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BHB Testing in Postmortem Blood to Differentiate Between Solvent Ingestion and DKA

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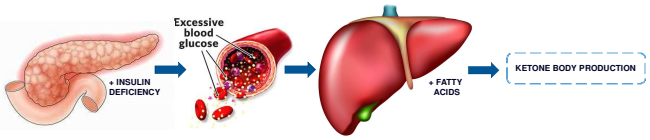
Diabetic Ketoacidosis (DKA)

- Characterized by hyperglycemia, ketoacidosis, ketonuria
- Ketone overproduction causes accumulation in the blood



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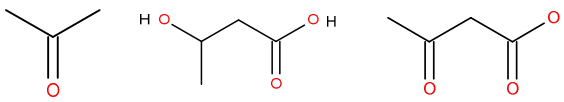
Ketone Bodies



acetone | β-hydroxybutyric acid (BHB) | acetoacetate

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How else can you get Acetone/IPA positive results?

Ingestion

minor metabolic pathway (reduction)

alcohol dehydrogenase

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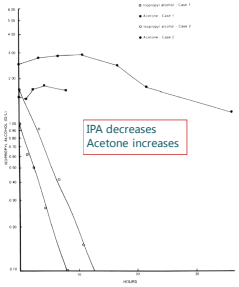
How else can you get Acetone positive results?

Isopropyl Alcohol Metabolism After Acute Intoxication in Humans

D.R. Daniel, B.H. McAnalley, and **J.C. Garriott**
Southwestern Institute of Forensic Sciences at Dallas, Post Office Box 35728, Dallas, Texas 75235

Journal of Analytical Toxicology, Vol. 5, May/June 1981



IPA decreases
Acetone increases

Figure 1. Isopropyl alcohol and acetone concentrations in two cases of acute overdose of isopropyl alcohol. Concentrations are averages of triplicate analyses of each sample.

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TOXICOLOGY – DKA PRESENTATION

Detailed Findings:

Analysis and Comments	Result	Units	Rpt. Limit	Specimen Source
Isopropanol	8.1	mg/dL	5.0	001 - Peripheral Blood
Acetone	34	mg/dL	5.0	001 - Peripheral Blood
Benzoylecgonine	330	ng/mL	50	001 - Peripheral Blood
Delta-9 Carboxy THC	7.1	ng/mL	5.0	001 - Peripheral Blood
Delta-9 THC	1.5	ng/mL	0.50	001 - Peripheral Blood
Isopropanol	Confirmed	mg/dL	5.0	001 - Peripheral Blood
Acetone	Confirmed	mg/dL	5.0	001 - Peripheral Blood
Creatinine (Vitreous Fluid)	2.3	mg/dL	0.050	002 - Vitreous Fluid
Sodium (Vitreous Fluid)	141	mmol/L	80	002 - Vitreous Fluid
Potassium (Vitreous Fluid)	>20	mmol/L	1.0	002 - Vitreous Fluid
Chloride (Vitreous Fluid)	105	mmol/L	70	002 - Vitreous Fluid
Glucose (Vitreous Fluid)	>500	mg/dL	35	002 - Vitreous Fluid
Urea Nitrogen (Vitreous Fluid)	61	mg/dL	3.0	002 - Vitreous Fluid

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TOXICOLOGY - DKA?

Analysis and Comments	Result	Units	Limit	Specimen Source
Acetone	10	mg/dL	5.0	001 - Femoral Blood
Delta-9 Carboxy THC	12	ng/mL	5.0	001 - Femoral Blood
Delta-9 THC	6.1	ng/mL	0.50	001 - Femoral Blood
Acetone	Confirmed	mg/dL	5.0	001 - Femoral Blood
Creatinine (Vitreous Fluid)	0.88	mg/dL	0.050	003 - Vitreous Fluid
Sodium (Vitreous Fluid)	138	mmol/L	80	003 - Vitreous Fluid
Potassium (Vitreous Fluid)	14	mmol/L	1.0	003 - Vitreous Fluid
Chloride (Vitreous Fluid)	114	mmol/L	70	003 - Vitreous Fluid
Glucose (Vitreous Fluid)	None Detected	mg/dL	35	003 - Vitreous Fluid
Urea Nitrogen (Vitreous Fluid)	18	mg/dL	3.0	003 - Vitreous Fluid
Acetone	11	mg/dL	5.0	003 - Vitreous Fluid

Not stable

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Acetone Concentrations – Are they helpful?

