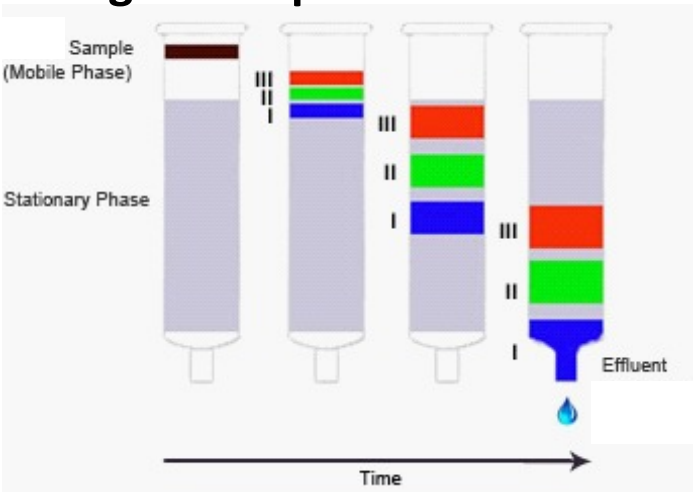
What was the Analytical Chemistry technique used by VCU-MCV and VA-DFS?

Chromatography: a SEPARATION technique. Can be gas or liquid.



Identity is ***inferred*** from time taken to pass through the column, not directly measured.

What is GC typically used for?

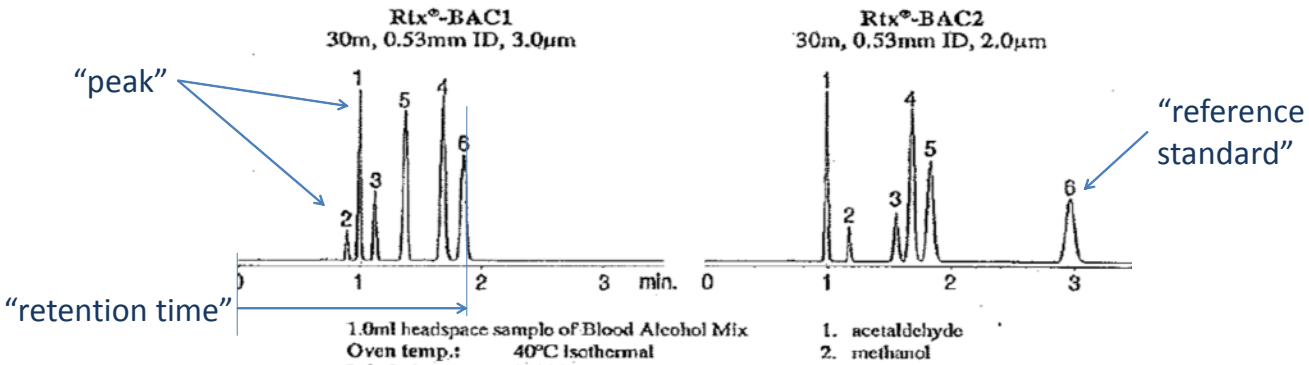
- Analysis of gas mixtures. (gases in air, gases dissolved in liquid)
- Considered a “confirmatory” test.
- Sometimes used in tandem with mass spectrometry (MS), especially when the sample has unknowns.

What are crucial factors for reliability?

- Baseline controls (“What consistutes NORMAL?”)
- Validity of specific separation desired (in-going assumptions are vital)
- Reference standards (“How do we perform the measurement?”)

What does a chromatogram look like?

Figure 1 - Achieve baseline resolution of blood alcohols using dual columns in less than 3 minutes.



1.0ml headspace sample of Blood Alcohol Mix
Oven temp.: 40°C Isothermal
Inj. & det. temp.: 200°C
Carrier gas: helium
Linear velocity: 80cm/sec. set @ 40°C
FID sensitivity: 1.28 x 10^{-10}AFS

1. acetaldehyde
2. methanol
3. ethanol
4. acetone
5. isopropanol
6. n-propanol

Chromatograms produced using a model 7000 static headspace autosampler on loan courtesy of The Tekmar Company.

RESTEK

An Advanced Base Deactivated Capillary Column for analysis of Volatile amines Ammonia and Alcohols.

