6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring acetone, its metabolites, and other biomarkers of exposure and effect to acetone. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and 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 modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Some of the more recent methods for determining the presence of acetone in different biological media are reported in Table 6-1. Prior to the 1960s acetone was considered to be present in only insignificant amounts in hyperketonic states in healthy people. Therefore, the concentration of acetone in biological fluids was rarely measured. The effort to develop analytical methods for the determination of acetone in body fluids increased when it was found that the level of acetone in diabetic patients with severe hyperketonaemia may indeed be significant (Trotter et al. 1971). The parent acetone or its reduction/derivatization product is almost exclusively determined by GC/FID method, although derivatized products can be determined by high-performance liquid chromatography with ultraviolet or fluorescence detectors (Brega et al. 1991; Vairavamurthy et al. 1992). Since the reference acetone values for healthy unexposed people are 0.034-0.120 mmol/L, with a mean of 0.075 mmol/L in plasma, and 0.034-0.095 mmol/L, with a mean value of 0.052 mmol/L in urine (Brega et al. 1991), the available methods have adequate sensitivity to determine the levels of acetone in biological samples of both healthy and diabetic people (Gavin0 et al. 1986; Kobayashi et al. 1983; Phillips and Greenberg 1987; Trotter et al. 1971). The determination of acetone in blood is difficult because acetone is generated as a metabolite from acetoacetate (major metabolite is βhydroxybutyrate), and the quantity produced depends on storage time even when the blood samples are stored at 4°C (Trotter et al. 1971). In addition, acetylacetone present in the plasma is thermally degraded to

TABLE 6-1. Analytical Methods for Determining Acetone in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Whole blood	Sample centrifuged to obtain plasma; plasma deproteinized with $HClO_4$	GC-FID	6.6 ng/mL (0.023 nmol)	No data	Preinado et al. 1987
Whole blood	Deproteinized with $HClO_4$ and subjected to purge-and-trap	GC-FID	23.2 ng/mL (0.4 μM)	90–92%	Mangani and Ninfali 1988
Whole blood	Purge-and-trap	GC-MS	0.2 μg/mL	87–107%	Ashley et al. 1992
Serum	Deproteinized with sodium tungstate and cupric sulfate	HRGC-FID	<58 μg/mL (<1 mmol/L)	No data	Smith 1984
Plasma	Acetylacetone in sample enzymatically converted to ß-hydroxybutyrate and sample distilled with H_2SO_4	Automated coulometric with salicylaldehyde in KOH	1.16 μg/mL (0.02 μmol/mL)	95%	Haff and Reichard 1977
Serum	Sample centrifuged and clear filtrate injected into GC	GC-FID	5.8 ng/mL (0.1 nmol/mL)	No data	Cheung and Lin 1987
Urine	Diluted sample derivatized with pentafluorobenzyloxylammonium chloride and extracted with hexane	GC-FID	0.2 μg/mL (3.45 μmol/mL	No data	Kobayashi et al. 1983
Liver	Liver perfusion medium reduced with $NaBH_4$ and an aliquot of reduced solution injected into GC	GC-FID	3.78 μg/mL in perfusate (65 μM)	No data	Holm and Lundgren 1984

TABLE 6-1. Analytical Methods for Determining Acetone in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Liver, kidney, lung, and adipose tissue	Purge-and-trap	GC-FID	No data	52% (lung) 98% (other tissue)	Holm and Lundgren 1984
Breath	Direct injection into GC	GC-FID	No data	98%	Trotter et al. 1971; Jansson and Larsson 1969

H_2SO_4 = sulfuric acid; FID = flame ionization data; GC = gas chromatography; $HClO_4$ = perchloric acid; HRGC = high resolution gas chromatography; KOH = Potassium hydroxide; MS = mass spectrometry; $NaBH_4$ = sodium borohydride

acetone inside the injection port and on the column, if a gas chromatographic method is used for quantification (Gavino et al. 1986; Trotter et al. 1971).

