

# Vitreous Humor in Forensic Toxicology: a Literature Review

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## ABSTRACT

**Introduction:** the vitreous humor (VH) is a semi-liquid matrix composed of collagens, hyaluronic acids, water and electrolytes that make up the interior of the eyeball, behind the light-catching structures, and has a structural and physiological function for eye homeostasis. Being irrigated by the blood flow, it is capable of receiving exogenous substances of forensic interest, such as licit or illicit drugs, thus being an important matrix in detection in postmortem forensic toxicology.

In this sense, we address scientific articles available on PubMed NCBI in English and open access published in the last 5 full years, in order to obtain an overview of how this matrix has been studied in the toxicological forensic field. 12 valid articles were found for this review, out of a total of 64 articles published in the period, with 4 being classified as literature reviews, 4 case reports, 2 descriptive and retrospective observational studies and 2 experimental research on the development of an analytical method. The other articles were also analyzed, but through access via the institutional network of the University of São Paulo (USP).

The results indicate a small number of articles available in open access in the last 5 years (mostly bibliographic reviews and case reports) but with the use of VH in the detection of other intoxicating molecules and also biomarkers of interest, such as illicit drugs. Articles with university institutional access indicated a similar classification pattern, 18 being experimental research on the development of an analytical method and 15 being case reports.

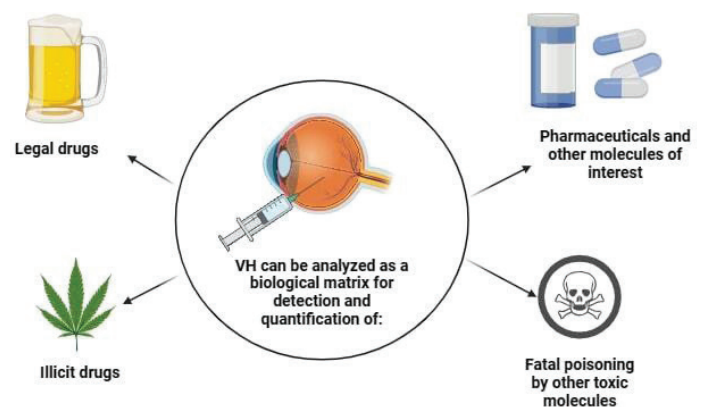
**Keywords:** Vitreous mood; Forensic toxicology; Review; Open access.

## Introduction

The study of toxic substances in forensic sciences is defined as Forensic Toxicology, having several subfields according to: the type of sample (biological or non-biological), the forensic field covered and the instrumental methods used. These substances are investigated to: determine which molecule is present in the analyzed matrix, identify the type of molecule, its classification in toxicology, and finally its quantification, which is the measurement of how much of this substance is found in the sample <sup>1</sup>.

As samples are classified as biological or non-biological by the expert service, in the forensic approach, the focus on the person is imperative. Therefore, not only the analytical approach in the type of sample must be highlighted, but also in which forensic area the results must be interpreted, such as the use of postmortem vitreous humor (VH) in estimating the postmortem interval (PMI) or in forensic toxicology<sup>2,3</sup>.

This semi-liquid substance that fills the eyeball, allowing the passage of light, contributes to the hydration, nutrition and support of the eye <sup>4</sup>. Composed mainly of collagen fibers, hyaluronic acid, water and electrolytes, the VH within the structure of the eyes, allows the its use as a biological matrix in forensic toxicology in the detection of several molecules of interest, shown in Figure 1.



**Figure 1.** Applications of vitreous humor in forensic toxicology. Created with Biorender.com.

For example, a toxicological examination of the peripheral blood of an individual approached on suspicion of drunkenness carried out by the police biochemical laboratory is a procedure that responds to these three areas: the peripheral blood is a biological sample, the biochemical test for measuring the alcohol percentage is the technique used and legal medicine is the field of forensic sciences. This progression begins from a sample processing chain, in the case of VH illustrated by Figure 2. Even in this example, the effect of contextual information can also impact the forensic toxicology methodology as well as in other forensic scenarios<sup>3</sup>.

