

Article

Uncertainty of Postmortem Time Estimation Based on Potassium Ion Determination in Vitreous Humor Using Potentiometric Ion-Selective Electrode and Microwave-Induced Plasma with Optical Emission Spectrometry Methods

Sonia Zięba^{1,†}, Marek Wiergowski^{1,*,†}, Bartłomiej Michał Cieślak², Jacek Sein Anand^{3,4}
and Marta Krzyżanowska¹

¹ Department of Forensic Medicine, Faculty of Medicine, Medical University of Gdańsk, M. Skłodowskiej-Curie 3a Str., 80-210 Gdańsk, Poland

² Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, Narutowicza 11/12 Str., 80-233 Gdańsk, Poland

³ Division of Clinical Toxicology, Faculty of Health Sciences with the Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, M. Skłodowskiej-Curie 3a Str., 80-210 Gdańsk, Poland

⁴ Pomeranian Center of Toxicology, Kartuska 4/6 Str., 80-104 Gdańsk, Poland

* Correspondence: marek.wiergowski@gumed.edu.pl

† These authors contributed equally to this work.



Citation: Zięba, S.; Wiergowski, M.; Cieślak, B.M.; Anand, J.S.; Krzyżanowska, M. Uncertainty of Postmortem Time Estimation Based on Potassium Ion Determination in Vitreous Humor Using Potentiometric Ion-Selective Electrode and Microwave-Induced Plasma with Optical Emission Spectrometry Methods. *Separations* **2023**, *10*, 201. <https://doi.org/10.3390/separations10030201>

Academic Editor: Victoria Samanidou

Received: 12 February 2023

Revised: 6 March 2023

Accepted: 10 March 2023

Published: 14 March 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: There is a need for a reliable and independent evaluation and confirmation of the post-mortem interval (PMI) based on objective factors other than only postmortem changes or temperature measurements. Estimating the PMI by examining the concentration of potassium ions in the vitreous humor (VH) has a tradition in forensic toxicology dating back to the mid-20th century. So far, the methods for determining the presence of potassium ions have not been characterized in terms of the measurement uncertainty of types A and B, which directly affect the estimation of time of death uncertainty. The study evaluated the uncertainty of the determination of potassium ion concentrations using potentiometric ion-selective electrode (ISE) and microwave-induced plasma with optical emission spectrometry (MIP-OES) methods. In addition, the influence of the estimated measurement uncertainty on the results of the analysis of potassium ions in the VH was investigated. The estimated values of the expanded uncertainty determined by the type A experimental method indicate lower uncertainty in the determination of potassium ion concentration in the case of MIP-OES than ISE; that is, for concentrations of 2, 10, and 25 mg/L of potassium ions, the expanded uncertainties by MIP-OES were 1.2%, 2.2%, and 2.5% and the uncertainties by ISE were 12.2%, 6.1%, and 3.1%, respectively. Similarly, in the case of estimating the uncertainty of type B, the MIP-OES method compared to ISE was characterized by almost two times lower expanded uncertainty; that is, for MIP-OES, it was 2.53%, and for ISE, it was 4.75%. Both methods of uncertainty estimation, types A and B, can be used interchangeably, as they provide comparable results.

Keywords: uncertainty; potassium ions; vitreous humor; postmortem interval; time since death; ion-selective electrode; microwave plasma atomic emission spectroscopy

1. Introduction

Estimating the postmortem interval (PMI, also called “time of death” [TOD] or “time since death” [TSD]) is an important element in prosecution proceedings (e.g., in the case of searching for a person suspected of murder). Witness testimonies are often associated with a high risk of inaccuracy, manipulation, or even forgery. Witnesses may also have prejudices against the suspect. However, PMI determination can verify the course of events, including limiting the number of suspects or verifying their alibi [1,2].

There are many methods for estimating the PMI. Nowadays, more and more often attempts are made to use microRNAs as useful tool to estimate TSD [3]. The frequently used ones include temperature measurement (which is usually performed in the anus), evaluation of rigor mortis, and assessment of livor mortis. Among these, the temperature measurement method has the widest scope of application. Unfortunately, the time window for the PMI in which it can be used is 36 h [1]. Methods with longer time windows for the PMI include biochemical processes in which the determination of the TOD is based on the correlation of the concentration of substances (e.g., potassium ions) in biological material (most often in the vitreous humor [VH]). The method of assessing potassium concentration in the VH enables us to determine the TOD within 120 h [4]. The VH is a valuable postmortem material because it is composed primarily of water and the autolysis process takes place much slower in it than in peripheral blood. However, this type of material also has disadvantages, such as low volume (1–2 mL) and relatively high viscosity. The analysis of the concentration of potassium ions concerning the PMI dates back to the 1960s [5–7]. Since then, researchers have proposed more than a dozen formulas that can be used to estimate the PMI (the uncertainty of this time estimate was difficult to determine) (Table 1).

Table 1. Formulas for estimating the PMI based on the concentration of potassium ions ($[K^+]$ in mmol/L).

No.	Author(s) and Year	Formula Proposed
1	Adelson et al. (1963) [8]	$PMI = 5.88 [K^+] - 31.53$
2	Coe (1969) [9]	$PMI = 6.15 [K^+] - 38.10$
3	Henßge and Madea (1989) [1]	$PMI = 5.26 [K^+] - 30.90$
4	Ross et al. (1997) [10]	$PMI = 4.32 [K^+] - 18.35$
5	Muñoz Barús et al. (2002) [11]	$PMI = 3.63 [K^+] - 17.33$
6	Zhou et al. (2007) [12]	$PMI = 5.88 [K^+] - 32.71$
7	Jashnani et al. (2010) [13]	$PMI = 1.08 [K^+] - 2.82$
8	Bortolotti et al. (2011) [14]	$PMI = 5.77 [K^+] - 13.28$
9	Mihailovic et al. (2012) [15]	$PMI = 2.75 [K^+] - 11.98$
10	Siddhamsetty et al. (2014) [16]	$PMI = 4.75 [K^+] - 27.9$
11	Bohra et al. (2014) [17]	$PMI = 3.75 [K^+] - 16.22$
12	Foster et al. (2016) [18]	$PMI = 6.42 [K^+] - 40.94$
13	Murthy et al. (2019) [19]	$PMI = 5.26 [K^+] - 30.9$
14	Focardi et al. (2020) [20]	$PMI = 6.16 [K^+] - 32.49$

The most frequently used method to measure the concentration of potassium ions in the VH is direct potentiometry in the form of an ion-selective electrode (ISE) and, less frequently, the method of flame photometry, for example, in the form of microwave-induced plasma with optical emission spectrometry (MIP-OES) (Table 2).

To the best of the authors' knowledge, both ISE and MIP-OES methods have not yet been characterized in terms of measurement uncertainty of types A and B, which directly affect the estimation of PMI uncertainty. Measurement uncertainty is the numerically expressed dispersion of the measured quantity, in which it is expected, with a certain probability, to find the true measurement value. Measurement uncertainty can be determined using two methodologies: type A and type B. The type A method most often uses a series of measurements and determines the variation of the results (e.g., standard deviation), considering the coverage factor equal to 2 and the corresponding confidence interval. The type B method uses data contained in the specifications of measuring devices, safety data sheets for reagents, laboratory equipment, and previous tests described in publications and test reports. The feature that distinguishes the type B from the type A method is the lack of necessity to conduct experimental tests to estimate the uncertainty.

