Amanda LA Mohr, MS, D-ABFT-FT

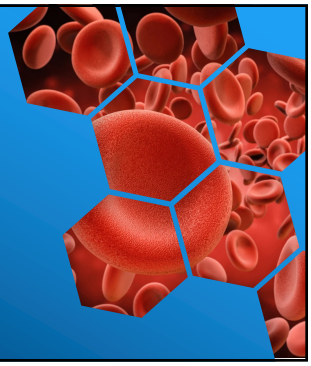
1. Director of Forensic Toxicology and Forensic Chemistry at the CFSRE
2. Adjunct Professor
   - Arcadia University
   - Thomas Jefferson University
3. Outgoing Section Chair of AAFS; Vice Chair of AAFS/SOFT Oral Fluid Committee
4. Member of several professional organizations and highly active in the field
   - Over 35 peer reviewed publications
   - Numerous national and international presentations

DRUG IMPAIRMENT.COM

1

DRUG IMPAIRMENT.COM

Blood Alcohol Testing



Amanda LA Mohr, MS, D-ABFT-FT

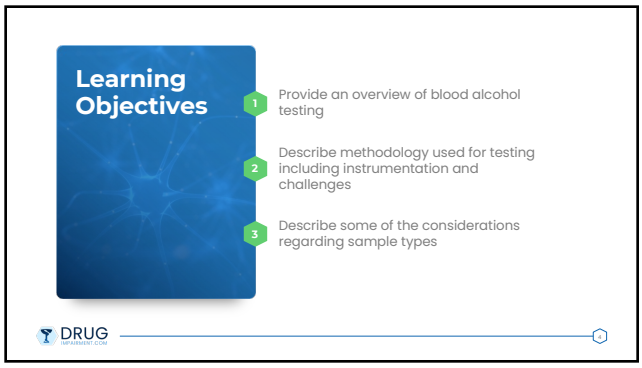
2

Disclosures

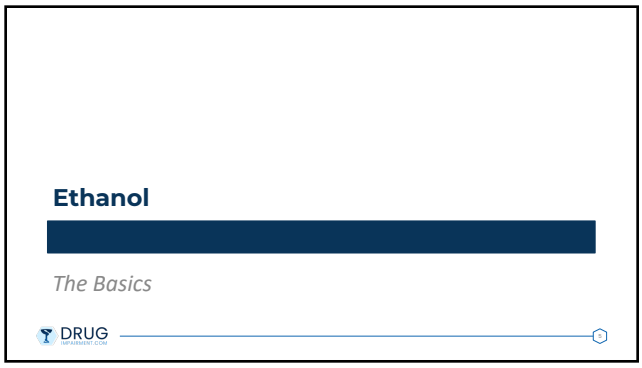
The speaker has no disclosures.

DRUG IMPAIRMENT.COM

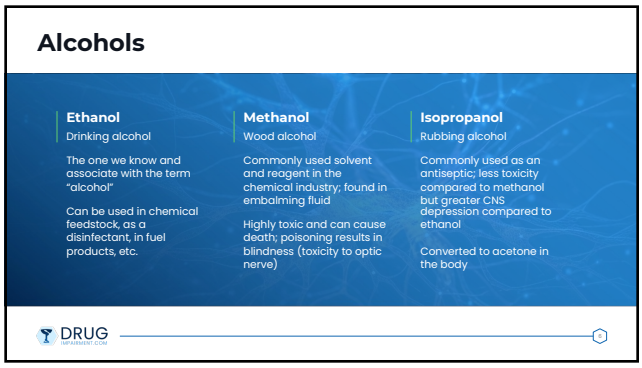
3


Learning Objectives
1
Provide an overview of blood alcohol testing
2
Describe methodology used for testing including instrumentation and challenges
3
Describe some of the considerations regarding sample types
DRUG

4


Ethanol
The Basics
DRUG

5


Alcohols
Ethanol
Drinking alcohol
The one we know and associate with the term "alcohol"
Can be used in chemical feedstock, as a disinfectant, in fuel products, etc.
Methanol
Wood alcohol
Commonly used solvent and reagent in the chemical industry; found in embalming fluid
Highly toxic and can cause death; poisoning results in blindness (toxicity to optic nerve)
Isopropanol
Rubbing alcohol
Commonly used as an antiseptic; less toxicity compared to methanol but greater CNS depression compared to ethanol
Converted to acetone in the body
DRUG

6

Ethyl Alcohol (Ethanol)

- Produced via a fermentation process
- Simple organic molecule that is water miscible
- Does not have its own receptor but acts on a variety of others
- Net effect is CNS depression

DRUG

7

ADME of Alcohol

Absorption

100% of ethanol is absorbed when taken orally; small intestine is major site

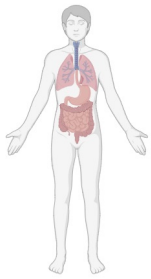
Factors influencing absorption

Heavily influenced by type of beverage, alcohol concentration, diseases, medications, food, etc.

