SeQuant Technical Note TN-004

Rapid Identification of Diethylene Glycol in a Mass Poisoning Epidemic using ZIC®-HILIC Chromatography

The detection and quantification of Diethylene Glycol in pharmaceutical preparations can be performed in less than three minutes using a ZIC®-HILIC column and mass spectrometric detection. The assay requires no sample pretreatment except for dilution. The rapid identification of the toxin allowed prevention of a more serious epidemic.

Introduction

A number of mass poisoning epidemics have occurred from the ingestion of pharmaceutical preparations containing toxic substances. A typical example is the accidental use of diethylene glycol (DEG) in place of glycerin or propylene glycol; there have been several epidemics due to the substitution of DEG in the past fifteen years. In a recent case, 51 deaths occurred in Panama when DEG was present in an antihistamine/expectorant preparation.

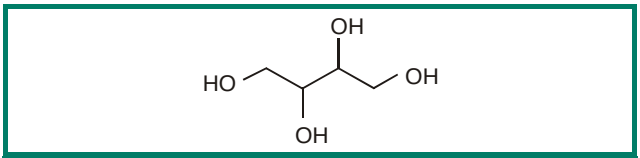

Figure 1: Structure of Diethylene Glycol, Cause of Mass Poisoning Epidemics

When an epidemic of this nature occurs, a rapid and sensitive analytical protocol is required to determine the cause so that the appropriate corrective action can be initiated as rapidly as possible. A rapid protocol for the detection of DEG in pharmaceutical products which requires no sample pretreatment has recently been developed by Barr and co-workers at the National Center of Environmental Health of the US Centers for Disease Control. This assay is very straight forward and requires no sample preparation except for a dilution of the sample. It is based on a HPLC separation using a ZIC®-HILIC column with atmospheric pressure chemical ionization mass spectrometric detection.

Hydrophilic interaction liquid chromatography (HILIC) is a very powerful technique for the separation of complex mixtures of polar compounds. HILIC separates compounds using a mostly organic hydrophobic mobile phase with a hydrophilic stationary phase. The solutes elute in order of increasing hydrophilicity, which is the opposite of the elution order in reverse phase chromatography and is especially useful for the separation of polar compounds that are poorly separated by reverse phase.

ZIC®-HILIC chromatography is a unique form of HILIC that involves the bonding of zwitterionic sulfobetaine groups to a silica or polymer backbone of the stationary phase and thus allows for a significant aqueous fraction in the mobile phase. This allows greater solubility of polar analytes in the mobile phase and therefore provides greater sensitivity.

Experimental

Samples: Liquid medicines which were believed to be involved in the epidemic were obtained from public health facilities in Panama. The samples included expectorants, antihistamines/expectorants, antacids and a vitamin preparation. A CVS Tussin DM cough formula (CVS Corporation, Woonsocket RI) was employed as the blank and was spiked to serve as a positive control.

Sample Handling: Samples were diluted 1:10 with methanol/water (1:10) prior to testing. Deuterated DEG (Cambridge Isotope Laboratory, Andover, MA) was added to the samples.

Instrumentation: A Surveyor HPLC system (Thermo Electron, Waltham, MA) was interfaced to a Quantum triple quadrupole mass spectrometer (Thermo Electron).

Separation: DEG was separated from the diluted sample using a ZIC®-HILIC column (150 x 2.1 mm packed with 5 µm particles) and a mobile phase consisting of acetonitrile/water (84.5:15.5) modified with 5 mM ammonium acetate at a flow rate of 200 µL/min and a column temperature of 35 °C.

Detection: The mass spectrometer was operated in the selected reaction monitoring mode (SRM) using atmospheric pressure chemical ionization. Nitrogen was used for the sheath gas (50 psi) and auxiliary gas (5 psi) and argon was used for the CID gas (1 mTorr).

Quantification: The samples were diluted 100:1 and 20 µL of the internal standard solution (40 µg/mL) was added to each sample to yield a 1 mL sample with an ISTD concentration of 0.8 µg/mL in each sample. A 1 µL sample was injected into the LC.

Confirmatory Techniques: Fourier transform (FT)-MS was used to provide accurate mass measurements, gas chromatography-time of flight (GC-TOF) MS and two dimensional GC (GC-GC)-TOF MS was used to confirm the presence of DEG.

Reference

1: This application note is condensed from the scientific paper *"Identification and quantification of diethylene glycol in pharmaceuticals implicated in poisoning epidemics: An historical laboratory perspective"* by D. B. Barr et al., *Journal of Analytical Toxicology*, Vol. 31, July/August 2007, 295-303. Figure 2 is reprinted with permission from Preston Publications, © (2007).

Merck SeQuant AB · Box 7956 · S-907 19 Umeå · Sweden
Tel: +46-90-154880 · Fax: +46-90-154883
info@sequant.com · www.sequant.com

