THE “MAKING A MURDERER” CASE: A BRIEF DESCRIPTION ON HOW EDTA IS MEASURED IN BLOOD

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Abstract

In the State of Wisconsin vs. Steven Avery trial, Mr. Avery was convicted of murdering Terasa Halbach. The case has been the subject of immense controversy, and has recently been documented in the popular mini-series Making a Murderer. Avery has insisted on his innocence, and the Avery legal team claimed that the defendant’s blood was “planted” at the crime scene. Local police forces are the target of this suspicion, as they had access to Avery’s blood, from a collection tube containing the preservative ethylenediamine-tetraacetic acid (EDTA). FBI forensics experts rejected this claim; they concluded that the blood found at the crime scene was not from an EDTA tube, as EDTA was not detected in the samples analyzed.

The procedures around this analysis have been passionately discussed and criticized on social media and other forums. However, few non-chemists are aware of how the technique used for measuring EDTA works.

Here, we present a short and relatively informal explanation of the central technique used for analyzing Avery’s blood, namely liquid chromatography-mass spectrometry (LC-MS). Beyond forensics, LC-MS is actually a big part of our lives, as it is also used for e.g. medical diagnostics, monitoring the environment and quality control of everyday products.
Introduction

In 2007, Steven Avery was sentenced to prison for the murder of Terasa Halbach. What made this case stand out was that Avery had recently been released from prison, after wrongfully spending 18 years incarcerated on a rape charge. Advances in DNA analysis had been used to prove Avery’s innocence, resulting in his release. Avery quickly filed a lawsuit against Manitowoc County and former officials, whom Avery believed had framed him. In the midst of these procedures, Avery was again arrested for murdering Halbach. His nephew, Brendan Dassey, was also arrested and convicted as an accomplice.

The handling of the Halbach murder case was highly controversial. Steven Avery and his lawyers argued that they had he had once again been “set up” by his judicial adversaries. One central piece of evidence incriminating Avery was the presence of his blood at the crime scene. Avery’s attorney’s argued that the blood had been planted, arguing that the source was a test tube containing Avery’s blood, which local police forces had access to, from the sexual assault case.

But how could one determine whether the crime scene blood was from a sample tube? This standard blood collection tube contained ethylenediamine-tetraacetic acid (EDTA), which prevents blood coagulation and degradation. EDTA is not naturally present in human blood, and the defense argued that if EDTA was found in the blood present at the crime scene, it would serve as proof that the blood had been planted. The results of this analysis has generated vast discussion on social media and elsewhere, particularly following the documentary Making a Murderer [1], which describes the life and trials of the Avery family. However, tools of forensics and chemical analysis are often “black boxes” to non-scientists, and perhaps early-stage science students as well. And even for the most curious and inquisitive, it can be difficult to learn more about these techniques as they are mostly described in scientific papers that cost considerable amounts of money to access.

Therefore, I would like to here describe the central technique used for determining EDTA in blood spots in the Avery case, namely liquid chromatography-mass spectrometry (LC-MS). I here attempt to describe it in relatively simple terms. Please note that I will not discuss the judicial aspects of the case, but merely describe a central scientific technique used in a trial that has captivated and concerned millions of people.
**LC-MS: The measuring apparatus**

LC-MS is a technique/apparatus that can be used for finding and measuring specific molecules in a sample. These molecules can be big (e.g. proteins) or small (e.g. amino acids), fatty or water-soluble. In other words, LC-MS is a versatile technique that can be used to search for a great variety of molecules.

Starting with the MS part: MS is an advanced instrument that is typically the size of a washing machine. The MS measures the mass of the molecules in the sample [2]. A human being can weigh e.g. 75 kilos, but a single amino acid molecule “weighs” about 0.000000000000000000000002 kilos. Hence, this is an extremely precise and accurate instrument that can pinpoint a molecular mass with great accuracy and precision. Therefore, if the MS detects a molecule with the mass of EDTA (see Figure 1), there is already a fair chance that may be EDTA is present in the sample.

![Figure 1: The chemical structure of EDTA.](image)

There are many kinds of MS instruments [2]. Some instruments determine the mass of a molecule by measuring the speed in which it flies in the MS, or measuring the frequency and radius in which it circles an electrode inside the MS (Figure 2).