Distribution

Greater the water content, the greater the ethanol concentration

Male v. Female
Serum v. Blood



DRUG

8

Widmark

Men vs. Women

Widmark determined total body weight that is water differs by an average 68% in men and 55% in women

Effect

If the same amount of ethanol is consumed, women end up with a higher ethanol concentration

If two men of different weights consumed the same amount, the heavier man of the two will have a lower ethanol concentration

Widmark's Formula

$$\frac{A=WRC}{0.8}$$

A = Volume of pure alcohol
W = Body weight
C = Blood alcohol concentration
R = Distribution ratio
0.8 = Specific gravity of ethanol

DRUG

9

Distribution in Other Fluids and Tissues

- Differences between the distribution of ethanol between serum and plasma and RBCs
  - Serum contains more water than whole blood (12-20%)
  - **Serum ethanol concentrations are higher than blood sample**
- After equilibrium has been reached, ethanol concentrations are higher in salvia, vit, and spinal fluid relative to blood
- Cannot use urine to estimate blood concentrations
  - Highly variable ratios in the post absorptive phase

Specimen	Ratio
Salvia	1.1
Vitreous Humor	1.2
Bile	1.0
Spinal Fluid	1.1
Liver	0.6
Kidney	0.7
Brain	0.8
Urine	1.3

Adapted from Levine, "Principles of Forensic Toxicology"

DRUG IMPAIRMENT.COM

10

Sample Collection

01 The Draw
Cubital vein of the forearm

02 The Prep
Appropriate antiseptic – prep pad considerations
- Winek and Eastly. 1977 PMID: 865218
- Carter and McConnell PMID: 2304404
- McIvor and Cosbey PMID: 2206816
- Kilic et. al. PMID: 38813189

03 Collection
Glass or plastic tubes
- Karinin *et. al.* 2010 PMID: 20822681
- Jones and Ericsson 2016 PMID: 28856195

04 Tube Contents
Preservatives
Anticoagulants

DRUG IMPAIRMENT.COM

11

Sample Collection

01 The Draw
Cubital vein of the forearm

02 The Prep
Appropriate antiseptic – prep pad considerations

03 Collection
Glass or plastic tubes

04 Tube Contents
Preservatives
Anticoagulants

DRUG IMPAIRMENT.COM

12

Sample Collection

Tube	Additive
Gray	Sodium Fluoride and Potassium Oxalate
Green	Sodium or Lithium Heparin
Lavender	Potassium EDTA
Red	No additive
Red/Grey	Serum tube/clot activator or gel
Light Blue	Sodium citrate
Royal Blue	No additive

References for Expired Tubes

- Zittle and Hardin 2006 PMID: 16839468
- Booker 2009 Canadian Society of Forensic Science Journal Volume: 42, Issue: 4

DRUG IMPAIRMENT.COM

13

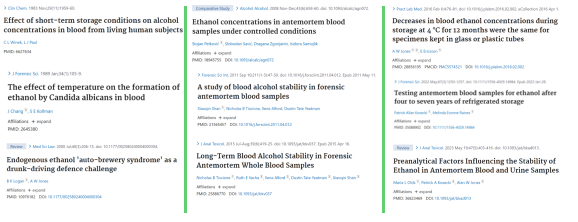
Specimen Storage

- No change or small decreases over time in antemortem blood with NaF (sterile conditions)
- No studies have shown an increase in BAC with NaF (sterile)
- RBCs + O_2 convert alcohol to acetaldehyde and decrease BAC (temp. dependent manner)
- Absence of NaF causes whole blood glucose to decrease thus removing one component of alcohol formation

DRUG IMPAIRMENT.COM

14

The Literature



Hundreds of articles available on ethanol production/loss and stability

DRUG IMPAIRMENT.COM

15

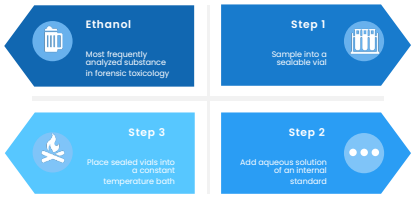
Alcohol Testing

Sample Preparation

DRUG

16

Volatile Liquids



DRUG

17

Alcohol Testing

Sample Analysis

DRUG

18

Sample Analysis Overview

Sample	Isolation	Identification	Report
Blood is drawn and collected into specimen containers, labeled and sealed before sending	In lab, the sample is separated into different components to be analyzed	Analytical techniques identify compounds using their chemical & physical properties	Identified substances are quantified and reported