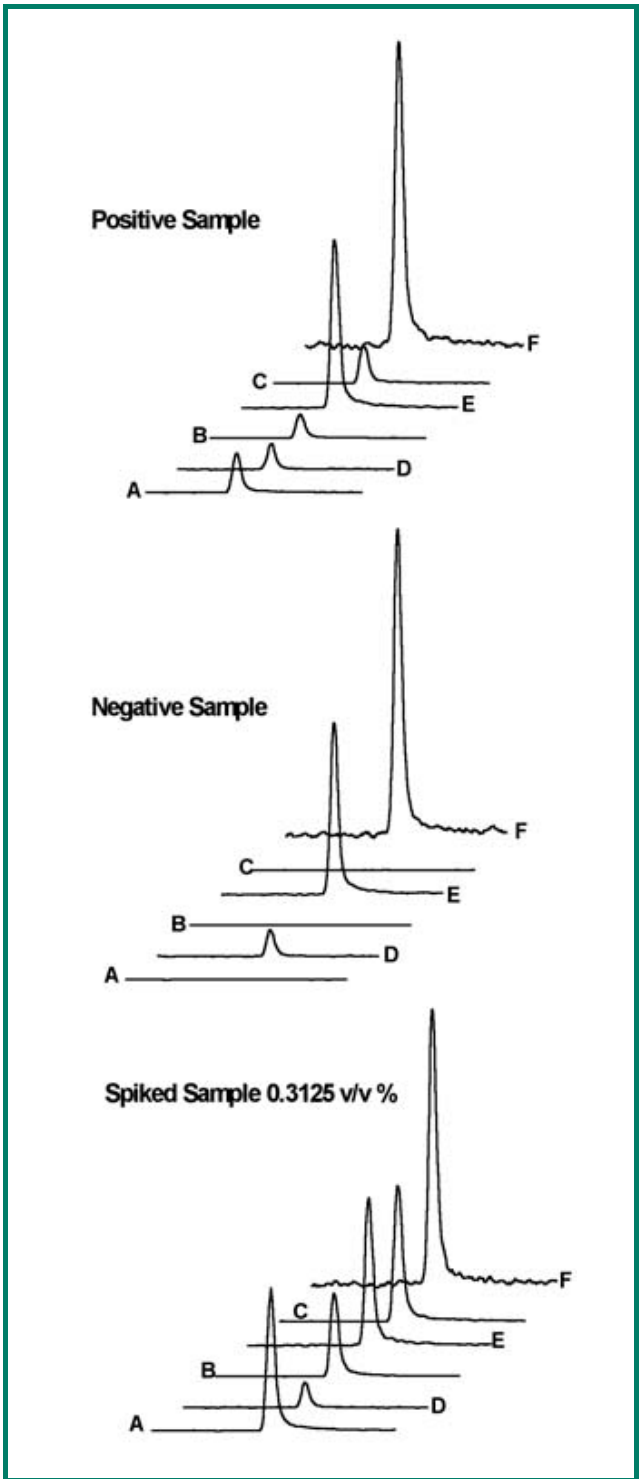

Figure 2: Chromatograms for a Positive Sample, Negative Sample and Spiked Sample, respectively. Trace A is for the m/z corresponding to the molecular ion of DEG, trace B is for the m/e corresponding to the m/z for the ammonium adduct, trace C is for the m/z corresponding to the acetonitrile adduct, while trace D-F correspond to the m/z for the same peaks for the internal standard (reprinted with permission).

Results

A series of chromatograms for a sample which was implicated in the Panama epidemic (an antihistamine/ expectorant syrup) is shown in Figure 2. Similar chromatograms were collected for a negative sample and for a spiked sample (Figure 2).

The LOD of the quantitiative analysis in implicated syrup was approximately 1% with a standard deviation of approximately 10 %. DEG was measured in the samples which were believed to be implicated in the epidemic with a concentration of 8.1 +/- 1% DEG. In samples that were with the same lot number from a given manufacturer, the DEG content was found to be 7.6 +/- 0.2%.

Conclusion

If diethylene glycol, a solvent used in anti-freeze is used in place of ethylene glycol in a pharmaceutical preparation, the individual ingesting the preparation may suffer severe illness or death. A number of mass poisoning epidemics have occurred due to this inadvertent substitution and a timely and rapid analytical protocol is required to determine if the solvent is present in the preparation.

A selective and sensitive analytical protocol based on HPLC with an MS-MS detector using a ZIC®-HILIC column and an acetonitrile-ammonium acetate buffer was developed to provide a separation in less than 3 minutes. The analysts were able to identify the toxin within 24 h of the receipt of the samples, thus preventing a more serious epidemic.

About ZIC®-HILIC Chromatography

The ZIC®-HILIC stationary phase is based on the covalently bonded permanent zwitterionic sulfobetaine group indicated in Figure 3. It is available with a silica support in 3.5, 5 and 10 µm particle sizes in various column dimensions from capillary to semi-preparative (75 µm up to 20 mm ID). In addition, it is available with a polymeric support in 5 µm particles (ZIC®-*p*HILIC). Merck SeQuant also publishes the tutorial booklet A Practical Guide to HILIC, which is available free of charge.

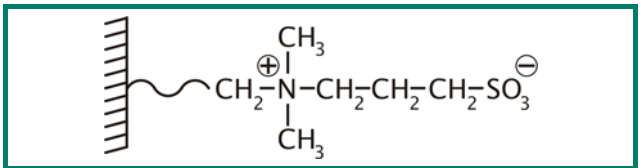

Figure 3: The Bonded Zwitterionic Sulfobetaine Group of ZIC®-HILIC.

About Merck SeQuant

Merck SeQuant is a Swedish company that develops a broad range of innovative products for separation and purification of complex samples via chromatography. The company was founded in 1987 by researchers from Umeå University. Products include ZIC®-HILIC columns for HPLC, Membrane Suppressors and Suppressor Regeneration Systems for Ion Chromatography, Proteomic Calibration Kits, and Post Column Reaction Delay and Mixing Coils which are marketed and supported around the world by a series of distributors.

Merck SeQuant AB · Box 7956 · S-907 19 Umeå · Sweden
Tel: +46-90-154880 · Fax: +46-90-154883
info@sequant.com · www.sequant.com