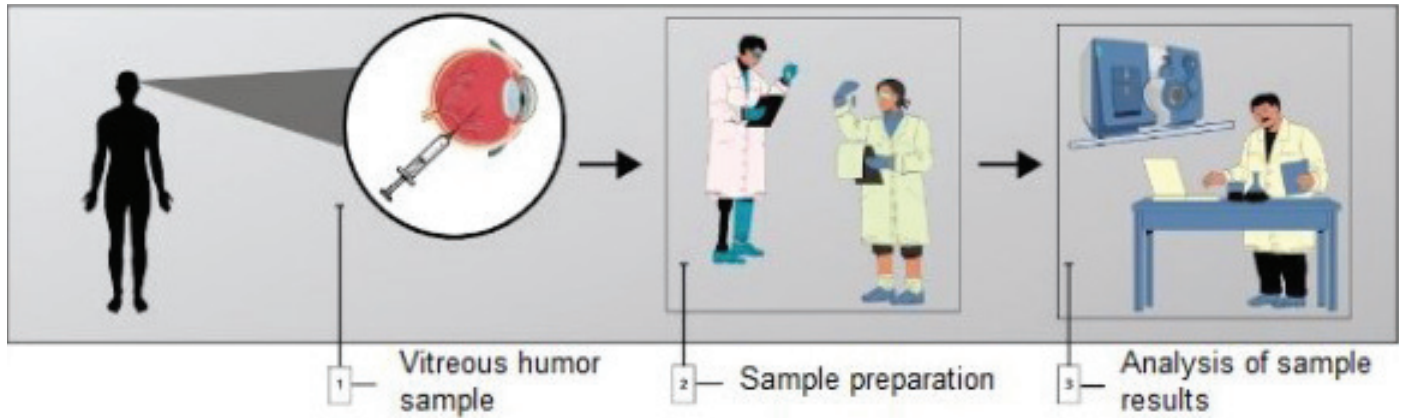


Figure 2. Vitreous humor processing chain as a biological matrix. Created with Biorender.com.

Since different biological matrices are used for forensic toxicological analysis, those different from conventional ones, usually peripheral blood, such as vitreous humor, are little studied. Therefore, this literature review article proposes to address how the vitreous humor in human forensic toxicology is currently studied by different research groups around the world, in order to obtain an overview of how the three aspects of each forensic situation are responded to through of scientific articles indexed in PubMed NCBI.

**Material and Methods**

The keywords “forensic toxicology vitreous humor” were used in the search field of scientific article repositories indexed in PubMed NCBI in the last five full years (September 2018 to September 2023). Articles on patents, clinical trials, theses, dissertations and book chapters on the topic were not analyzed in this review, only scientific review articles, experimental research, descriptive observational research and case reports available with open access in English in international journals were revised. Only works published within the scope of human forensic toxicology were analyzed.

In this sense, the forensic fields of veterinary medicine or zootechnics have not been studied. In the search for defined terms, 64 scientific articles published in international journals from 2018 to 2023 were found, including literature reviews, case reports and experimental research.

Of these, only 12 are open access. The others vary in their reading availability between just the open summary or complete introduction with objectives, methodology, results, discussion and conclusions partially available for reading. We list the title, first author, year of publication, magazine and main idea of each article.

Thus, using the university institutional network to access the full text of the other articles, we analyzed within the same scope 50 articles divided according to their category (review, case report, retrospective and/or descriptive study, experimental optimization of analytical detection method). Two of them did not fall into these categories and were therefore not reviewed (article comment and position paper). The pros and cons of each article, based on its main idea, were also listed. The methodology used in this review is outlined in Figure 3.