DRUG

19

Analysis

Forensic Testing

Requires two tests with two independent samplings

→ Two samplings on two analytical columns

Types of Analysis

Enzymatic

Headspace-Gas Chromatography/Mass Spectrometry

Headspace-Gas Chromatography/Flame Ionization Detector

DRUG

20

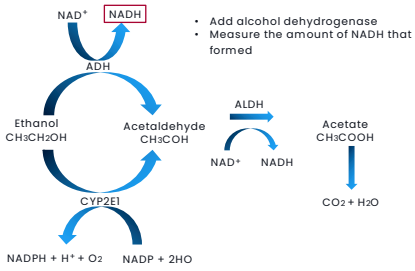
Enzymatic Alcohol Testing

1. Commonly used in clinical applications
2. Testing uses specific enzymes that react with alcohol to produce a measurable signal
3. Most common enzymatic methods use alcohol dehydrogenase

DRUG

21

Enzymatic Assays



- Add alcohol dehydrogenase
- Measure the amount of NADH that is formed

22

Understanding Hyphenated Techniques

Chromatography

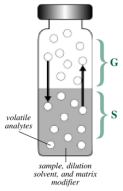
- A separation technique
  - Separating components of a mixture
  - Separation based on chemical and physical properties of a compound
  - Coin star analogy
- Gas Chromatography – a separation technique useful for separating volatile organic compounds
  - Robust
  - High specificity

Detectors

- Identification/detection
  - Multiple types of detectors available
- Mass Spectrometry – a detection technique that identifies compounds based on mass and fragmentation pattern
  - High sensitivity
  - Versatile
- Flame Ionization detector – measures electrical conductivity resulting from the combustion of organic compounds
  - High sensitivity
  - Universal detector

23

Headspace Gas Chromatography



G = The gas phase

The gas phase is commonly referred to as the headspace and sits above the condensed sample phase

S = The sample phase

The sample phase contains the analyte(s) of interest in solid or liquid form

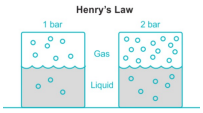
Sample phase also contains a diluent or other matrix modifier

24

Henry's Law

C = kP

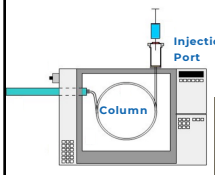
At a constant temperature, the amount of a given gas dissolved in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium in that liquid



DRUG

25

Gas Chromatography



Mobile Phase

Helps move the sample through the instrument

Injection Port

How the sample is introduced into the instrument (sample is volatilized)

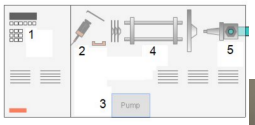
Column

Where the separation of analytes occurs; located in a heated oven

DRUG

26

Mass Spectrometry



Electron Impact Ionizer

High energy electrons (70 eV) bombard the sample

Quadrupole

Used to separate ions with various m/z produced in the source

Electron Multiplier Tube

Ions from quadrupole hit a dynode that releases two electrons for every ion

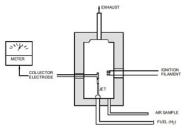
Detector

Amplified signal is read and generates a mass spectrum

DRUG

27

Flame Ionization Detector (FID)



Detector that measures analytes in a gas stream

Detection is based on ions forum during the combustion of organic compounds in a hydrogen flame

Principle

Analytes elute off GC column and are ionized by the flame

Flame is supported by mix of air and hydrogen

Nitrogen also used as a makeup gas to reduce noise produced by the flame

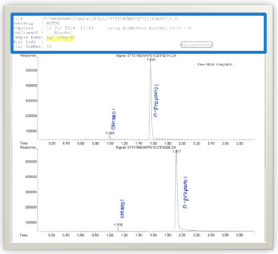
Ions produced in the flame are drawn toward a collector electrode

Ions striking the electrode releases electrons which creates an electrical current that is registered as a response in the chromatogram

DRUG

28

The Output

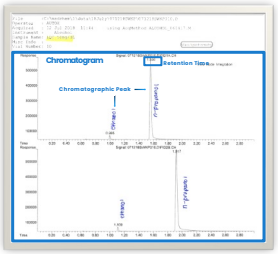


Header	Description
File	Data file storage location
Operator	Individual loading instrument
Sample Name	What sample is (cal, QC) or unique descriptor
Vial Position	Location in autosampler tray

DRUG

29

The Output



Header	Description
Chromatograms	Visual output of the data 2 because instrument is a dual column
Chromatographic Peak	Graphical representation of the detection of a specific analyte
Retention Time	Amount of time it took for analyte to travel through the system – from injection to detection