This study aimed to estimate the uncertainty of potassium ion determination using ISE and MIP-OES methods and to examine the impact of the estimated measurement uncertainty on the analysis of potassium ions in the VH and, consequently, on the PMI estimation.

Table 2. Methods applied to determine the postmortem concentration of potassium ions in the VH.

No.	Volume [mL]	Sample Preparation	Instrumental Analysis (Commercial Name of Instrument)	Ref.
1	0.10	Samples were stored at $-20\text{ }^{\circ}\text{C}$ and centrifuged at 16,000 rpm for 10 min; only the supernatant was decanted	Photometric, potentiometric, turbidimetric (ARCHITECT 8000)	[7]
2	0.10	Samples were stored at $-20\text{ }^{\circ}\text{C}$ and diluted at 1:20 with a 40-mg/mL aqueous solution of barium chloride (internal standard)	Capillary electropherogram with a UV absorbance detector (A PACE MDQ; capillary column: 75 mm ID \times 50 cm, 60 cm)	[17]
3	n/a	Samples were centrifuged	Ion-selective electrode (ADVIA 2400 Chemistry System)	[9]
4	0.20	Samples were not pre-treated (without dilution, centrifugation, or sonication)	Ion-selective electrode (ABL 625 radiometer with UniCel Dx C 800)	[21]
5	1.50–2.00	Blood was collected in test tubes with EDTA, the VH was placed in sterile plain vials, and samples were centrifuged at 3500 rpm for 10 min	Ion-selective electrode (Analyser AU680)	[11]
6	0.10	Samples were stored at $-70\text{ }^{\circ}\text{C}$	Turbidimetric (Humalyzer Junior)	[22]
7	2.00	Samples were centrifuged at 4500 rpm and the supernatant was transferred to another container	Ion-selective electrode, flow-through, liquid membrane electrode (Roche 9180 Electrolyte Analyzer)	[23]
8	0.15	n/a	Indirect potentiometry (Advia 2400).	[24]
9	3.00–4.00	Samples were stored at $4\text{ }^{\circ}\text{C}$ and centrifuged at 2000 rpm for 5 min	Flame photometry (FLM3, Biolyte 2000)	[25]
10	1.50–2.00	Samples were centrifuged at 3500 rpm for 10 min	Ion-selective electrode (indirect potentiometry method)	[26]
11	n/a	Samples were stored at $4\text{ }^{\circ}\text{C}$ and centrifuged at 3000 rpm for 10 min	Indirect potentiometry (BM/747)	[27]
12	n/a	Samples were centrifuged at $13,000 \times g$ for 10 min, supernatant solutions were stored at $-80\text{ }^{\circ}\text{C}$ and vortexed for 10 s, and viscous VH samples were diluted with deionized water	Photometric, potentiometric, turbidimetric (ARCHITECT c16000)	[15]
13	2.00	Samples were stored at $-18\text{ }^{\circ}\text{C}$ and $-70\text{ }^{\circ}\text{C}$ and centrifuged at 3000 rpm for 10 min	Ion-selective electrode (Beckman auto-analyzer)	[28]
14	2.00	Samples were stored at $-80\text{ }^{\circ}\text{C}$, vortexed for 30 s using the highest level, and centrifuged at $1650 \times g$ for 8 min; the supernatant was divided into four aliquots	Potentiometric method (VLYTE1 Integrated Multisensor K800A)	[20]

UV, ultraviolet detection; EDTA, ethylenediaminetetraacetic acid.

2. Materials and Methods

2.1. Sample Collection

During the medicolegal autopsy in the Department of Forensic Medicine of the Medical University of Gdańsk in 2021–2022, VH samples were initially collected from 42 deceased individuals. The deceased with head injuries (risk of VH contamination by peripheral blood) and those who died more than 120 h before dissection were excluded from the study. After sampling, the material was transferred to 2 mL microcentrifuge tubes (Eppendorf-type vials) and clearly labeled. The collected VH samples were centrifuged shortly after dissection for 10 min at 3500 rpm and transferred to 2 mL Eppendorf vials, which were then stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

Table 3 summarizes the data of the deceased: gender, age of death, blood alcohol content and other biological materials, cause of death, TOD or disclosure of the body, and time of postmortem examination.

Table 3. Selected data of the deceased individuals autopsied in 2021–2022.

No	Sex ¹	Age (y)	Circumstances, Mode, and Possible Cause of Death	Concentration of Ethanol (‰)	Estimated Time of Death	Time of Autopsy	PMI (h)
1	M	40	Acute circulatory and respiratory failure, cirrhosis, ethanol poisoning	0.92 (blood), 1.13 (VH)	6 June 2020 19:47	8 June 2020 12:00	40
2	M	39	Acute circulatory and respiratory failure, asphyxiation (by hanging), ethanol poisoning	3.18 (blood), 3.77 (urine)	19 July 2020 21:20	21 July 2020 09:00	36
3	M	50	Acute cardiorespiratory failure, urosepsis, acute bacterial interstitial nephritis	Negative ²	26 July 2020 05:35	30 July 2020 08:00	98
4	M	32	Acute circulatory and respiratory failure, asphyxiation (by hanging), ethanol poisoning	0.69 (blood) 0.22 (urine)	26 July 2020 22:30	28 July 2020 09:30	35
5	F	58	Acute circulatory and respiratory failure, acid–base disorders, ethanol addiction, cachexia	Negative	3 August 2020 13:59	7 August 2020 12:00	94
6	M	63	Acute circulatory and respiratory failure, asphyxiation (blood aspiration), oral cancer	Negative	6 August 2020 18:45	10 August 2020 10:30	88
7	M	57	Acute circulatory and respiratory failure, bleeding from esophageal varices, cirrhosis, ethanol addiction, ethanol poisoning	1.01 (blood)	24 August 2020 11:00	27 August 2020 10:00	71
8	M	41	Acute circulatory and respiratory failure, alcohol-induced liver damage, hygiene negligence	Negative	6 September 2020 14:50	10 September 2020 09:45	91
9	M	54	Acute circulatory failure, hypertrophic and dilated cardiomyopathy, condition after aortic valve implantation, obesity	Negative	14 September 2020 14:20	16 September 2020 10:30	44
10	M	42	Acute heart failure, hypertrophic cardiomyopathy, arrhythmia	Negative	14 September 2020 11:00	16 September 2020 09:15	46
11	M	55	Acute circulatory and respiratory failure, disturbances in acid–base and water–electrolyte balance, suspected alcohol ketoacidosis, ethanol addiction	Negative	15 September 2020 01:10	17 September 2020 09:00	56
12	M	37	Acute circulatory and respiratory failure, bleeding from the duodenum ulcer, alcohol-induced liver damage	Negative	16 September 2020 22:20	21 September 2020 09:00	107