Two methods that have been used to eliminate the interference from acetylacetone are enzymatic conversion of acetoacetate to 3-hydroxybutyrate and chemical reduction of acetone to 2-propyl alcohol (Gavin0 et al. 1986; Haff and Richard 1977). Another factor that makes determination of acetone unreliable is the increased evaporative loss of acetone from the samples as the time of storage is prolonged (Trotter et al. 1971). Acetone was detected in several brands of heparin (Powell et al. 1985). Therefore, determination of acetone in heparinized blood may give erroneous results if the heparin is contaminated with acetone.

6.2 ENVIRONMENTAL SAMPLES

Some of the analytical methods for determining acetone in environmental samples are reported in Table 6-2. In addition to these methods, personal sampling methods have been developed in Japan for the determination of acetone in the workplaces (Uchida et al. 1990). There is no unique method that can be used for the determination of low levels of acetone in unpolluted environmental samples. As in the case of biological samples, the sensitivity of acetone detection in environmental samples can be enhanced by derivatization. Such a method, which uses 2,4-dinitrophenylhydrazine as a derivatizing agent, has been applied for the determination of extremely low levels of acetone in open ocean sea water (Kieber and Mopper 1990). A new method based on the reaction of mercuric oxide with carbonyl compounds has been applied for the determination of low levels of acetone in unpolluted atmosphere (O'Hara and Singh 1988). A good review of the methods presently used for the determination of acetone in the atmosphere is available (Vairavamurthy et al. 1992). Unlike in biological samples, acetylacetone may not be present in most environmental samples. If acetylacetone is detected in an environmental sample, thermal degradation inside the gas chromatographic system (injection port and column oven) may produce erroneous results unless this interference is eliminated. As in the case of biological samples, proper precautions in sample handling during collection and analysis, and storage of environmental samples should be taken to minimize contamination and evaporative loss. It has been suggested that ambient water containing low levels of acetone be sampled upwind of sources of contamination (e.g., smoke stack or other sources of emissions) and that the operator stand downwind of the samples while withdrawing them (to minimize contamination from operator's breath) (Kieber and Mopper 1990).

TABLE 6-2. Analytical Methods for Determining Acetone in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (occupational)	Air passed through charcoal and components disorbed with CS_2	GC-FID (NIOSH method 1300)	0.007 mg/L	No data	NIOSH 1990
Air (occupational)	Air passed through Porapak N and components thermally desorbed	GC-FID	<0.01 ppm (v/v)	110% at 0.52 ppm	Campbell and Moore 1977
Air (occupational)	Air passed through charcoal and components desorbed with CS_2	GC-FID	<0.05 mg/L	74–92%	Otson et al. 1983
Air (ambient)	Air passed through a cryogenic trap and the trapped component injected into GC	GC-RGD	10 ppt	No data	O'Hara and Singh 1988
Air	Air passed through a 1% sodium bisulfite solution and absorbed acetone reacted with alkaline vanilla solution	Spectrophotometry	<0.5 ppm (in solution)	No data	Amlathe and Gupta 1990
Rural air	Air passed through silica gel coated with 2,4-dinitrophenyl-hydrazine and eluted with acetonitrile	HPLC-UV	No data	<90%	Shepson et al. 1991
Water	Sample reacted with alkaline diazotized anthranilic acid solution	Spectrophotometry	0.5 mg/L	98.5–102%	Rahim and Basir 1981

TABLE 6-2. Analytical Methods for Determining Acetone in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Sample reacted with sodium bisulfite	IC/conductivity detection	No data	No data	DuVal et al. 1985
Fresh and seawater	Sample derivatized with 2,4-dinitrophenylhydrazine passed through a C_{18} cartridge and adsorbed compound eluted with acetonitrile	HPLC-UV detection	0.5 nM (0.03 μg/L)	No data	Kieber and Mopper 1990
Waste water, soil or sediment	Sample or sample mixed with reagent water subjected to purge-and-trap	GC-MS (EPA method 8240)	100 μg/L (water) 100 μg/kg (sediment and soil)	No data	EPA 1986
Fresh fruit	Vacuum distillation followed by solvent extraction of pulp	HRGC-MS	No data	No data	Takeoka et al. 1988