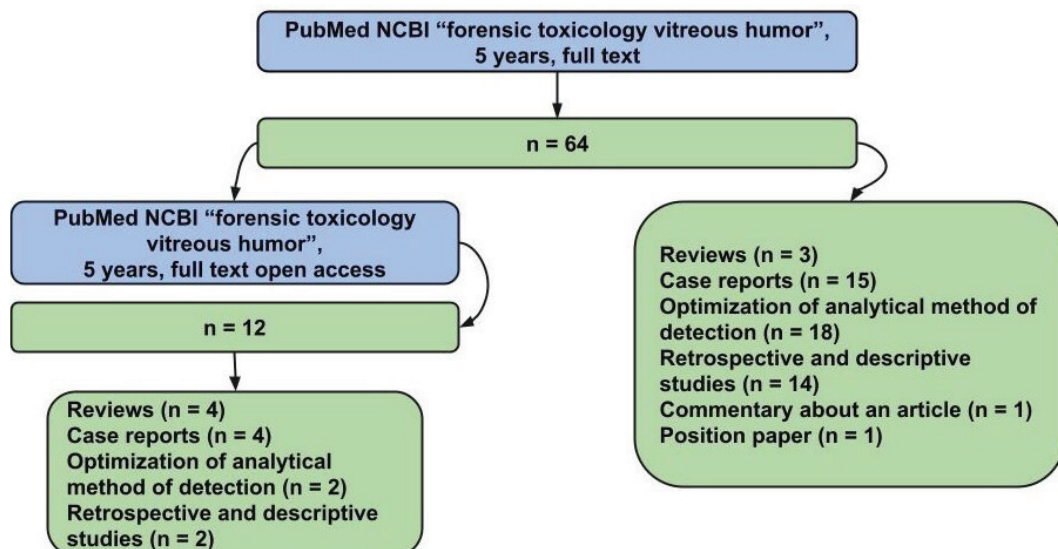


Figure 3. Methodology used.

## Results

Given the small number of articles with open access, we included the remaining articles found that dealt with VH as a biological matrix in forensic toxicology, listing them according to the pros and cons of their main idea, including the 12 articles

with open access. The pros and cons of this idea are linked to the type of article, namely: literature review, case report, descriptive and/or retrospective observational studies and experimental research to develop an analytical method for VH as a biological matrix, listed in Table 1.