Table 3. *Cont.*

No	Sex ¹	Age (y)	Circumstances, Mode, and Possible Cause of Death	Concentration of Ethanol (‰)	Estimated Time of Death	Time of Autopsy	PMI (h)
13	M	46	Acute circulatory and respiratory failure, myocardial infarction, pulmonary edema, chronic coronary artery disease, ethanol poisoning	2.82 (blood) 4.15 (urine)	20 September 2020 05:50	24 September 2020 08:30	99
14	M	56	Acute circulatory and respiratory failure, asphyxiation (by hanging)	Negative	19 September 2020 20:40	23 September 20 08:30	84
15	M	63	Acute circulatory failure, hypertrophic and dilated cardiomyopathy, generalized atherosclerosis	Negative	4 October 2020 12:10	7 October 2020 09:30	69
16	M	49	Acute circulatory failure, chronic coronary artery disease, arrhythmia	Negative	5 October 2020 15:00	8 October 2020 10:30	68
17	M	33	Acute circulatory and respiratory failure, asphyxiation (by hanging), ethanol poisoning	1.73 (blood), 2.85 (urine)	17 October 2020 14:40	20 October 2020 10:30	68
18	M	57	Acute circulatory and respiratory failure, cirrhosis, ascites, chronic pancreatitis, COPD (chronic obstructive pulmonary disease), ethanol addiction	Negative	25 October 2020 18:00	27 October 2020 10:00	40
19	F	61	Acute circulatory and respiratory failure, myocardial infarction	Negative	13 December 2020 00:00	17 December 2020 08:30	104
20	M	59	Acute circulatory and respiratory failure, ethanol poisoning	4.18 (blood), 3.96 (urine)	11 April 2021 14:30	14 April 2021 09:00	67
21	F	50	Acute circulatory and respiratory failure, venous hemorrhage, ethanol poisoning	2.62 (blood), 2.57 (urine)	21 April 2021 15:05	23 April 2021 09:00	42
22	F	99	Acute circulatory and respiratory failure, death from natural causes	Negative	10 October 2021 11:55	14 October 2021 08:15	92
23	M	67	Acute circulatory and respiratory failure, arrhythmia, vascular cardiomyopathy	Negative	31 October 2021 14:15	3 November 2021 11:00	69
24	M	34	Acute circulatory and respiratory failure	Negative	1 November 2021 12:20	4 November 2021 10:00	70
25	M	42	Acute cardiorespiratory failure, ethanol poisoning, liver damage, pancreatic damage, ethanol addiction	3.55 (blood) 3.48 (urine)	4 November 2021 11:30	9 November 2021 08:15	117
26	M	45	Acute circulatory and respiratory failure, asphyxiation (by hanging), ethanol poisoning	0.33 (blood) 0.56 (urine)	7 November 2021 12:30	10 November 2021 08:15	68

¹ M, male; F, female. ² Regarding Polish legal limits, “negative” means below 0.2‰ of ethanol.

The results of the deceased who died due to hanging, who may also rupture the blood vessels of the eyes, should be interpreted with great care. Samples from 26 deceased individuals were taken for further analysis.

From the TOD to the autopsy examination, the time usually did not exceed a few days. During the time preceding the autopsy, the bodies were stored at +4 °C. In the case of analytical methods, the time from the moment of collecting the vitreous humor during autopsy to the moment of taking measurements is important. The storage time of the vitreous humor samples at −20 °C is also essential, as we assume no significant changes in the chemical composition during this time, which is not necessarily accurate.

2.2. VH Preparation

The potassium ion determination procedure was developed based on the research results of Mihailovic et al. [15] and Yang et al. [27] (Table 2). Below, we present the methodology optimized to our conditions. Samples with the centrifuged VH (supernatant) were left at room temperature for about 1 h after removal from the −20 °C freezer. Each sample was vortexed for 15 s. Then, 50 µL of the supernatant was collected twice into two 5 mL Eppendorf vials and refilled (completely) with deionized water. As a result, an 80-fold dilution was achieved. Each sample was vortexed for 15 s. Depending on the method, the remaining steps of the procedure differed from each other and are described in Table 4.

Table 4. Procedure for determining potassium ion concentration in VH by ISE and MIP-OES methods.

Step	ISE	MIP-OES
1	Take a sample of VH during medicolegal autopsy (1–2 mL) using a metal, pointed (35 × 2 mm) injection needle.	
2	Centrifuge VH at 3500 rpm for 10 min.	
3	Pipette 50 µL of the supernatant twice in two 5 mL Eppendorf tubes.	
4	Fill Eppendorf tubes to 4 mL (i.e., 3950 µL) with deionized water.	
5	Mix on a vortex shaker for 15 s.	
6	Pour the contents into an intermediate vial (5 mL).	
7	Add 80 µL of ionic strength adjuster (ISA) solution (NaCl solution) and mix.	Quantitatively pour the entire beaker into the mineralizer tube.
8	Rinse the ion-selective electrode membrane with deionized water and dry it.	Rinse the test tube with nitric acid (3–4 mL) and pour it into the mineralizer.
9	Place the electrode in the beaker and wait for the measurement to stabilize for 1 min.	Perform microwave-assisted mineralization for 40 min at 180 °C.
10	Write down and save the result.	After mineralization and reaching the room temperature of the solution, pour the mineralization tube contents quantitatively into 10 mL measuring flasks and then wash the tube with deionized water.
11	After the measurements, place the ISE in a solution with a concentration of 100 mg/L of K ⁺ .	Pour the contents of the beaker into a 15 mL plastic test tube and provide MIP-OES measurements.
12	After analysis, store VH samples at −20 °C.	After analysis, store VH samples at −20 °C.

2.3. Instrumentation

Measurements were carried out using two methods: direct potentiometry (ISE) and plasma-excited optical emission spectrometry (MIP-OES). Measurements by direct potentiometry were conducted using a combination of ISE (HI 4114, Hanna Instruments) and a pH meter (S220, Mettler Toledo SevenCompact). When performing measurements using the MIP-OES method with plasma excitation, the Agilent 4210 MP-AES (the older and trade name derived from “microwave plasma–atomic emission spectroscopy”) device was applied, and mineralization was performed with the Multiwave GO Plus by Anton Paar.

3. Results

Calibration curves obtained for potassium ion concentrations ranging from 2 to 100 mg/L by the ISE method in three measurement series (performed on three different days) carried out with six replications for each concentration are presented in Figure 1.