HEALTH STATUS	ACETONE (mg/dL)	REFERENCE(S)
Controlled Diabetics	Blood: < 3.0	• Jones AW., et al. J Anal Toxicol. 1993 May-Jun;17(3):182-5.
DKA	Blood: 14.5 – 74.8 (27 patients)	• Sulway MJ, Malins JM. Acetone in diabetic ketoacidosis. Lancet. 1970 Oct 10;2(7676):736-40.
Acetone Ingestions	Serum: 83.6 and 390 (2 cases) Blood: 200 and 250 (2 cases)	• Kallenberg K, et al. MR imaging-based evidence of vasogenic brain edema in a case of acute acetone intoxication. AJNR Am J Neuroradiol. 2008 Apr;29(4):e16. • Kostusiak V, et al. Survival after drinking lethal dose of acetone. Intensive Care Med. 2003 Feb;29(2):339. • Ramu A, et al. Disposition of acetone following acute acetone intoxication. West J Med. 1978 Nov;129(5):429-32. • Zettinig G, et al. [Survival after poisoning due to intake of ten-times lethal dose of acetone]. Dtsch Med Wochenschr. 1997 Nov 28;122(48):1489-92.

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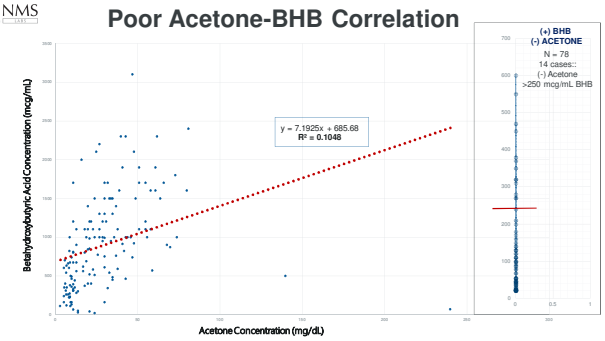
Poor Acetone-BHB Correlation



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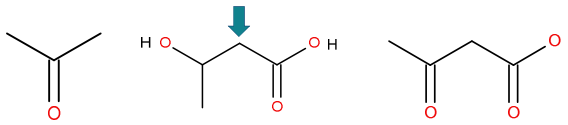
Ketone Bodies

The most stable and specific indicator of ketoacidosis
Can be used to differentiate between ketoacidosis and solvent ingestion



acetone
Least abundant

β-hydroxybutyric acid (BHB)

acetoacetate
Not stable

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BHB Sample Preparation

1) **Sample + ISTD**
   - 100 µL Blood, S/P, Fluid, Urine
   - 3-Hydroxybutyric Acid-D4 (ISTD)
2) **Solvent Crash**
   - 1mL Acetonitrile
   - -20°C for 10 min
   - 3800 rpm, 15 min, 4°C
3) **Dry Down**
   - 45 ± 5°C
4) **Derivatize**
   - BSTFA + 1% TMS
   - Transfer to autosampler vial

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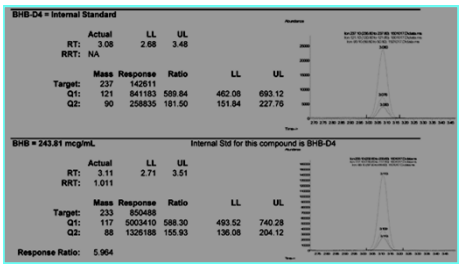
SIM-GC/MS Data

- Agilent 5973 GC/MS
- DB-17 (Agilent) 15M x 0.25 mm
- Helium carrier gas
- Selected Ion Monitoring
- 6 point calibration; area
- 1/x weighting



BHB-D4 = Internal Standard

	Actual	LL	UL
RT:	3.08	2.68	3.48
RRT:	NA		

	Mass	Response	Ratio	LL	UL
Target:	237	142611			
Q1:	121	841183	589.84	462.08	693.12
Q2:	90	258835	181.50	151.84	227.76

BHB = 243.81 mcg/mL — Internal Std for this compound is BHB-D4

	Actual	LL	UL
RT:	3.11	2.71	3.51
RRT:	1.011		

	Mass	Response	Ratio	LL	UL
Target:	233	850488			
Q1:	117	5003410	588.30	493.52	740.28
Q2:	88	1326188	155.93	136.08	204.12

Response Ratio: 5.964

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Alaska Department of Health and Social Services
Division of Public Health

Case History

- 46-year old male with history of ethanol abuse
- During hotel stay found incoherent at 12 PM
- Assisted back to room and police notified
- Discovered unresponsive at 12:52 PM
- Death pronounced
- Multiple empty Vodka bottles at scene