**Table 1.** Pros and cons of the reviewed articles.

Title	Authors and year	Pros	Cons
Acylcarnitines in Ophthalmology: Promising Emerging Biomarkers	Konstantinos Theodoridis <i>et al</i> <sup>5</sup> . 2022	Carnitines and their metabolites are promising molecules as biomarkers in ophthalmological diseases, mainly because they are multifunctional.	There is a lack of more specific literature for each disease regarding the identification and quantification of this molecule, although the vitreous humor is an important matrix for its detection.
Alternative matrices in forensic toxicology: a critical review	Eduardo Geraldo de Campos <i>et al</i> <sup>6</sup> . 2022	Alternative biological matrices are important for forensic toxicology, as one or more biological samples may be too degraded for collection.	It is necessary to choose the best biological matrix, since the molecule of interest may be more widespread in one sample than another, with the vitreous humor being no different, especially new psychoactive substances (NPS)
Forensic biomarkers of lethal traumatic brain injury	Johann Zwirner <i>et al</i> <sup>7</sup> . 2022	17 articles were found that deal with biomarkers of traumatic brain injury (TBI), among the molecules of interest, LDH was identified in the vitreous humor in this scope	The vitreous humor as a biological matrix showed no statistically relevant difference in LDH level, survival time and microtubule-associated protein TAU (molecule of interest in TBI)
A Rapid Method for Postmortem Vitreous Chemistry-Deadside Analysis	Brita Zilg <i>et al</i> <sup>8</sup> . 2021	Casuistry of 50 cases and collection from different regions of the eyeball, something different in the collection of this biological matrix. The use of a blood gas analyzer was also a differentiator, since LC-MS/MS is the most common method in forensic toxicology	No difference was found between the concentrations of metabolites of interest in the left and right eyeballs and also in the vitreous humor collection region.
Frequency of postmortem ethanol formation in blood, urine and vitreous humor - Improving diagnostic accuracy with the use of ethylsulphate and putrefactive alcohols	Katja Oshaug <i>et al</i> <sup>9</sup> . 2022	Casuistry of 2504 cases for Ethylsulfate (EtS) and 8001 cases for putrefied alcohols (PA), with detection being made in samples of blood, urine and vitreous humor. These molecules are important biomarkers for fatal ethanol poisoning	The vitreous humor as a biological matrix for these molecules of interest showed lower detection than peripheral blood, making it necessary to optimize the analytical method used.
Fatal poisoning by ingestion of a self-prepared oleander leaf infusion	Anna Carfora <i>et al</i> <sup>10</sup> . 2021	Case report of the detection of toxic substances extracted from oleander leaves, using LC-MS/MS in post-mortem samples. The vitreous humor showed quantifiable levels of oleandrin	No odoroside and neritaloside were found in the vitreous humor and blood, but these molecules were found in urine, liver, stomach contents and in the infusion itself, indicating the need to optimize the analytical method
Ethanol Determination in Post-Mortem Samples: Correlation between Blood and Vitreous Humor Concentration	Fabio Savini <i>et al</i> <sup>11</sup> . 2020	Validation of a new and rapid analytical method for ethanol detection in post-mortem biological matrices using gas chromatography-ionized flame detector (GC-FID) in 31 samples	Only two matrices were used, blood and vitreous humor, although other matrices are also collected in forensic toxicology, mainly urine.
Interpreting $\gamma$ -hydroxybutyrate concentrations for clinical and forensic purposes	Francesco Paolo Busardò <i>et al</i> <sup>12</sup> . 2019	Bibliographic review on the detection of gamma-hydroxybutyrate (GHB) in biological matrices, with GHB being identified in the vitreous humor in all articles reviewed	Low casuistry of articles reviewed on the vitreous humor
Findings of the neuromuscular blocking agent rocuronium in blood from deceased subjects several months after exposure: A report of two cases	Joachim Frost <i>et al</i> <sup>13</sup> . 2023	Report of two cases of necropsies with detection of rocuronium (muscle analgesic) and other substances (such as ethanol, tetrahydrocannabinol, fentanyl) in biological matrices (blood, urine and vitreous humor)	Compared to blood as the matrix in these reports, the vitreous humor presented lower levels of the molecule of interest, while the other substances analyzed were not identified.