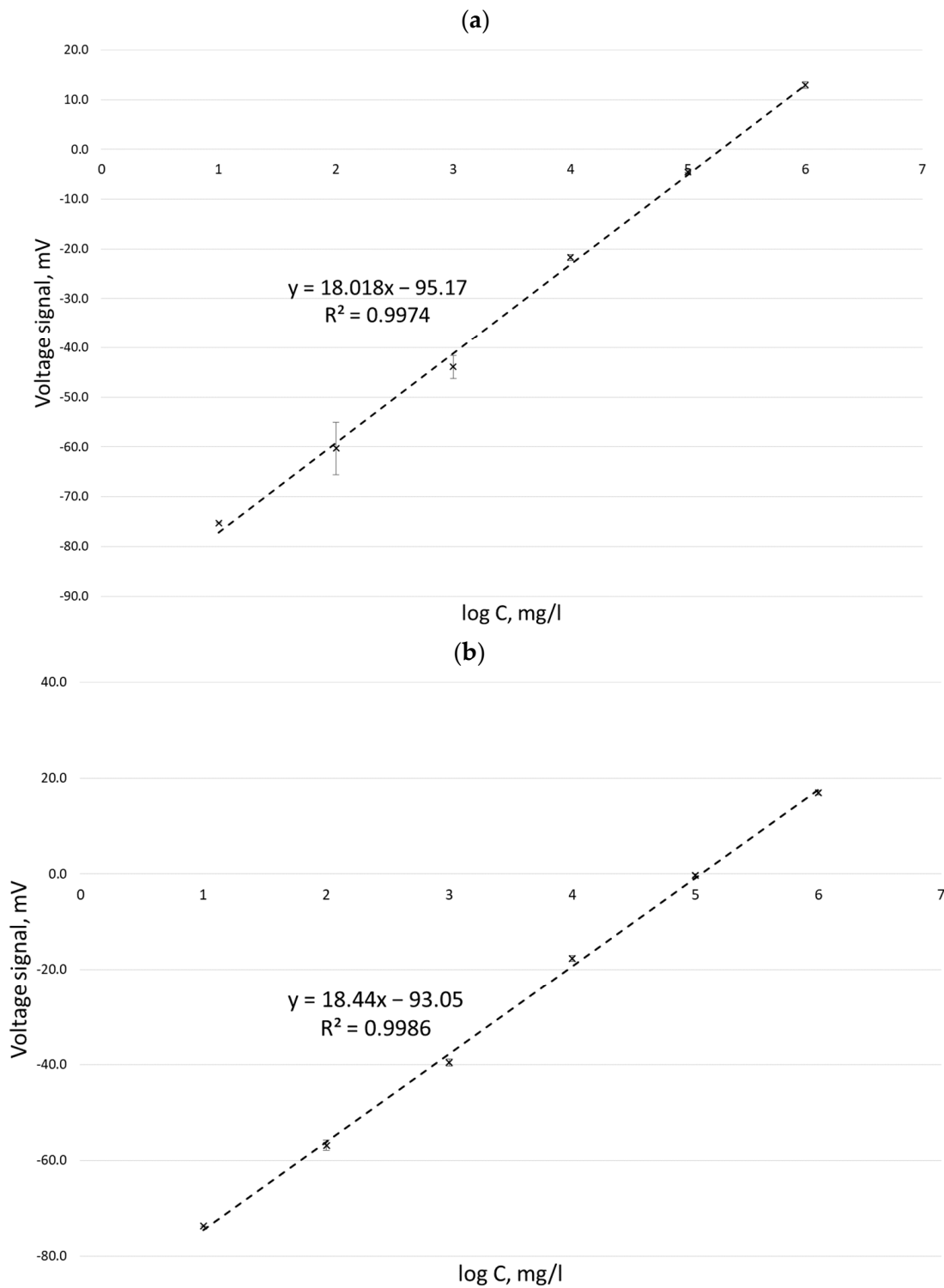


Figure 1. Cont.

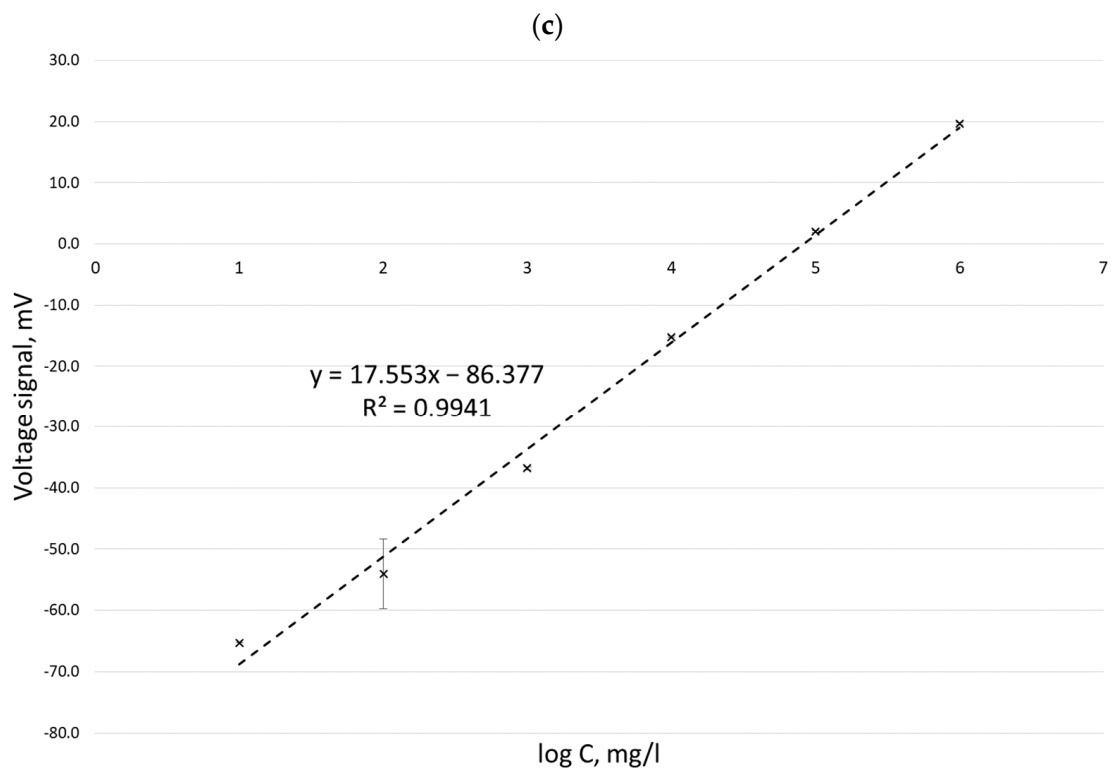


Figure 1. Calibration curves for the determination of potassium ion concentration by the ISE method in three different measurement series: 10 March 2022 (a), 11 March 2022 (b), and 14 March 2022 (c).

Calibration curves obtained for potassium ion concentrations ranging from 1 to 25 mg/L by the MIP-OES method for two different wavelengths are shown in Figure 2.