However, other molecules present in a sample may have the same mass as EDTA (just like many humans weigh 75 kilos; how could a balance tell them apart?). This presents a risk of a false positive, i.e. believing you are measuring one molecule, while in fact measuring another. Therefore, more steps can be taken to ensure a valid analysis. One is that the MS instrument can feature a collision cell, where molecules are bombarded with gas, breaking the molecule into smaller pieces. The masses of the pieces/fragments are then measured, and a computer visualizes the fragments of the shattered molecule. This visualization (called an “MS/MS spectrum”) can serve as a “fingerprint” of the molecule. If a mass fingerprint is for a particular molecule is observed, it serves as strong evidence that the molecule of interest is indeed present.
Figure 2: Very simplified illustrations on some principles for measuring molecular mass with MS. Top: The mass of a molecule can be calculated based on the speed in which it flies through a chamber in the MS unit (“time of flight” MS variant). Bottom left: The mass can be calculated based on the radius and frequency in which it moves around an electrode system inside the MS unit (“Orbitrap” MS variant). Bottom right: An MS can be tuned to only measure one mass, by e.g. sending molecules through a set of four electrodes that are tuned to only permit one mass exit to be measured (“quadrupole” variant).

One problem with MS instruments is that if you put a very complicated sample in it (e.g. blood), it gets “too crowded”, and the MS might not be able to tell one molecule from the
other, or have trouble detecting molecules [3]. In addition, one can be so unfortunate that two slightly different molecules may have virtually the same fingerprint [4]!

This is where the LC part comes in; LC [5] is a technique that introduces different molecules from the sample to the MS at different time points, so the MS have fewer molecules to measure at a time. For example, the LC system may first permit very water-soluble molecules to enter the MS, followed by semi-soluble molecules, followed by poorly-soluble molecules, and so on. The LC sort of forces different molecules to “stand in a queue, to wait for their turn for entering the MS” (Figure 3). The time points in which a molecule enters the MS from the LC (called the retention time), is very precise, and can be used as additional grounds for identifying a molecule in a sample. An LC-MS analysis typically lasts for a few minutes or several tens of minutes, depending on e.g. the sample’s complexity. If EDTA is present in a sample, its “mass fingerprint” should be detected, and at a specific retention time.

Figure 3: Very simplified illustration of the LC process.
The Avery samples

Regarding the Avery samples, the FBI was unable to detect EDTA using LC-MS [6]. It was concluded that EDTA was not in the samples analyzed.

When reading the FBI reports regarding the analysis of Avery’s blood [6,7], it seems that the LC-MS part has been suitably performed, with a number of steps taken to ensure that false positives and negatives would not occur (use of “mass fingerprint”, retention time, and several other steps). The method does not appear to be “thrown together”, but is based on a previous method reported some years earlier [8]. However, it would have been desirable if control samples absorbed to variety of absorbents (metal surfaces, wall paper, etc.) had been investigated, to demonstrate the validity and robustness of the total method.

Some limitations of the method are stated in the FBI reports. One weakness is that the system can “only” detect 0.013 milligrams of EDTA molecules per milliliter blood. In contrast, other compounds can be readily identified at 1,000 times lower concentration or less. On the other hand, EDTA concentrations are is expected to be about 100 times higher than 0.013 milligrams of EDTA per milliliter blood [8].

The LC-MS spectra (the “fingerprint”) is not presented, making it difficult for viewers to acknowledge that there is a 100 % certainty that EDTA was not present in the Avery blood stains. If these spectra were released for external inspection, it could relieve suspicions of contextual bias [9].

Beyond Making a Murderer: LC-MS and our daily life

I would also like to take this opportunity to tell you that LC-MS is not only a technique that is used in forensics. It is used for e.g. measuring pollutants in the environment [10], scouting for molecules that can predict diseases [11], and measuring the levels of nutrients in food [12]. In other words, society as a whole is dependent on the application and development of LC-MS and other analytical techniques. However, it is imperative that professionally trained personnel handle these techniques, as pitfalls regarding false negatives/positives are always present, calling for rigorous validation regarding the analysis. Moreover, excellent instrumentation cannot make up for poor handling of samples; Technology cannot make up for human errors prior to analysis.
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