A fatal case of poisoning with a cathinone derivative: $\alpha$ -PiHP and its postmortem distribution in body fluids and organ tissues	Paulina Wachholz <i>et al</i> <sup>14</sup> . 2023	Case report of fatal poisoning by $\alpha$ -PiHP, an illicit drug of the cathinone class, with the detection of this substance in samples of vitreous humor, blood, cerebrospinal fluid and organs	The detection of other illicit substances of interest was not carried out, as this was an addict, other drugs could have been evaluated in the same matrices
Characteristics of post-mortem beta-hydroxybutyrate-positive cases - A retrospective study on age, sex and BMI in 1407 forensic autopsies	Stina Ahlström <i>et al</i> <sup>15</sup> . 2021	Retrospective study with a series of 1407 necropsies associating the identification and quantification of BHB with clinical factors such as alcoholism, body mass index and age, identifying a relationship between BHB and age and gender	Vitreous glucose collected showed high levels of BHB in all groups, requiring comparison with the blood matrix, inferring that perhaps for this cohort, it is not the best matrix for a single analysis
Putatively lethal ingestion of isopropyl alcohol-related case: interpretation of postmortem isopropyl alcohol and acetone concentrations remains challenging	Carine Dumollard <i>et al</i> <sup>16</sup> . 2021	Case report of a suicide by ingestion of isopropyl alcohol, with its detection being made in samples of vitreous humor, urine, bile, gastric contents and cardiac blood	The samples were collected 5 days after the identification of death, therefore peripheral blood could not be collected due to clotting and only isopropyl alcohol was identified, with other substances not being identified (such as other drugs of abuse)
The Impact of Donor Preparation for Tissue Procurement on Postmortem Vitreous Isopropanol Concentration	Sarah E Avedschmidt <i>et al</i> <sup>17</sup> . 2023 It is not open access (full text only on the university institutional network)	Retrospective study of 50 cases of necropsies for donation for collection of vitreous humor with positive detection of isopropanol in 8 cases	This study was considered Not Regulated according to the institutional review board
A Fatal Case Report of Barium Chloride Toxicity	Caroline S Copeland <i>et al</i> <sup>18</sup> . 2023 It is not open access (full text only on the university institutional network)	Case report of fatal barium chloride poisoning, detected in the vitreous humor and femoral blood	The vitreous humor had a lower concentration of barium chloride compared to blood
Effects of postmortem interval, putrefaction, diabetes, and location of death on the analysis of ethyl glucuronide and ethyl sulfate as ethanol biomarkers of antemortem alcohol consumption	Ahmed I Al-Asmari <i>et al</i> <sup>19</sup> . 2022 It is not open access (full text only on the university institutional network)	Detection of biomarkers for alcohol intake in 52 necropsy cases in biological matrices (blood, urine, vitreous humor)	The study had limitations with the type of sample collected per necropsy case, with some cases being possible to collect blood and vitreous humor but not urine.
Homicidal paraquat poisoning: Poisoned while drinking	Zihao Liu <i>et al</i> <sup>20</sup> . 2022 It is not open access (full text only on the university institutional network)	Case report of detection of paraquat in several post-mortem samples, including vitreous humor, in a homicide caused by ingestion of this substance with wine	The vitreous humor presented a low concentration of paraquat compared to other tissues, indicating low accumulation of this substance in this matrix.
Sodium nitrite detection in costal cartilage and vitreous humor - Case report of fatal poisoning with sodium nitrite	Marcin Tomsia <i>et al</i> <sup>21</sup> . 2021 It is not open access (full text only on the university institutional network)	Case report of suicide due to fatal poisoning with sodium nitrate, detected in samples of vitreous humor, urine, blood, liver, kidneys, stomach contents and cartilage	Blood and urine samples showed lower levels of sodium nitrate compared to the vitreous humor as it was already being metabolized
Methamphetamine-related postmortem cases in Jeddah, Saudi Arabia	Ahmed I Al-Asmari <sup>22</sup> . 2021 It is not open access (full text only on the university institutional network)	Detection of methamphetamine and amphetamine in biological samples, including blood, urine, bile, stomach contents and vitreous humor in 47 necropsy cases	Of the biological matrices used, the vitreous humor had a lower average detection rate than urine, stomach contents and bile
Analysis of Diglycolic Acid after Mass Poisoning by Diethylene Glycol	Cristiano OL Goulart <i>et al</i> <sup>23</sup> . 2022 It is not open access (full text only on the university institutional network)	Detection of diethylene glycol (DEG) in biological samples due to accidental contamination of this substance in beer in Minas Gerais, Brazil in 39 cases	Only 9 cases of the total series had vitreous humor collected, due to the need for postmortem collection, with 5 cases negative for DEG