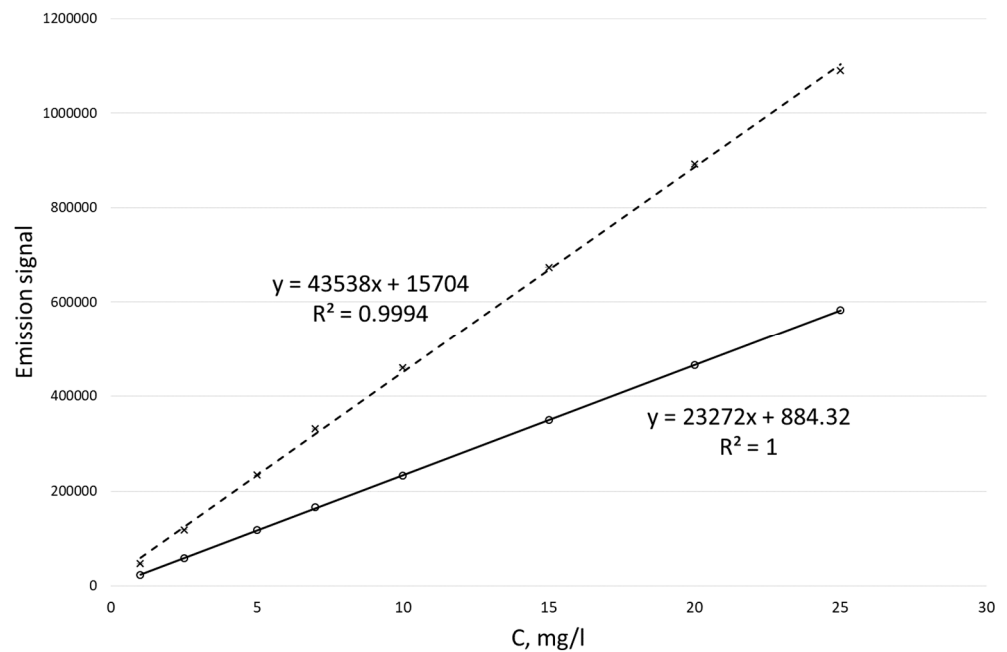


Figure 2. Calibration curves for the determination of potassium ion concentration by the MIP-OES method for two wavelengths at 766.49 nm (dotted line) and 769.89 nm (solid line).

The measurement uncertainty, and hence the reliability of PMI estimation, is influenced by several factors, including the properties of the biological material, the measurement

method applied, the process of sample preparation, the sample concentration, and the cause of death (Figures 3 and 4).

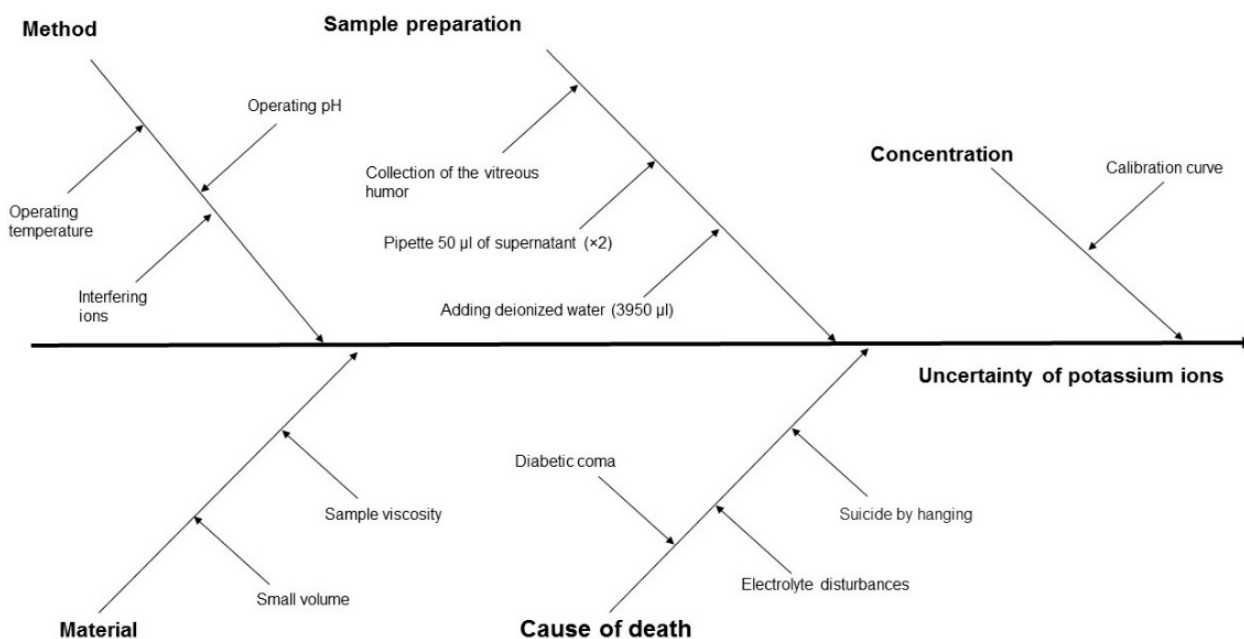


Figure 3. Cause-and-effect diagram for ion potassium concentration in VH using the ISE method, showing the key components that contribute to measurement uncertainty.

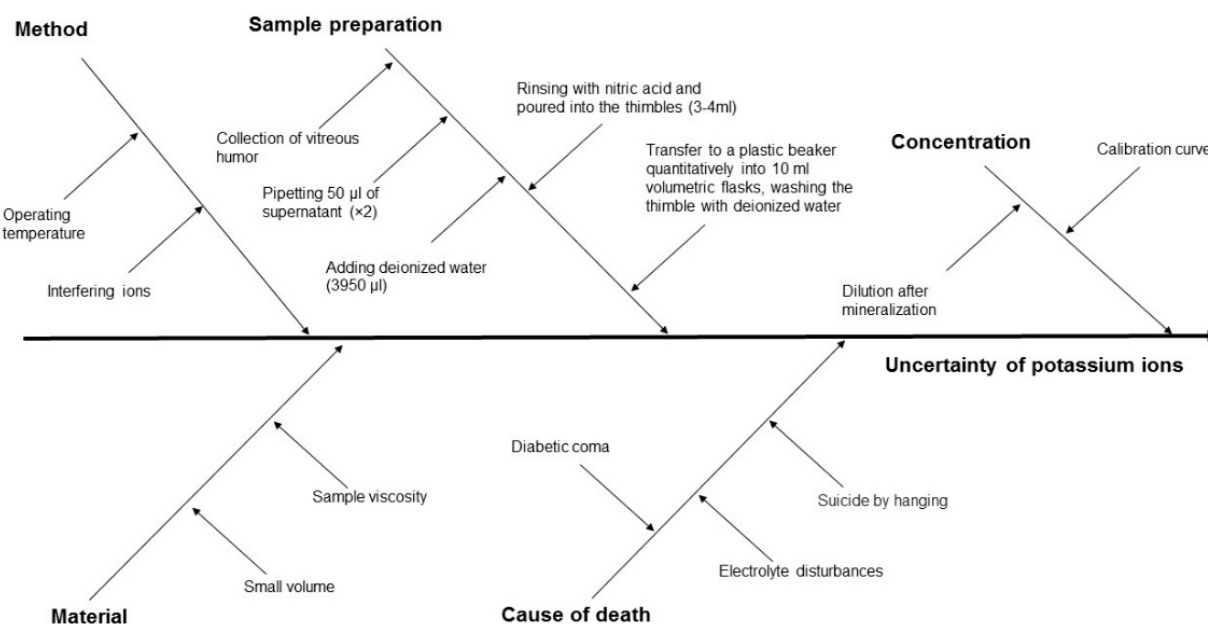


Figure 4. Cause-and-effect diagram for ion potassium concentration in VH using the MIP-OES method, showing the key components that contribute to measurement uncertainty.

To obtain the A-type uncertainty, water samples with low (2 mg/L), medium (10 mg/L), and high (25 mg/L) potassium ion concentrations were tested using the ISE and MIP-OES methods, and the results with the estimated uncertainty are presented in Table 5.

