6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring propylene glycol in biological samples or in environmental media. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify propylene glycol. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect propylene glycol in environmental samples are the methods approved by federal organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Table 6-1 is a summary of some of the most commonly used methods reported in the literature for detecting propylene glycol in biological samples. The primary method for measuring propylene glycol in biological samples is derivatization followed by gas chromatography (GC) using either a flame ionization detector (FID) or mass spectrometry (MS) for quantification. GC is the preferred analytical method because of the ease of sample preparation and the accuracy of the quantification of sample concentrations. Alkali flame ionization detectors have also been used for ethylene glycol analysis and give a response ratio of 3:l compared with PID (Bogusz et al. 1986). Capillary gas chromatography with a constant current ^{63}Ni electron capture detector (ECD) has also been used successfully to detect propylene glycol (Needham et al. 1982).

Sample preparation for GC is important and proceeds through several steps: acidification, esterification, and extraction into an organic solvent. The use of internal standards is necessary for quantification. In clinical cases involving ethylene glycol poisoning, propylene glycol should not be used as an internal standard for quantitation because certain sedatives (Valium and Ativan) may contain propylene glycol (Apple et al. 1993).

Detection of propylene glycol in biological samples using GC with either FID or MS is very sensitive, with detection limits ranging from sub to low ppm. The coefficient of variation (CV) varies with the concentration of glycol used but typically ranges from 0.4% to 27% and is usually less than 10%. In gas chromatographic procedures, the glycols and their acid metabolites are derivatized to form esters in order

Table 6-1. Analytical Methods for Determining Propylene Glycol in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human plasma	Deproteinization with acetic acid; vortex; centrifugation; supernatant spiked with internal standard; reaction with butyl-boronic acid; neutralize with NH_4OH, extraction with dichloromethane; concentration.	HRGC/MS	1 ppm	94–106	Giachetti et al. 1989
Human serum	Acetonitrile with internal standard added to sample; centrifugation; concentration; extraction with *p*-bromo-phenyl boric acid in ethyl acetate.	HRGC/ECD	0.38 ppm	>90	Needham et al. 1982
Human blood	Deproteinization with $HClO_4$; centrifugation; pH adjustment; centrifugation	GC/MS	0.6 ppm	NR	Sisfontes et al. 1986
Human serum and urine	Internal standard added; centrifugation; derivatization with phenylboronate in methanol.	HRGC/FID	1.0 ppm	89–98	Houźe et al. 1993
Human serum (glycolic acid)	Colorimetric: precipitation of protein with trichloroacetic acid followed by centrifugation, addition of chromotropic acid, heating, and dilution; gas chromatographic: addition of internal standard and acetone followed by centrifugation, addition of NaOH, evaporation to dryness, and formation of methyl ester.	Absorbance at 580 nm or GC/FID as appropriate	1.0 mmol/L (60 ppm, w/v) for both methods; 3–6% RSD	NR	Fraser and MacNeil 1993
Humans serum (glycolic acid)	Extraction from salted, acidified serum using methyl ethyl ketone followed by removal of organic phase and evaporation to dryness and derivatization with PNBDI.	HPLC/UV	0.05 mmol/L (3 ppm, w/v); 1% RSD	NR	Hewlett et al. 1986

Table 6-1. Analytical Methods for Determining Propylene Glycol in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human plasma, urine (oxalate)	Heparinized blood deproteinated by addition of acetonitrile and phosphate buffer (pH=7), centrifugation, removal of solvent and evaporation to dryness; derivatization as for urine; urine acidified and derivatized using 1,2-diaminobenzene, adjustment of pH to 5-6, centrifugation.	HPLC/UV	Plasma: 0.15 mg/L (ppm, w/v); 7.5% RSD; urine: 0.5 mg/L (ppm, w/v); 5% RSD.	85	Brega et al. 1992

ECD = electron capture detector; FID = flame ionization detector; GC = gas chromatography; $HClO_4$ = chloroform; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; MS = mass spectrometry; NH_4OH = ammonium hydroxide; NR = not reported; PNBDl = O-*p*-nitrobenzyl-N,N'-diisopropylisourea; RSD = relative standard deviation; UV = ultraviolet detector; w/v = weight:volume

to facilitate quantitative elution from the chromatographic columns (see Table 6-1). Simple and rapid methods are also available for the quantitation of the glycols in urine, serum, or deproteinated whole blood. These methods use direct sample injection without prior solvent extraction and derivatization (Aarstad et al. 1993; Edinboro et al. 1993; Jonsson et al. 1989). However, such methods, particularly those that use packed columns may misidentify propionic acid (found in patients with methylmalonic acidemia) as ethylene glycol (Shoemaker et al. 1992).

High-resolution proton nuclear magnetic resonance spectroscopy has potential use in the identification and quantification of propylene glycol and other chemicals in cerebrospinal fluid (CSF) and serum (Petroff et al. 1986). The technique has two advantages: 1) it requires no pretreatment of the specimens prior to analysis and no advance knowledge of possible compounds present in fluids and 2) results are extremely rapid. Propylene glycol was detected at 1 ppm in CSF (Petroff et al. 1986).

No information was located on detecting propylene glycol in feces, adipose tissue, or human milk.