CS_2 = carbon disulfide; FID = flame ionization detector; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; IC = ion chromatography; MS = mass spectrometry; RGD = reduction gas detector; UV = ultraviolet; v/v = volume per volume

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 acetone is available. Where adequate information is not available, ATSDR, in conjunction with the 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 acetone.

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. The concentration of acetone in expired air, blood, and urine can serve as indicators of recent exposure to acetone (Ghittori et al. 1987; Pezzagno et al. 1986; also see Section 2.5.1.). Concentrations of acetone similar to those produced from occupational exposure are also produced in blood and urine as a result of inhalation and ingestion of isopropyl alcohol (may be present as a contaminant in some alcoholic beverages) (Kawai et al. 1990b) and in patients with diabetes mellitus (Dobson et al. 1968; Trotter et al. 1971). Among urine, alveolar air, and blood, the strongest correlation was found between the time-weighted average breathing zone air concentration and urinary acetone level when the concentration of acetone in breathing zone air exceeded 15 ppm (v/v) (Fujino et al. 1992; Kawai et al. 1992). Analytical methods of adequate sensitivities are available for determining acetone in blood, urine, and expired air at background levels in the population and levels at which biological effects occur (Gavin0 et al. 1986; Kobayshi et al. 1983; Phillips and Greenberg 1987; Trotter et al. 1971). Although the precision and accuracy of the analytical methods have not always been reported (e.g., Robayashi et al. 1983), these values are satisfactory in cases where they have been reported (Haff and Reichard 1977; Holm and Lundgren 1984; Mangani and Ninfali 1988; Trotter et al. 1971).

Biological monitoring has not been used to associate acetone exposure levels with specific effects that can be quantified in humans (see Section 2.5.2). Clinical tests for nonspecific effects of acetone exposure (see Section 2.9.2) are available (Johansson et al. 1988; Lupulescu et al. 1972, 1973; Tosti et al. 1988). Since acetone exposure itself can be quantified in biological tissues, there does not appear to be a need to improve existing clinical tests or develop additional test procedures to detect effects of acetone exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining low levels of acetone found in ambient air are available (Ants and Meeks 1981; Cavanagh et al. 1969; O'Hara and Singh 1988; Shepson et al. 1991; Snider and Dawson 1985). Methods are also available for determining low levels of acetone found in natural waters (Kieber and Mopper 1990). The concentrations of acetone found in volatile components of several foods have also been determined (Bartley and Schwede 1989; Day and Anderson 1965; Takeoka et al. 1988). The degradation products formed as a result of photochemical reactions of acetone in air in the presence or absence of nitrogen oxides have been identified (Altshuller and Bufalini 1971; Boule et al. 1987; Hanst and Gay 1983; Meyrahn et al. 1986). However, products from the reaction of acetone with hydroxyl radicals and $O(^3P)$ remain unidentified (Cox et al. 1980; Lee and Timmons 1977), although it has been postulated that methylglyoxal may be the principal product of the reaction with hydroxyl radicals (Altshuller 1991). It would be helpful to determine the products formed from the gas phase reaction of acetone with hydroxyl radicals in order to assess the potential health effect implications of these products.

6.3.2 On-going Studies

No on-going studies that could free the data gap regarding the products formed from the reactions of acetone with atmospheric oxidants (e.g., hydroxyl radicals, $O[^3P]$) were found.

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health and Injury Control, Centers for Disease Control, is developing methods for the analysis of acetone and other volatile organic compounds in blood. These methods use purge-and-trap methodology, high resolution gas chromatography, and magnetic sector mass spectrometry which gives detection limits in the low parts per trillion (ppt) range.