlet Temp.	240 ºC
Wash Vials	MeOH
Inlet Liner	Restek Split w/ wool, 4.0-mm x 6.3 x 78.5
Run Time	14.1 min

Mass spectrometer conditions
Instrument: Triple quadrupole mass spectrometer (Agilent) or equivalent

Mass Spectrometry Settings:

MSD Transfer Line	250 ºC
Solvent Delay	3 minutes
MSD Off	8.2 min
SIM m/z	31,33,43,75,113

Analyte	Quantitation Ion	Qualifier Ion	Extracted Ion
EG	31	33	31
DEG	75	43	75
IS	31	113	31

Injection Sequence

Inject Blank (methanol) at least twice at the beginning of a sequence.
Inject the methanol with internal standard blank 1.
Inject the Working QC Standard six consecutive times.
Inject the blank (methanol) once.
Inject samples (maximum of twelve).
Inject the blank (methanol) once.
Inject the QC Standard once every twelve injections of the samples and at the end of a sequence.

Example:

Order	Solution	No. of Injections
1	Blank (methanol)	2
2	Methanol with Internal Standard	1
3	Working QC Standard	6
4	Blank (methanol)	1
5	Sample 1	1
6	Sample 2	1
7	Sample 3	1
8	Sample 4	1
9	Sample 5	1
10	Sample 6	1
11	Sample 7	1
12	Sample 8	1
13	Sample 9	1
14	Sample 10	1
15	Sample 11	1
16	Sample 12	1
…	…	…

System Suitability Criteria:

System Suitability Parameter	Acceptance Criteria
Injection Precision: % RSD of the EG/IS and DEG/IS peak area ratios for the 6 injections of the working QC Std	≤ 5.0%
Calibration Curve (linear fit)	$R^2 \geq 0.99$

% Recovery for Working QC Std (Bracketing Standard)	100.0 ± 15.0%
Global Precision: % RSD of the EG/IS and DEG/IS peak area ratios for all injections of the working QC Std (Injection Precision and all Bracketing Standards)	≤ 10.0%

Calculation:

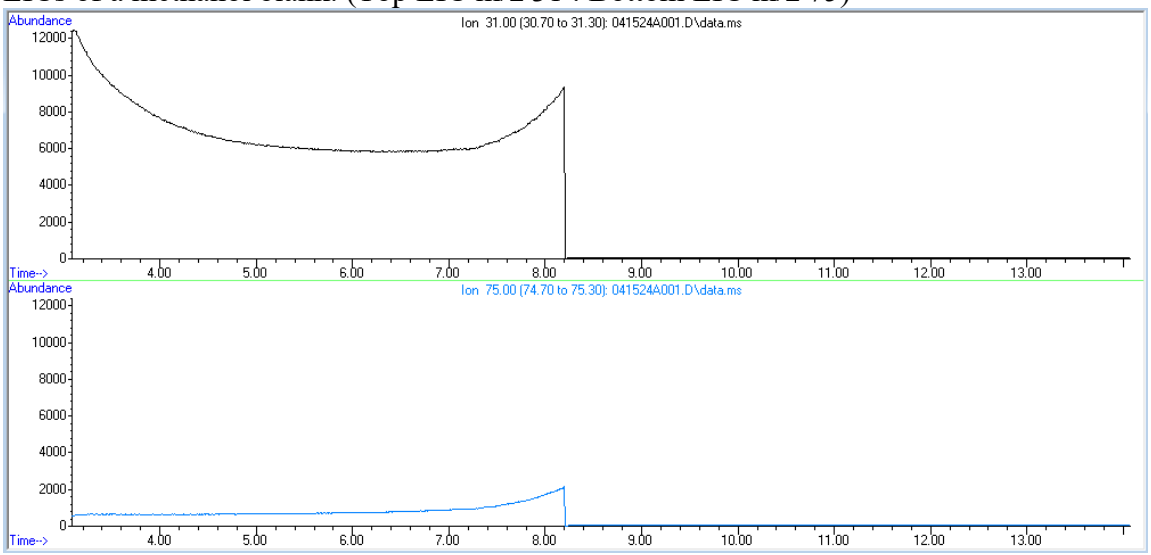
Analyte Concentration in Sample (µg/g) or ppm (w/w):