Jaap de Zeeuw, Ron Stricek and Gary Stidsen
Restek Corp Bellefonte, USA

Standard deactivation with non-polar phase
sample: Trimethylamine and impurities

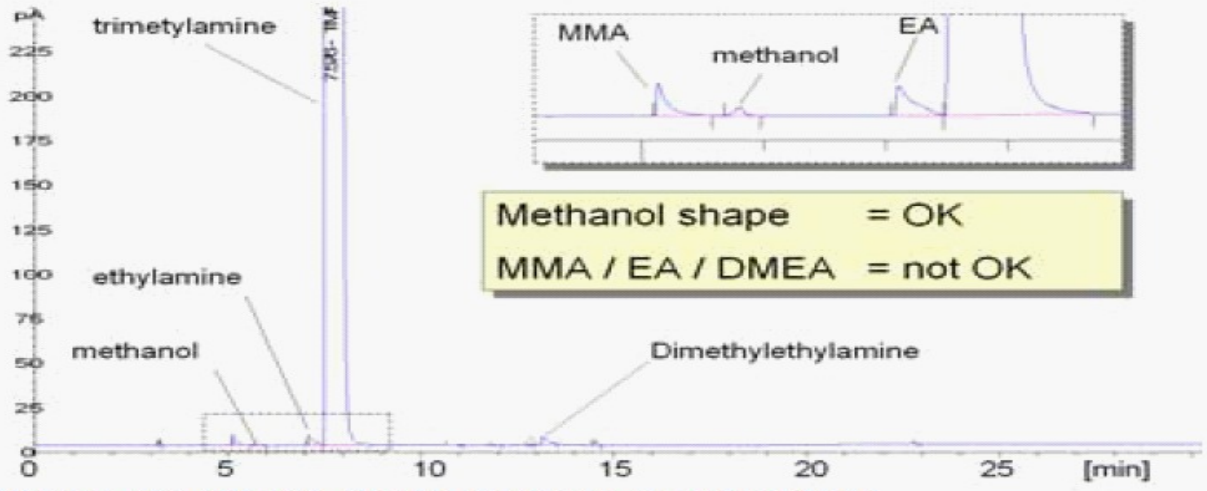


Figure 2: Short chain amines on standard deactivated column; conditions, table 1;

What do other government guidelines say about methanol analysis by GC?

OSHA – fact sheet on MeOH
https://www.osha.gov/dts/sltc/methods/organic/org091/org091.html
Methyl Alcohol
Related Information: Chemical Sampling - Methyl Alcohol in AIR

3.6. Interferences (analytical)

3.6.1. Any compound which produces an FID response and has a similar retention time as methyl alcohol or the internal standard is a potential interference. Potential interferences which were reported when the samples were submitted for analysis should be considered before desorbing the samples.

3.6.2. Retention time on a single column is not proof of chemical identity. Confirmation of suspected identity should be performed by **GC/mass spectrometry** when necessary.

What do FDA guidelines say about analytical chemistry methods?

From **FDA**
Guidance for Industry
Bioanalytical Method Validation

Selective, sensitive, and validated analytical methods for the quantitative evaluation of drugs and their metabolites (analytes) and biomarkers are critical for the successful conduct of nonclinical and/or biopharmaceutics and clinical pharmacology studies. Validating bioanalytical methods includes performing all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix (e.g., blood, plasma, serum, or urine) is reliable and reproducible for the intended use. Fundamental parameters for this validation include the following:

- Accuracy
- Precision
- Selectivity
- Sensitivity
- Reproducibility
- Stability

Are all tests "confirmatory?" NO

Examples of situations resulting in samples sent for alcohol analysis:

- Inebriated driver, need to verify blood alcohol is over limit. **Confirmation.**
- Unconscious patient in industrial situation, need to verify exposure by inhalation. **Confirmation.**
- Patient with emergency, need to identify unknown toxic substance. ***Discovery. One test is insufficient.***

What is the difference between a "Defined Matrix" versus "Undefined Matrix" and why is that relevant to analytical chemistry?

- QA/QC: Co makes chemicals X, Y, needs to verify quality: **Defined.**
- Inebriated driver: Patient admits to drinking alcohol, the only issue is how much: **~Defined.**
- ER patient: Patient does not know toxic ingestion: ***Undefined. Method validation required.***

What is the VA-DFS method for methanol analysis by GC?

See VA-DFS Procedures Manual, chapter seven.

