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	Document Manager: Cheryl Lozen	Approved By: Jeffrey Nye

10.14 Sugar Analysis

10.14.1 Sugar Analysis - Introduction

10.14.1.1 Common types of sugars

- Sucrose (table sugar, saccharose, cane or beet sugar) $C_{12}H_{22}O_{11}$
- Fructose (levulose, fruit sugar, D-fructose) $C_6H_{12}O_6$
- Dextrose (glucose, grape sugar, corn sugar) $C_6H_{12}O_6 * H_2O$
- Mannitol (manna sugar, mannite) $C_6H_8(OH)_6$
- Maltose (malt sugar, maltobiose) $C_{12}H_{22}O_{11} * H_2O$
- Lactose (milk sugar, saccharum lactis) $C_{12}H_{22}O_{11} * H_2O$

10.14.1.2 Sugar in gasoline

NOTE: According to published data, the solubility of sugar is 1.5 mg/L. This means that the total amount of sucrose that would go into solution in a 15 gallon tank of gas would be on the order of 90 mg. When a 100 ml. sample is submitted to the lab, the analyst would not expect to get more than 150 μ g of sucrose. It is important, therefore, that any investigation of cases of motor fouling caused by the suspected addition of sugar to the gasoline should include a sampling of any solid residues in the fuel tank.

10.14.2 Chemical Reactivity Tests - Sugar Screening Tests

This procedure includes chemical reactivity tests that may be performed to determine the possible presence of sugar. These tests are general tests for saccharides and is based on the sulfuric acid-catalyzed dehydration of sugars to furfural and its derivatives, which are then condensed with α -naphthol and subsequently are oxidized to give a violet (purple) colored product. A third chemical reactivity test uses the Tollen's reagent which will also give positive results for aldehydes and ketones.

10.14.2.1 Safety Considerations

Standard Laboratory Precautions

10.14.2.2 Preparations

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10.14.2.2.1

Molisch's Test Reagent (15% ethanol solution of α -Naphthol): Dissolve 7.5g of 1- naphthol in 50 ml. of EtOH.

10.14.2.2.2

Concentrated Sulfuric Acid.

10.14.2.2.3

Tollen's reagent: To a 10 ml. solution of 10% AgNO_3 , add 10 ml of 10% NaOH. AgO is precipitated out. The precipitate is then dissolved by the dropwise addition of a 1:1 solution of NH_4OH .

10.14.2.2.4

Spot plate.

10.14.2.2.5

Test tube.

10.14.2.3 Procedure or Analysis

10.14.2.3.1

Procedure 1

10.14.2.3.1.1

Place two drops of the test solution on a spot plate.

10.14.2.3.1.2

Add one drop of a 15% ethanol solution of 1-naphthol and two drops of conc. sulfuric acid.

10.14.2.3.1.3

A blue or purple-blue color indicates the presence of sugar. This color is obtained from partly charred sugar.

10.14.2.3.1.4

A purple color will result from uncharred sugar.

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10.14.2.3.2

Procedure 2

10.14.2.3.2.1

Place approximately 0.5 ml. suspected sugar sample in a small test tube.

10.14.2.3.2.2

Add two drops of the Molisch's test reagent.

10.14.2.3.2.3

Incline the tube and, with a dropper, carefully add approximately 1 ml. of concentrated sulfuric acid so that it flows down the side of the tube and forms a layer beneath the aqueous solution.

10.14.2.3.2.4

After a few minutes a red-violet color will be seen at the interface which indicates the presence of sugar.

10.14.2.3.2.5

Gentle agitation, but not enough to mix the layers, will cause the violet color to diffuse throughout the lower layer.

10.14.2.3.3

Procedure 3

The suspected sugar sample is dissolved in water. Equal volumes of Tollen's reagent and sample are mixed in a small test tube. The test tube is immersed in a hot water bath (70-80°C) for approximately thirty seconds. If a sugar is present, a thin film of shiny metallic silver will be deposited on the walls of the test tube.

10.14.2.3.4

Compounds other than carbohydrates will react with the reagents to give a positive test. A negative test, however, usually indicates that there is no carbohydrate.

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10.14.3 Sugar Analysis General Extraction

This procedure includes various techniques that may be used to process and extract sugar from evidence.

10.14.3.1 Preparations

The following materials are needed:

- distilled water
- chloroform
- petroleum ether
- acetone
- filter paper (Whatman # 41)
- hot plate

10.14.3.2 Instrumentation

- Gas Chromatograph/Mass Spectrometer (GC/MS)
- Fourier Transform Infrared Spectrometer (FTIR)

10.14.3.3

Procedure or Analysis

10.14.3.3.1

Extract sample (suspected sugar) with distilled water.