Table 5. Type A uncertainty estimation for the determination of potassium ion concentrations of 2 mg/L (low), 10 mg/L (medium), and 25 mg/L (high) using the ISE and MIP-OES methods.

Concentration of K ⁺	Standard Deviation (SD) of ISE (mg/L)	Standard Deviation (SD) of MIP-OES (mg/L)	Expanded Uncertainty (U _A) ¹ by ISE (%)	Expanded Uncertainty (U _A) by MIP-OES (%)
Low (2 mg/L)	±0.12	±0.012	±12.2	±1.2
Medium (10 mg/L)	±0.30	±0.111	±6.1	±2.2
High (25 mg/L)	±0.39	±0.308	±3.1	±2.5

¹ Expanded uncertainty is defined as $U_A = \frac{2 \cdot SD}{\text{concentration}} \cdot 100\%$.

The components of the uncertainty budget of ISE and MIP-OES methods to estimate type B uncertainty are presented in Tables 6 and 7, respectively.

Table 6. Components of the uncertainty budget of the ISE method [28,29].

Procedure Stage and Comments	Volume (V)	Precision (P)	Accuracy (A)
Pipette 50 µL twice into Eppendorf tubes; variable-capacity pipette 10–100 µL, precision and accuracy for 50 µL	0.05 mL	±0.7%	<0.3%
Fill an Eppendorf tube to 4 mL (i.e., 3950 µL) with deionized water; variable-capacity pipette 500–5000 µL, precision and accuracy for 2500 µL	3.95 mL	±0.6%	<0.15%
Add 80 µL of ISA solution (NaCl solution) and mix; variable-capacity pipette 10–100 µL, precision and accuracy for 50 µL	0.08 mL	±0.8%	<0.2%
Influence of temperature fluctuations on ISE measurement	n/a	n/a	<2%

Table 7. Components of the uncertainty budget of the MIP-OES method [28,29].

Procedure Stage and Comments	Volume (V)	Precision (P)	Accuracy (A)
Pipette 50 µL twice into Eppendorf tubes; variable-capacity pipette 10–100 µL, precision and accuracy for 50 µL	0.05 mL	±0.7%	<0.3%
Fill an Eppendorf tube to 4 mL (i.e., 3950 µL) with deionized water; variable-capacity pipette 500–5000 µL, precision and accuracy for 2500 µL	3.95 mL	±0.6%	<0.15%
After mineralization and reaching the room temperature of the solution, pour the thimble contents quantitatively into 10 mL volumetric flasks and then wash the thimble with deionized water.	10 mL	±0.08 mL	n/a

Using the components of the uncertainty budget listed in Tables 6 and 7, the expanded uncertainty using the ISE and MIP-OES methods was estimated (Table 8).



Table 8. Results of the B-type expanded uncertainty estimation for the determination of potassium ion concentration using ISE and MIP-OES methods.

Concentration of K ⁺	U _B for ISE (mg/L)	U _B for MIP-OES (mg/L)	Expanded Uncertainty (U _B) ¹ by ISE (%)	Expanded Uncertainty (U _B) by MIP-OES (%)
Low (2 mg/L)	±0.24	±0.051	±4.75	±2.53
Medium (10 mg/L)	±0.48	±0.253	±4.75	±2.53
High (25 mg/L)	±1.19	±0.633	±4.75	±2.53

¹ Expanded uncertainty of type B (U_B) was defined as $U_B = k \cdot C \cdot \sqrt{\sum P^2 + \sum A^2}$, where $k = 2$, C is the nominal concentration of potassium ions, P is precision, and A is accuracy.

Table 9 presents 26 cases for which the PMI was determined (based on the data from Table 3), and the concentration of potassium ions was determined using the ISE and MIP-OES methods. The concentration units were converted from mg/L to mmol/L.

Table 9. Results of the PMI assessment (based on the data from Table 3) and the determination of potassium ion concentration in the VH using ISE and MIP-OES methods in 26 deceased individuals.

No.	Concentration of K ⁺ by ISE (mmol/L)	Concentration of K ⁺ by MIP-OES (mmol/L)	PMI (h)
1	17.0	15.1	40
2	22.4	44.2	36
3	19.2	31.4	98
4	19.9	19.7	23
5	18.6	21.9	82
6	18.7	24.4	88
7	10.5	16.9	71
8	30.8	27.7	91
9	21.3	35.6	44
10	7.59	27.0	46
11	10.4	16.8	72
12	24.2	21.0	107
13	23.8	22.0	99
14	16.6	23.0	84
15	23.5	29.4	70
16	12.2	12.7	57
17	11.0	21.9	68
18	7.91	7.42	40
19	37.1	39.0	104
20	15.8	23.3	67
21	16.6	25.5	42
22	16.0	24.8	93
23	23.3	21.7	69
24	28.2	25.7	70
25	18.7	18.3	117
26	25.6	29.4	81



4. Discussion

4.1. Properties of Biological Material

VH is characterized by a much lower rate of decomposition changes (or increased resistance to autolysis) than blood. However, the described type of material has limitations related to its relatively high viscosity and small volume (up to 2 mL). Because of the above aspects, the samples were diluted to reduce the matrix effect. The first factor influencing measurement uncertainty is interfering ions, which include NH_4^+ , Li^+ , Na^+ , and Ca^{2+} . To reduce the interference, the samples were diluted 80-fold.

It is important to keep the temperature constant during the entire test (in the range of 20–25 °C) because, depending on the temperature, the measurement results may fluctuate. According to the manufacturer, the temperature range of the ISE operation is equal to 0–40 °C. Maintaining the pH of the measuring environment also influences the measurement, and the environment is stable in the range of 1.5–12.00 pH.

VH samples were collected using a metal, pointed (35 × 2 mm) injection needle. The time the samples were collected varied according to the duration of the autopsy. The bodies of the deceased were stored in autopsy refrigerators (4 °C) at various times.

4.2. Measurement Uncertainty by ISE and MIP-OES

In this study, an attempt was made to estimate the uncertainty components (A and B types) related to sample preparation and instrumental analysis using ISE and MIP-OES (Table 10).

Table 10. Summary of the uncertainty budget results of types A (experimental) and B (nonexperimental) for determining potassium ion concentration using ISE and MIP-OES methods.

Concentration of K^+	Expanded Uncertainty (%)			
	ISE		MIP-OES	
	U_A	U_B	U_A	U_B
Low (2 mg/L)	±12.2	±4.75	±1.2	±2.53
Medium (10 mg/L)	±6.1	±4.75	±2.2	±2.53
High (25 mg/L)	±3.1	±4.75	±2.5	±2.53

The estimated values of the expanded uncertainty determined by the experimental method A (U_A) indicate a few to even ten-times lower uncertainty in the determination of potassium ion concentration in the case of the MIP-OES method. Similarly, in the case of estimating the expanded uncertainty with the B (U_B) method, the MIP-OES method is characterized by twice the lower uncertainty. Based on our experience in determining the concentration of potassium ions using the ISE and MIP-OES methods, it is worth considering their advantages and disadvantages before implementing them into diagnostic practice (Table 11).