DRUG

30

Alcohol Testing

Sample Quantitation

DRUG

31

Types of Testing

Qualitative Analysis

Determines the presence of absence of a drug or poison in a submitted sample

- Yes or no type answer
- Hard to interpret or draw definitive conclusions from

Quantitative Analysis

Determines the amount of drug or poison that is present in the submitted sample

- Can assist a toxicologist with interpretation within the context of a case

DRUG

32

Sample Quantitation

Using known references to determine unknowns

1. Prepare calibrators, controls and sample
2. Analyze sample via analytical instrument
3. Plot Calibration Curve
4. Verify accuracy and precision of curve
5. Determine concentration of sample using curve

DRUG

33

Drug Standards

Drug standards

Certificate of Analysis (COA)

Materials containing a know concentration of an analyte (drug)

DRUG IMPAIRMENT.COM

34

Calibrators

Solutions of known concentrations of drug in your matrix (e.g., blood, urine, etc.)

- Prepared using drug standards
- Calibrators at different concentrations are prepared
  - Concentrations determined using reference material (Online, In Literature, Baselt, Clarkes, etc.)
- At least 5 calibrators are required for a curve
  - Prepare 6
- Don't want to exceed two orders of magnitude
- Points in between multiples of two or three
  - 10, 25, 80, 100, 200, and 400 mg/dL
- Contains internal standard

DRUG IMPAIRMENT.COM

35

Internal Standard

- Analyte chemically and structurally similar to your analyte of interest
- Should not be something expected to appear in the sample
  - Example: Caffeine or Other drugs
- Added during sample preparation at a specified concentration
  - Usually in the middle of calibration range
- Used in quantitative analysis to account for efficiencies or variation in recovery or behavior of the analyte in the matrix extraction or analytical processing
  - Response ratio

DRUG IMPAIRMENT.COM

36

Controls

Solutions of known concentrations of drug in your matrix treated as unknowns

- Ensures the accuracy of the curve
- Evaluated at the low and high ends of the calibration curve range
  - Curve: 10, 25, 80, 100, 200, and 400 mg/dL
  - Controls: 50 and 300 mg/dL
- Must also contain internal standard at the same concentration as what's in the calibrators
- Established means and acceptance criteria for performance
  - ±5% of target concentration
  - Tighter controls due to *per se*

DRUG

37

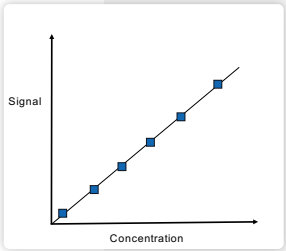
Calibration Curve

What is it?

The graphical representation of the instrument's response plotted against the concentration

How do we use it?

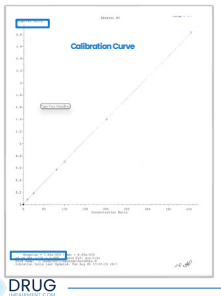
Determine unknown concentration by comparing to a set of standard samples of known concentrations



DRUG

38

The Output

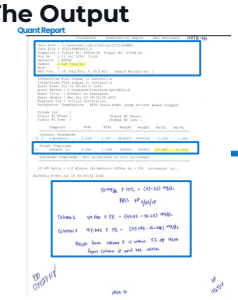


Header	Description
Response ratio	Drug response/internal standard response
Coefficient of Determination (r^2)	How close calibrators are to line of best fit – ***precision***

DRUG

39

The Output



Header	Description
File information	Should correlate with the information from the chromatogram
Ethanol Concentration	Concentration determined from calibration curve and reported via software
Calculations	Assessment of QCs – ***accuracy***
	Check known concentration to computer calculated concentration – established performance
	Check calculations from two columns – required to be within 5% of each other

DRUG IMPAIRMENT.COM

40

Reporting Results

Concentration	**Multiple analyses –** Report lowest, average, earliest?
Sig Figs	**Three –** Results are reported with 3 sig figs (e.g. 0.082 g/100 mL)
Review	**Independent –** Independent review of data and report
UOM	**Report Requirement –** Is not error; inherent variation associated with the measurement process

DRUG IMPAIRMENT.COM

41

Conclusions

Alcohol testing is one of the most common forensic tests

- Forensically defensible results come from 2 independent test
- Analysis is performed using sound scientific techniques that have been validated
- System checks
- Reporting results is guided by standards
- Labs are checked; proficiency tests and audits

DRUG IMPAIRMENT.COM

42

Acknowledgements

- Barry Logan
- Laura Labay
- Curt Harper

Thank you!

DRUG IMPAIRMENT.COM

43

CONTACT ME

Amanda LA Mohr, MS, D-ABFT-FT

mandi.mohr@cfsre.org

DRUG IMPAIRMENT.COM

44