10.14.3.3.2

Filter using Whatman #41 filter paper and evaporate to dryness on a hot plate.

10.14.3.3.3

Perform spot test for screening and/or use one or more of the identification techniques e.g. GC/MS or IR.

10.14.3.3.4

Sugar in gasoline

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10.14.3.3.4.1

Extract with distilled water

10.14.3.3.4.2

Remove and evaporate the aqueous phase

10.14.3.3.4.3

Perform screening and/or identification techniques

10.14.3.3.5

Sugar residue from gasoline

10.14.3.3.5.1

Sugar is not soluble in chloroform, acetone, or petroleum ether.

10.14.3.3.5.2

Wash gasoline residue with water, filter, and let filtrate evaporate to dryness.

10.14.3.3.5.3

Wash the filtrate evaporated residue with chloroform. Remove liquid and discard.

10.14.3.3.5.4

Wash the filtrate evaporated residue with petroleum ether and/or acetone to remove any remaining water. Remove liquid and discard.

10.14.3.3.5.5

The filtrate evaporated residue may be dried in oven.

10.14.3.3.5.6

Perform screening and/or identification techniques.

10.14.4 Minimum Standards & Controls

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10.14.4.1 Definitions

Blank - an analysis performed on a laboratory-prepared sample which includes all components of the unknown sample except for the material of interest. All reagents, preparatory steps, and analysis conditions will be identical to that used on the unknown sample.

Control - a test performed on a known sample under identical conditions as that used on the unknown sample.

Verification - a test performed on a known sample as a check of an instrument's output.

Minimum Standard of Analysis - the minimum required analytical tests to be performed in order to report a conclusive finding.

Preliminary Test - an analytical procedure that yields information about a sample but by itself cannot be used as a basis for a conclusive finding.

Confirmatory Test - an analytical procedure that will specifically identify an unknown sample.

Standards File - a collection of spectra or chromatograms of known materials to be used as a reference for comparison to an unknown sample.

Reagent - any substance used in a reaction for the purpose of detecting, measuring, or analyzing other substances.

10.14.4.2 Analytical Techniques

10.14.4.2.1

Chemical Reactivity Tests

- All reagents will be properly labeled, initialed by the preparer and dated.
- Controls and blanks will be run each time a prepared reagent is used on a case. The results will be noted on the worksheet.

10.14.4.2.2

Instrumentation

10.14.4.2.2.1

Blanks must be run at the same conditions as the samples.

10.14.4.2.2.2

All instrument hard copies generated from case exhibits must be properly identified.

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10.14.4.2.2.3

Pertinent instrument parameter used for an analysis should be documented.

10.14.4.2.2.4

If applicable, verification and calibration data must be documented and contain the analyst's initials and date.

10.14.4.2.2.5

Identification or classification made by comparison to other than in-house standards must be noted in the case record.

10.14.4.2.2.6

Identification or classification made via computer search must be confirmed by visual comparison to computer's standard and noted in the case record.

10.14.4.2.2.7

Fourier Transform Infrared Spectrometer (FTIR)

- Run a polystyrene calibration standard each day the instrument is in use.
- Blanks - appropriate blanks will be run.
- IR Spectra must be properly identified.
- IR Spectra of case exhibits must be preserved in the case record.

10.14.4.2.2.8

Mass Spectrometer

- Run function verification test each day the instrument is in use.

10.14.4.3 Documentation

10.14.4.3.1

All exhibits/containers must be properly identified.

10.14.4.3.2

Worksheets

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- State all tests performed and their results.
- All non-routine tests will be fully detailed.
- An accurate description of the evidence will be recorded.
- If the sample is consumed in analysis, a notation will be made.
- An accurate description of how the evidence is repackaged will be noted.

10.14.4.4

General

Any of the procedures may be modified to enhance the quality of the data.

10.14.5 Safety Considerations

10.14.5.1

This protocol involves hazardous materials, operations, and equipment. This protocol does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this protocol to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. Proper caution should be exercised and the use of personal protective equipment should be considered to avoid exposure to dangerous chemicals. Consult the appropriate MSDS for each chemical prior to use.

10.14.5.2

NFPA hazard ratings for chemicals used in this protocol:

Chemical	Health	Flammability	Reactivity
Alpha (a)-Naphthol (1-Naphthol)	2	1	1
CAUTION! Avoid skin contact			
Ethyl Alcohol	0	3	0
Silver Nitrate	2	0	0
Sodium Hydroxide	3	0	1
Sulfuric Acid	3	0	0
Pyridine	2	3	0

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Chemical	Health	Flammability	Reactivity
Chloroform	2	0	0
Acetone	1	3	0
Petroleum Ether	1	4	0

10.14.6 References

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