Specimens Received:

ID	Tube/Container	Volume/ Mass	Collection Date/Time	Matrix Source
001	Gray Top Tube	5.5 mL	01/14/2019	Femoral Blood
002	Homogenate Container	Not Given	01/14/2019	Femoral Blood
003	Gray Top Tube	4.75 mL	01/14/2019	Heart Blood
004	Gray Top Tube	9.25 mL	01/14/2019	Subclavian Blood
005	Red Top Tube	2.5 mL	01/14/2019	Vitreous Fluid
006	Red Top Tube	9.75 mL	01/14/2019	Postmortem Urine

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Case – First Round of Toxicology Testing

Isopropanol	43	mg/dL	5.0	002 - Femoral Blood
Acetone	170	mg/dL	5.0	002 - Femoral Blood

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Case – Second Round of Toxicology Testing

Isopropanol	43	mg/dL	5.0	002 - Femoral Blood
Acetone	170	mg/dL	5.0	002 - Femoral Blood
Creatinine (Vitreous Fluid)	1.2	mg/dL	0.050	005 - Vitreous Fluid
Sodium (Vitreous Fluid)	141	mmol/L	80	005 - Vitreous Fluid
Potassium (Vitreous Fluid)	11	mmol/L	1.0	005 - Vitreous Fluid
Chloride (Vitreous Fluid)	125	mmol/L	70	005 - Vitreous Fluid
Glucose (Vitreous Fluid)	None Detected	mg/dL	35	005 - Vitreous Fluid
Urea Nitrogen (Vitreous Fluid)	27	mg/dL	3.0	005 - Vitreous Fluid

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Case – Third Round of Toxicology Testing

Isopropanol	43	mg/dL	5.0	002 - Femoral Blood
Acetone	170	mg/dL	5.0	002 - Femoral Blood
Creatinine (Vitreous Fluid)	1.2	mg/dL	0.050	005 - Vitreous Fluid
Sodium (Vitreous Fluid)	141	mmol/L	80	005 - Vitreous Fluid
Potassium (Vitreous Fluid)	11	mmol/L	1.0	005 - Vitreous Fluid
Chloride (Vitreous Fluid)	125	mmol/L	70	005 - Vitreous Fluid
Glucose (Vitreous Fluid)	None Detected	mg/dL	35	005 - Vitreous Fluid
Urea Nitrogen (Vitreous Fluid)	27	mg/dL	3.0	005 - Vitreous Fluid

Blood BHB: NONE DETECTED

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Case – COD & MOD Determination

Isopropanol	43	mg/dL	5.0	002 - Femoral Blood
Acetone	170	mg/dL	5.0	002 - Femoral Blood
Creatinine (Vitreous Fluid)	1.2	mg/dL	0.050	005 - Vitreous Fluid
Sodium (Vitreous Fluid)	141	mmol/L	80	005 - Vitreous Fluid
Potassium (Vitreous Fluid)	11	mmol/L	1.0	005 - Vitreous Fluid
Chloride (Vitreous Fluid)	125	mmol/L	70	005 - Vitreous Fluid
Glucose (Vitreous Fluid)	None Detected	mg/dL	35	005 - Vitreous Fluid
Urea Nitrogen (Vitreous Fluid)	27	mg/dL	3.0	005 - Vitreous Fluid

Blood BHB: NONE DETECTED

The cause of death was reported as acute acetone/isopropanol ingestion and the manner of death was accident.

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BHB – biomarker for DKA v. solvent ingestion

-INTERPRETATION TABLE-			
Acetone/IPA	Glucose (>200 mg/dL)	BHB (>250 mg/dL)	Interpretation*
+	+	+	DKA
+	-	+	DKA/EtoH ketoacidosis
+	-	-	Solvent Ingestion

* If someone has ketoacidosis and consumes acetone or IPA → I'm out ☺

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CONCLUSIONS

ACETONE

- Analytical testing only shows presence and quantity, not its source
- Acetone by itself is not a robust biomarker for determination of a pathologically significant ketoacidosis or to show its absence
- A low or negative acetone concentration does not preclude an elevated BHB

VITREOUS GLUCOSE

- While elevated concentrations are indicative of hyperglycemia, instability can mean the reported concentration is not representative of the antemortem concentration

BHB

- A strong indicator of ketoacidosis and can be used to substantiate or negate a diagnosis

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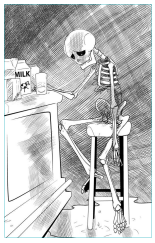

Questions

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