6.2 ENVIRONMENTAL SAMPLES

As with biological samples, GC is the major technique used to determine propylene glycol concentrations in environmental samples whether in air, water, food, drugs, or other substances. Capillary gas chromatography with FTD or ECD, possibly followed by MS, generally gives good quantitative results down to the ppm range with recovery usually greater than 80%. The determination of propylene glycol in air requires adsorption onto a surface and subsequent extraction. Water samples may be analyzed without preparation (EPA 1995a, 1995b). Detection of propylene glycol in foods and drugs may be accomplished by chromatography of the sample; for substances with a high fat content, extraction with hexane may be used to remove the fat. Table 6-2 is a summary of some of the most commonly used methods reported in the literature for detecting propylene glycol in environmental samples. The specific techniques used for each analytical method are listed in the table if that information was provided by the author(s).

The presence of propylene glycol in foods packaged with plastic films containing the compounds has been studied, as have ethylene glycol levels in drugs sterilized with ethylene oxide. Sample preparation is important because procedures vary depending on the fat content of the food sample. Foods with low fat content can be extracted with ethyl acetate, derivatized to a trimethylsilyl ether, and then injected into the gas chromatograph. For foods with a high fat content, hexane is used as the defatting agent prior to derivatization. Quantifying ethylene glycol or propylene glycol in wines requires no preparation of the

Table 6-2. Analytical Methods for Determining Propylene Glycol in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Sample adsorbed on Amberlite® XAD-2 with personal sampling pump; extraction with diethyl ether.	GC/FID	NR	75–98	Andersson et al. 1982
Water	Direct injection (Method 8015b).	GC/FID	NR	NR	EPA 1995a
Water	Direct injection (Method 8430).	GC/FTIR	120 mg/L (ppm, w/v)	NR	EPA 1995b
Plastics	Sample extraction from plastic with carbon disulfide.	GC/FID	16.5 ng	58–61	Muzeni 1985
Plastics	Sample extraction with solvent of ethylacetate-water-methanol.	GC/FID	2 ppm	NR	DeRudder et al. 1986
Cosmetics	Co-distillation with isooctane	GC/FID	NR	NR	Helrich 1990a
Ground tobacco	Extraction with anhydrous methanol.	GC/FID	NR	NR	Helrich 1990b
Aqueous solution	Sample concentration, then dilution with water; concentration with helium gas; redilution.	GC/FID	50 ppb	97–103	Kashtock and Breder 1980
Beer	Addition of ammonium sulfate and extract with ethyl acetate.	HRGC/FID	0.73 ppm	88	Williamson and Iverson 1993
Vanilla extract	Refluxing with heptane and addition of KIO_4, $NaHCO_3$, KI, and starch to aqeous phase followed by titration with $KasO_2$.	Titration	NR	NR	Helrich 1990c
Food	Addition of hot water to sample to obtain slurry; extraction with hexane; precipitation of sugars with calcium hydroxide; concentration; derivatization with BSTFA.	HRGC/FID; GC/MS	10 ppm	78–107	Castle et al. 1988b
Anchovies	Extraction with methanol and concentration.	HRGC/MS/ MS (PICI)	12.5 ppb	NR	Matusik et al. 1993

BSTFA = bis(trimethylsilyl)trifluoroacetamide; FID = flame ionization; GC = gas chromatography; HRGC = high resolution gas chromatography; MS = mass spectrometry; MS/MS = tandem mass spectrometry; PICI = positive ion chemical ionization

samples prior to analysis (Kaiser and Rieder 1987; Klaus and Fischer 1987). Drugs in aqueous solutions may be analyzed directly, water insoluble drugs should be extracted in water, and ointments may be dissolved in hexane and then extracted with water. Recovery is between 80 and 114%, with detection limits in the low-ppm range (Hartman and Bowman 1977; Manius 1979). The use of ion exchange chromatography with sulfuric acid as the mobile phase has also given good recovery (98-101%) with a detection limit of 5 µg/mL propylene glycol from pharmaceuticals (Iwinski and Jenke 1987). Although the use of TLC (Ballarin 1980) has been recommended, it has been superseded by GC.

Propylene glycol in cigarette smoke has been detected using electrostatic precipitation or filter pad, with extraction and separation with capillary gas chromatography (Borgerding et al. 1990).

No information was located on techniques for detecting and analyzing propylene glycol in soil.

6.3 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of ethylene glycol and propylene glycol is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of propylene glycol.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Methods for the determination of propylene glycol in blood and urine are available (Giachetti et al. 1989; House et al. 1993; Needham et al. 1982; Sifontes et al. 1986) with sensitivities in the sub-ppm range.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for the determination of propylene glycol have been reported for air (Andersson et al. 1982; NIOSH 1984), water or aqueous solutions (EPA 1995a, 1995b; Kashtock and Breder 1980), and

foods (Castle et al. 1988b; Matusik et al. 1993; Williamson and Iverson 1993). Methods have also been developed for the determination of glycols that leach from plastics (DeRudder et al. 1986; Muzeni 1985) and that can end up in foods stored in containers made from the plastics. An MRL of 0.009 ppm for intermediate inhalation exposure to propylene glycol has been defined and none of the methods reported would be adequate without modification. It is likely that the LODs of some of the methods could be reduced but this remains to be shown.

6.3.2 Ongoing Studies

No ongoing research on analytical methods for the determination of propylene glycol was found.