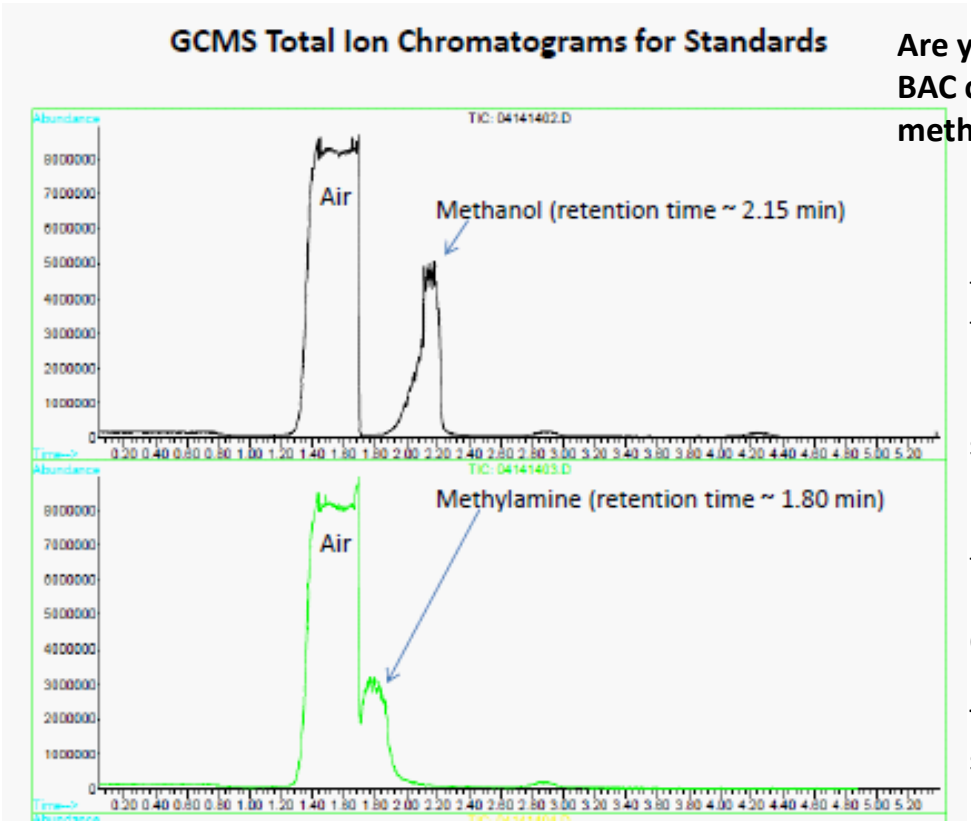
- Rtx-BAC column.
- Single, indirect reference standard.
- Ordinary donor blood as negative control.
- No explicit confirmatory step.
- No validation for amines.

What are the main problems with the methanol by GC method at VA-DFS?

According to OSHA & FDA Best Practices: Until a method is developed and validated to separate methylamine from methanol, the methanol finding by GC without MS, is spurious.

Other methodological problems:

- The GC protocol from VA-DFS does not recommend an orthogonal test to verify.
- No confirmatory analytes (such as blue dye or Bitrex) were demonstrated and no source of pure methanol was identified.
- The column used (Rtx-BAC) was a high-throughput device unsuited for discovery in an undefined matrix.
- The detector used was non-specific. A specific detector such as a mass spectrometer (MS) was not used.



Are you sure that the Rtx-BAC column should detect methylamine?

Methylamine is not voided by the Rtx-BAC column, according to tests done at Alera Labs

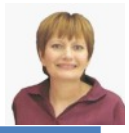
Retention time differs by just 20 seconds.

If the standard elutes at 5 mins, then this difference = 7%.

Operational variance = ~5%.

Thus, the separation is barely significant.

What is the summary of the Plaintiff Case?



	Methanol	Creatine	
Toxicology symptoms	Consistent with formaldehyde and formic acid.	Consistent with formaldehyde and formic acid.	Toxicology, alone cannot distinguish methanol from creatine.
Source	No viable source identified.	Numerous witnesses + powder still in evidence storage.	
Analytical chemistry	Inadequate to be definitive.	Untested.	**ABLE TO BE DEFINITIVE, BASED ON STORED BOTTLES**
DMPK	Definitely inconsistent.	Consistent.	

How Should Re-Testing Be Performed?

Some chemicals in the bottles are volatiles and some are non-volatile, yet soluble. Thus, two analytical methods are required to fully elucidate the contents:

- GC-MS Gas chromatography mass spectrometry
- LC-MS Liquid chromatography mass spectrometry

Or variation thereof, e.g. GC/MS/MS or Obitrap/GC-MS

Who & How – To any accredited lab who can retain the chain of custody. But first, *a method must be developed and validated* to separate these potential analytes:

- Methanol
- Methylamine
- Dimethylamine
- Paraformaldehyde *May require derivatization*

Other potential chemicals predicted by Plaintiff:

- Urea
- Sarcosine
- Glyoxylate
- Peroxide
- Formate
- Citrate
- Sucrose or sucralose

How can we rule out degradation of the Gatorade itself? Will methanol spontaneously degrade?

- Some Gatorade bottles tested *positive* for methanol. These are the ones the plaintiff claims actually contain degraded creatine.
- Some Gatorade bottles tested *negative*. These are internal controls for the stability of Gatorade itself.
- One bottle of wiper washer fluid was tested positive for methanol. This is also an internal control for the stability of methanol.