$$Analyte\ (ppm) = \frac{PAR_A - B}{M} \times \frac{V}{W_S}$$

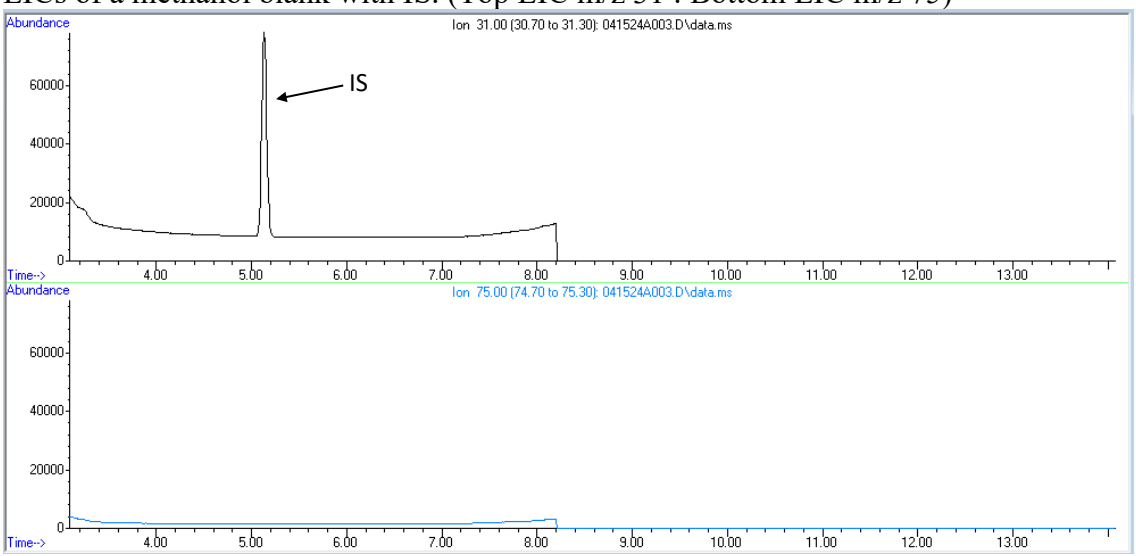
Where:
PAR_A = Peak area ratio of analyte (Peak area of analyte divided by peak area of IS)
B = Y intercept of calibration curve
M = Slope of calibration curve
V = Total volume (mL) of prepared sample solution
W_S = Weight (g) of sample

Example Chromatograms:

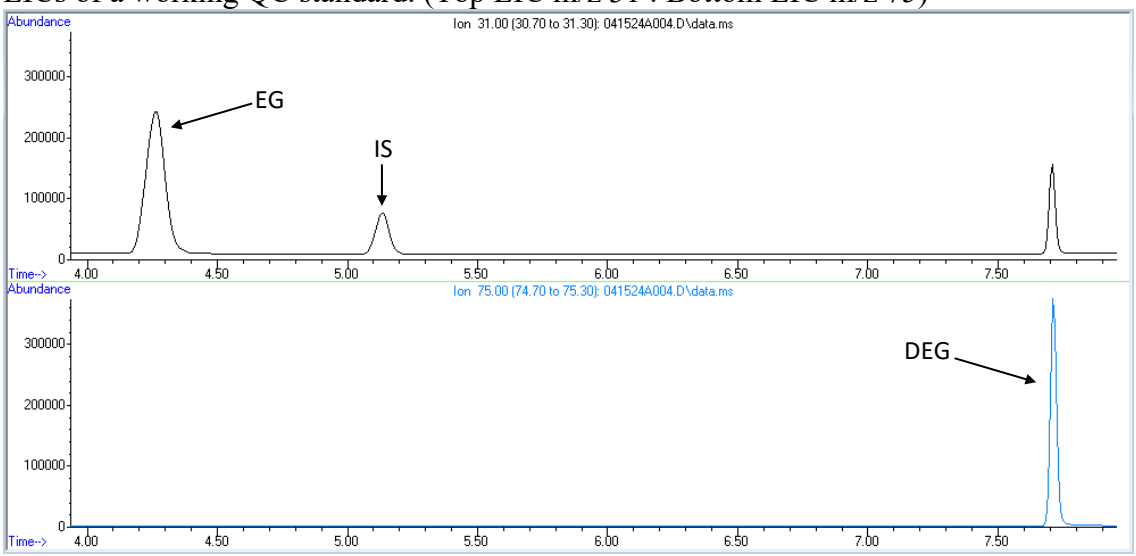
EICs of a methanol blank. (Top EIC m/z 31 : Bottom EIC m/z 75)



EICs of a methanol blank with IS. (Top EIC m/z 31 : Bottom EIC m/z 75)



EICs of a working QC standard. (Top EIC m/z 31 : Bottom EIC m/z 75)



Overlaid EICs from an un-spiked cough and cold product (Top EIC m/z 31 : Bottom EIC m/z 75)