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A Simple Method for Estimating the Time Of Death by Measurement of the Potassium Ion Recovered from Vitreous Humor by Ion-Exchange Chromatography and Determined Quantitatively by Acid-Base Titration

Armando Gamarra[1], Nancy González[1], Ileana Godoy[1], María Carla Rampulla[2], Andreas Melinato[2]

1 Catholic University of Salta

2 Istituto di Scienze Forensi (ISF)

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Abstract

It is well known that potassium ion concentration in vitreous humor increases gradually and linearly with Time After Death – TOD. [1] [2]. That is why vitreous humor has been used for several decades for postmortem biochemical investigations and for the estimation of the Time Of Death or Time Since Death. To determine quantitatively the concentration of potassium ions in the vitreous humor we will utilize a simple, well know, and accurate technique: ion exchange chromatography coupled with acid-base titration. Since ion exchange chromatography is a well-tested technique to recover ions quantitatively, we are sure it can produce, in a simple way, quantitative results with the same accuracy as the other used methods.

Armando Gamarra[a], **Nancy González**[a], **Ileana Godoy Asis**[a], **María Carla Rampulla**[b], **Andreas Melinato**[b]

[a] *Catholic University of Salta, Argentina,*

[b] *European Forensic Institute*

Corresponding author: Armando Gamarra, agamarra@ucasal.edu.ar

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Dating in Forensic Science

In this section, we follow Weyerman seminal studies on dating in forensic sciences[3]. A very important problem in criminalistics is locating events such as TOD and traces (fingerprints) in time. Forensic scientists routinely have to answer the following questions:

- Did a criminal event occur at a given time?
- Where was a suspect at a specific time?
- When did a death event occur?
- When was a fingerprint left on the crime scene?

Introduction

The estimation of the Time Of Death – TOD, also called Post Mortem Interval, (PMI), or Time since Death, is very important for the police, forensic scientists and forensic medicine. TOD has special relevance in criminal and civil cases. Many mistakes in court cases could have been avoided if the TOD had been established with acceptable precision.

Due to the advances in the instrumental methods of chemical analysis over the past decades, the determination of TOD has been based on the measurements of the post-mortem time evolution of some biochemical markers found in body fluids such as blood, cerebrum-spinal fluid, vitreous humor (HV) and synovial fluid.

There are many ways to estimate TOD by measurement of biochemical markers such as ARN, hypoxanthine and potassium ions in the vitreous humor. All these methods require sophisticated equipment, expensive reagents, and highly qualified personnel, which are often scarce or non-existent in developing countries and even in small and isolated developed cities.

Although the use of sensitive and advanced instrumentation is attractive in the field of forensic chemistry, there are other approaches with the same level of analytical precision, that should be seen as feasible alternatives to expensive instrumentation, that is often not available. One of these classic quantitative analytical approaches is ion exchange chromatography coupled with acid-base titration.

Advantages of Choosing the Vitreous Humor as the Body Fluid

Vitreous humor as a source of biochemical markers offers a number of advantages: it has a relatively large volume; it is easily obtainable, and it is usually free from interfering components. Moreover, it has been demonstrated that the time-change of biochemical markers in the VH takes place gradually and linearly [2].

Biochemical markers that are organic in nature are determined by HPLC-MS, or other equally expensive equipment. On the other hand, the potassium ion can be determined by AAS, Flame spectrophotometry, capillary electrophoresis, or potentiometry with ion-specific electrodes [4]. In developing countries, however, most of these instruments are not available outside the main urban centers.

With our method, in contrast, it is possible to determine potassium quantitatively and with sufficient precision without resorting to advanced instrumentation. All we need is an ion exchange column filled with a strong acid cation resin, an

Erlenmeyer flask, a color indicator, and a NaOH solution of known concentration.

Physicochemical Fundamentals of the Method

A cation exchange resin of the acidic type will take up the cations (potassium ions in our case) from the sample solution (VH suitably diluted and free of interferents) and an equivalent amount of the protons from the resin, H^+, will pass into solution.

The equilibrium that establishes is as follows:

Res- **H** + $K_{(ac)}^+$ = Res- **K** + $H_{(ac)}^+$

Where: Re- H is the cation-exchange resin in the hydrogen, strongly acidic, form,

Re- K is the cation-exchange resin with the potassium ion on it

Determination of Salts with Cation-Exchange Resins

By using a cation-exchange resin in the hydrogen form, the potassium ion content of a soluble salt, such as K from VH, may be determined [5]. The reaction is:

K $A_{(ac)}$ + Res- **H** = H $A_{(ac)}$ + Res −**K**

Where: **K**A is the potassium salt in the vitreous Humor, as KCl. $HA_{(ac)}$ is the titratable acid formed and Res-**K** is the potassium ion bonded to the resin.

We observe that for every mol of K^+ on the resin, one mol of the acid HA (HCl in this case) is formed. Therefore, the potassium content of the sampled vitreous humor is quantified by titrating the acid formed, H $A_{(ac)}$ with NaOH.

Materials and Methods

We will follow the procedure established by many authors[6], [7], [8]. At autopsy, approximately 1 mL of HV fluid will be obtained from each eye by inserting a needle into the globe at the lateral canthus. The vitreous humor will be aspirated with a syringe with an 18-gauge needle and will be conveniently diluted and stored. It is then poured into the ion exchange column.

The Origin of the Increase in the Concentration of Vitreous Humor After Death

After death, cell membranes become permeable. In this way, potassium ions, together with glucose, hyaluronic acid, collagen fibers and ascorbic acid, immediately begin to migrate from the interior of the retinal cells to the vitreous. In this

way, the potassium concentration increases with time [7], [8], [9].

Preliminary Pilot Study in Pigs

The vitreous humor of pigs is very similar to that of humans. Therefore, before working with a human cadaver, we can calibrate the entire procedure by determining the potassium ion content of freshly slaughtered pigs from a slaughterhouse. In this way, we will know the exact TOD of the cadaver.

Conclusions

Since the physicochemical basis of our method is well established and the experimental procedure is routinely described in any quantitative analytical chemistry book, we are confident, that once the corresponding protocol is established, our method could become a useful tool to improve the application of justice in many places.

Definitions

- Time Of Death or Time Since Death or Postmortem Interval: It is the time that has elapsed since a person has died.
- Dating: Can be defined as the attribution of a relative or absolute age of an event, an object or a trace.
- Ion-exchange chromatography: An ion exchanger is a solid or gel that has chemically bound charged groups to which ions are electrostatically bound; it can exchange these ions for ions in an aqueous solution. Cation exchangers have groups negatively charged; they will exchange positive ions.
- Vitreous humor: The clear gel that fills the space between the lens and the retina of the eyeball of humans and other vertebrates, such as pigs.
- Acid-base titration or acid-base volumetry: A method of quantitative analysis for determining the concentration of acid or base by exactly neutralizing it with a solution of known concentration of base or acid.

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