4.3. Causes of Death

The causes of death that affect the postmortem concentration of potassium ions in VH are hanging, diabetic coma, and electrolyte disturbances. The increased concentration of potassium ions in hanging cases is due to the tightness of the loops in the neck, which causes a rupture of the jugular system and a significant increase in venous pressure. The cause of death strongly influences the change in the concentration of potassium ions after death, which is mainly related to the possibility of blood contamination of the VH. Venous circulation can lead to capillary hyperemia and thus increase vascular leakage from the capillaries into the retina [23,30]. In addition, if metabolic acidosis occurs, hyperkalemia is

expected, which may make it impossible to reliably estimate the PMI from the concentration of potassium ions.

Table 11. Advantages and disadvantages of ion-selective electrode (ISE) and microwave-induced plasma with optical emission spectrometry (MIP-OES) methods.

Method	Advantages	Disadvantages
ISE	<ul style="list-style-type: none"> - High selectivity to potassium ions - Wide pH range in aqueous solutions (from 2.5 to 11) - Wide potassium ion concentration range (from 10^{-6} mol/L to 1 mol/L or from 0.04 mg/L to 39,000 mg/L) - Simple and direct analysis - Low costs of equipment and analysis - Nondestructive measurements - Relatively low volume of samples required 	<ul style="list-style-type: none"> - Limit of detection (LOD) and limit of quantification (LOQ) are not good as other techniques (e.g., optical emission spectrometry) - Problems due to interferences. If the electrode is exposed to high concentrations of interfering ions, drift and long response times may occur. Popular cations such as Cs^+, NH_4^+, Tl^+, H^+, Ag^+, Tris^+ (cation of tris (hydroxymethyl) aminomethane), Li^+, and Na^+ can cause an error of up to 10% for different levels of potassium ion concentration - Temperature changes affect the electrode potential. For this reason, both samples and standard solutions should not differ in temperature by more than ± 1 °C. At a potassium concentration level of 10^{-3} mol/L, a temperature difference of 1 °C causes an error of more than 2.5% - It cannot be used for a long time due to the deterioration of the membrane (the real-life time of the ISE module is about six months). If the membrane is damaged, the potentiometric response becomes extremely sluggish or the slope of the calibration curve decreases significantly (the ISE module should be replaced)
MIP-OES	<ul style="list-style-type: none"> - Despite the selectivity issues, the possibility of performing measurements in several spectral ranges - Possibility of performing analysis in a broader range for alternative wavelengths (from 20 mg/L up to 1000 mg/L for 404.414 nm, 344.738 nm, 404.721 nm, and 693.877 nm) - Low cost of routine analysis - Very simple and automatized analysis - Low volume of samples required (1–3 mL) - Moderately low influence of the temperature on the analysis result - Possibility of performing a wide range of elemental analyses during one measurement 	<ul style="list-style-type: none"> - Narrow range of analysis (from 0.1 mg/L up to 100 mg/L for regularly used wavelengths: 766.491 nm and 769.897 nm) in comparison to other plasma-based methods - Possible occurrence of spectral interferences from other elements (La—766.434 nm and Yb—769.949 for most often used wavelengths) - Moderately high costs of apparatus - Destructive measurement - Limitations connected to the determination of elements with high ionization potentials

Differences were observed in lethal diabetic coma. It is known that, in a diabetic coma, there is a potassium liberation from the cell to the outside due to insulin deficiency and acidosis [21,31].

Any sudden mechanical movement during or after death can introduce the breaking of blood vessels in the eyes and changes in the concentration of potassium ions. The uncertainty of determination related strictly to the ISE and MIP-OES methods is relatively small concerning the other factors influencing the concentration of potassium ions (cause of death, ambient temperature, and cadaver storage conditions). However, based on our studies, the uncertainty of type A (experimental) for low concentrations (2 mg/L) of potassium ions can be as high as 12.2% using the ISE method. Reducing the uncertainty of PMI estimation can be performed by introducing additional indicators that determine



the TOD zystkie surowe dane mogą być udostępnione na prośbę skierowaną do autora korespondencyjnego. (muscle degradation products by means of quantitative MRI).

5. Conclusions

The postmortem concentration of potassium ions in the VH has a limited PMI time range, which usually does not exceed four days, and may be influenced by interfering factors, including analytical factors (e.g., sample viscosity, the necessity of dilution, failures, and nonselectivity of the ISE) and nonanalytical factors (e.g., age, cause of death, and ambient temperature).

To determine the concentration of potassium ions in the VH, the analytical uncertainty of types A and B should be taken into account, as it generally has a significant effect on the estimation of PMI. Both A and B estimates give similar results, which may prove the usefulness of both methods in estimating the measurement uncertainty of the concentration of potassium ions, and thus PMI estimation.

The cause of death also influences the postmortem concentration of potassium ions in the VH. Caution should be exercised if death occurs due to hanging or electrolyte disturbances, as they may significantly affect the measurement results and thus the estimated TOD.

Author Contributions: Conceptualization, S.Z., M.W. and B.M.C.; methodology, S.Z., M.W., B.M.C. and M.K.; validation, S.Z., B.M.C. and M.W.; investigation, S.Z., M.W. and B.M.C.; resources, S.Z. and M.W.; writing—original draft preparation, S.Z., M.W., B.M.C., J.S.A. and M.K.; writing—review and editing, S.Z., M.W., B.M.C., J.S.A. and M.K.; supervision, M.W. and J.S.A. All authors have read and agreed to the published version of the manuscript.

Funding: The publication of the article was supported by the project POWR.03.02.00-00-I014/17-00 co-financed by the European Union through the European Social Fund under the Operational Programme Knowledge Education Development 2014–2020.

Informed Consent Statement: Patient consent was waived due to the consent of the bioethics committee of the Medical University of Gdańsk, dated 28 February 2020 (NKBBN/18/2020).

Data Availability Statement: All research data can be made available upon request send to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Henße, C.; Madea, B. Estimation of the time since death in the early post-mortem period. *Forensic Sci. Int.* **2004**, *144*, 167–175. [[CrossRef](#)]
2. Mathur, A.; Agrawal, Y.K. An overview of methods used for estimation of time since death. *Aust. J. Forensic Sci.* **2011**, *43*, 275–285. [[CrossRef](#)]
3. Maiese, A.; Scatena, A.; Costantino, A.; Di Paolo, M.; La Russa, R.; Turillazzi, E.; Frati, P.; Fineschi, V. MicroRNAs as useful tools to estimate time since death. A systematic review of current literature. *Diagnostics* **2021**, *11*, 64. [[CrossRef](#)] [[PubMed](#)]
4. Madea, B. Methods for determining time of death. *Forensic Sci. Med. Pathol.* **2016**, *12*, 451–485. [[CrossRef](#)] [[PubMed](#)]
5. Markowska, J.; Szopa, M.; Zawadzki, M.; Piekoszewski, W. Ciało szkliste oka—Rutynowy czy alternatywny materiał do badań w medycynie sądowej. *Arch. Med. Sądowej Kryminol.* **2017**, *67*, 201–213. [[CrossRef](#)] [[PubMed](#)]
6. Madea, B. Is there recent progress in the estimation of the postmortem interval by means of thanatochemistry? *Forensic Sci. Int.* **2005**, *151*, 139–149. [[CrossRef](#)]
7. McCleskey, B.C.; Dye, D.W.; Davis, G.G. Review of postmortem interval estimation using vitreous humor: Past, present, and future. *Acad. Forensic Pathol.* **2016**, *6*, 12–18. [[CrossRef](#)]
8. Adelson, L.; Sunshine, I.; Rushforth, N.B.; Mankoff, M. Vitreous potassium concentration as an indicator of the postmortem interval. *J. Forensic Sci.* **1963**, *8*, 503–514.
9. Coe, J.I. Vitreous potassium as a measure of the postmortem interval: An historical review and critical evaluation. *Forensic Sci. Int.* **1989**, *42*, 201–213. [[CrossRef](#)]
10. Ross, J.; Hoadley, P.; Sampson, B. Determination of postmortem interval by sampling vitreous humour. *Am. J. Forensic Med. Pathol.* **1997**, *18*, 158–162.
11. Barús, J.I.M.; Suárez-Peñaranda, J.M.; Otero, X.L.; Rodríguez-Calvo, M.S.; Costas, E.; Miguéns, X.; Concheiro, L. Improved estimation of postmortem interval based on differential behaviour of vitreous potassium and hypoxantine in death by hanging. *Forensic Sci. Int.* **2002**, *125*, 67–74. [[CrossRef](#)] [[PubMed](#)]

12. Zhou, B.; Zhang, L.; Zhang, G.; Zhang, X.; Jiang, X. The determination of potassium concentration in vitreous humor by low pressure ion chromatography and its application in the estimation of postmortem interval. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2007**, *852*, 278–281. [[CrossRef](#)] [[PubMed](#)]
13. Jashnani, K.D.; Kale, S.A.; Rupani, A.B. Vitreous humor: Biochemical constituents in estimation of postmortem interval. *J. Forensic Sci.* **2010**, *55*, 1523–1527. [[CrossRef](#)] [[PubMed](#)]
14. Bortolotti, F.; Pascali, J.P.; Davis, G.G.; Smith, F.P.; Brissie, R.M.; Tagliaro, F. Study of vitreous potassium correlation with time since death in the postmortem range from 2 to 110 hours using capillary ion analysis. *Med. Sci. Law* **2011**, *51* (Suppl. S1), 20–23. [[CrossRef](#)] [[PubMed](#)]
15. Mihailovic, Z.; Atanasijevic, T.; Popovic, V.; Milosevic, M.B.; Sperhake, J.P. Estimation of the postmortem interval by analyzing potassium in the vitreous humor: Could repetitive sampling enhance accuracy? *Am. J. Forensic Med. Pathol.* **2012**, *33*, 400–403. [[CrossRef](#)]
16. Siddhamsetty, A.K.; Verma, S.K.; Kohli, A.; Verma, A.; Puri, D.; Singh, A. Exploring time of death from potassium, sodium, chloride, glucose & calcium analysis of postmortem synovial fluid in semi arid climate. *J. Forensic Leg. Med.* **2014**, *28*, 11–14. [[CrossRef](#)] [[PubMed](#)]
17. Bohra, B.; Verma, R.; Mathur, I.B.; Sharma, Y.; Khangwal, V.P. Estimation of postmortem interval by measuring potassium level in vitreous humor. *J. Indian Acad. Forensic Med.* **2014**, *36*, 374–378.
18. Foster, S.N.; Smith, P.R.; Biggs, M.; Ruddy, G.N.; Hollingbury, F.E.; Morley, S.R. Estimation of postmortem interval using vitreous potassium levels in cases of fatal road traffic collision. *Arch. Med. Sadowej Kryminol.* **2016**, *66*, 71–82. [[CrossRef](#)]
19. Murthy, A.S.; Das, S.; Thazhath, H.K.; Chaudhari, V.A.; Adole, P.S. The effect of cold chamber temperature on the cadaver's electrolyte changes in vitreous humor and plasma. *J. Forensic Leg. Med.* **2019**, *62*, 87–91. [[CrossRef](#)]
20. Focardi, M.; Lanzilao, L.; Bonari, A.; Lazzeretti, M.; Lorubbio, M.; Ognibene, A.; Gualco, B.; Fanelli, A.; Pinchi, V. Improvement in estimation of time since death by albumin and potassium concentrations in vitreous humor. *Forensic Sci. Int.* **2020**, *314*, 110393. [[CrossRef](#)] [[PubMed](#)]
21. Zilg, B.; Bernard, S.; Alkass, K.; Berg, S.; Druid, H. A new model for the estimation of time of death from vitreous potassium levels corrected for age and temperature. *Forensic Sci. Int.* **2015**, *254*, 158–166. [[CrossRef](#)]
22. Rognum, T.O.; Hauge, S.; Øyasaeter, S.; Saugstad, O.D. A new biochemical method for estimation of postmortem time. *Forensic Sci. Int.* **1991**, *51*, 139–146. [[CrossRef](#)] [[PubMed](#)]
23. Chandrakanth, H.V.; Kanchan, T.; Balaraj, B.M.; Virupaksha, H.S.; Chandrashekar, T.N. Postmortem vitreous chemistry—An evaluation of sodium, potassium and chloride levels in estimation of time since death (during the first 36 h after death). *J. Forensic Leg. Med.* **2013**, *20*, 211–216. [[CrossRef](#)]
24. Cordeiro, C.; Ordóñez-Mayán, L.; Lendoiro, E.; Febrero-Bande, M.; Vieira, D.N.; Muñoz-Barús, J.I. A reliable method for estimating the postmortem interval from the biochemistry of the vitreous humor, temperature and body weight. *Forensic Sci. Int.* **2019**, *295*, 157–168. [[CrossRef](#)] [[PubMed](#)]
25. Hansson, L.; Uotila, U.; Lindfors, R. Potassium content of the vitreous body as an aid in determining the time of death. *J. Forensic Sci.* **1966**, *11*, 390–394.
26. Moriya, F.; Hashimoto, Y. Endogenous γ -hydroxybutyric acid levels in postmortem blood. *Leg. Med.* **2004**, *6*, 47–51. [[CrossRef](#)] [[PubMed](#)]
27. Yang, M.; Li, H.; Yang, T.; Ding, Z.; Wu, S.; Qiu, X.; Liu, Q. A study on the estimation of postmortem interval based on environmental temperature and concentrations of substance in vitreous humor. *J. Forensic Sci.* **2018**, *63*, 745–751. [[CrossRef](#)]
28. Stephen, R.J. Vitreous humor chemistry. The use of potassium concentration for the prediction of postmortem interval. *J. Forensic Sci.* **1987**, *32*, 503–509. [[CrossRef](#)]
29. Eppendorf.com. Eppendorf Research Instruction Manual. 2016. Available online: <http://www.sun-way.com.tw/Files/DownloadFile/Researchplus.pdf> (accessed on 28 February 2016).
30. Hanna Instruments. Instruction Manual Potassium Ion Selective Electrode HI 4114, MAN4114 07/06 REV3. 2007. Available online: <https://www.hannainst.com/hi4114-potassium-combination-ion-selective-electrode.html> (accessed on 23 November 2022).
31. Kitabchi, A.E. Hyperglycemic crises in diabetes. *Diabetes Care* **2004**, *27* (Suppl. S1), s94–s102. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.