Ethyl glucuronide and ethanol concentrations in femoral blood, urine and vitreous humor from 117 autopsy cases	Jasna Neumann <i>et al</i> <sup>24</sup> . 2021 It is not open access (full text only on the university institutional network)	Detection of Ethyl glucuronide (EtG) and ethanol in 117 necropsy cases using biological samples, with the vitreous humor matrix showing more positivity for EtG than urine and blood	Another metabolite of ethanol, ethyl sulfate (EtS), was quantified only in urine and not in the vitreous humor and the decomposition time of each body was not measured at the time of necropsy.
Study of the distribution of antidepressant drugs in vitreous humor using a validated GC/MS method	Panagoula-Stamatina A Ntoupa <i>et al</i> <sup>25</sup> . 2020 It is not open access (full text only on the university institutional network)	Development and optimization of a protocol for positive detection of several antidepressants in the vitreous humor of 43 blood necropsy cases positive for these substances	Due to the wide spectrum of maximum and minimum detection values in the vitreous humor and blood, the authors indicated that it cannot be determined whether the drugs were ingested days or weeks before death or months before
Vitreous humor endogenous compounds analysis for post-mortem forensic investigation	Nicola Pigaiani <i>et al</i> <sup>26</sup> . 2020 It is not open access (full text only on the university institutional network)	Bibliographic review of 210 articles on the use of vitreous humor in the detection of endogenous components for forensic applications such as postmortem interval determination (PMI)	The literature survey indicated poorly validated analyzes with vitreous humor, with the majority of reported applications related to clinical detection using different methodologies.
Acidic Drug Concentrations in Postmortem Vitreous Humor and Peripheral Blood	Jacqueline A Hubbard <i>et al</i> <sup>27</sup> . 2021 It is not open access (full text only on the university institutional network)	Detection of 7 acidic drugs in 89 necropsies in biological samples, including in regions other than the vitreous humor	Although it was detected in all cases, the concentration of drugs in the vitreous humor was lower compared to the blood analyzed from the cases.
Distribution of synthetic opioids in postmortem blood, vitreous humor and brain	Rachel Chesser <i>et al</i> <sup>28</sup> . 2019 It is not open access (full text only on the university institutional network)	Development of a detection method for 15 opioid drugs in the vitreous humor, blood and brain in 58 cases	Vitreous humor presented a lower drug quantification than blood and brain tissue
Drug concentrations in post-mortem specimens	Raimo A Ketola <i>et al</i> <sup>29</sup> . 2019 It is not open access (full text only on the university institutional network)	Meta-analysis of 388 studies with drug detection in biological samples, including vitreous humor	According to the results of the meta-analysis, the vitreous humor is the 3rd matrix of choice for toxicological analysis of drugs of interest
The Stability of 4-Chloromethcathinone in Blood and Vitreous Humor	Karolina Nowak <i>et al</i> <sup>30</sup> . 2020 It is not open access (full text only on the university institutional network)	Detection of the stability of 4-chloromethcathinone (4-CMC) in blood and vitreous humor from a necropsy	The antemortem history of the case was not analyzed, and samples were collected two days after death
Is insulin intoxication still the perfect crime? Analysis and interpretation of postmortem insulin: review and perspectives in forensic toxicology	Charline Bottinelli <i>et al</i> <sup>31</sup> . 2020 It is not open access (full text only on the university institutional network)	Review of 173 articles in the literature on suspected cases of insulin poisoning detected in biological samples, including vitreous humor	Insulin detection in non-diabetic patients using the vitreous humor is more difficult compared to diabetic patients
The effect of cold chamber temperature on the cadaver's electrolyte changes in vitreous mood and plasma	Abilash Srinivasa Murthy <i>et al</i> <sup>32</sup> . 2019 It is not open access (full text only on the university institutional network)	Detection of electrolytes in the vitreous humor and plasma of 100 cadavers at low temperatures in order to identify changes in quantification, with a significant change in potassium concentration in the vitreous humor	The analytes of interest were not quantified at the time of death confirmation. The ambient temperature was also not considered at the time of this confirmation and the first collection of the vitreous humor.
Unpredictable Behavior Under the Influence of "Magic Mushrooms": A Case Report and Review of the Literature	Emma Honyiglo <i>et al</i> <sup>33</sup> . 2019 It is not open access (full text only on the university institutional network)	Case report of the detection of psilocin in the vitreous humor and other samples in death induced by ingestion of hallucinogenic mushrooms and review of the literature on the dangers of using hallucinogenic mushrooms	The vitreous humor presented the lowest concentration of psilocin among the matrices analyzed (blood, urine, bile)
Postmortem Fluid Concentrations of Heroin Biomarkers and Their Metabolites	Ahmed I Al-Asmari <sup>34</sup> . 2020 It is not open access (full text only on the university institutional network)	Detection of 6-monoacetylmorphine (6-MAM), 6-acetylcodeine (6-AC), and metabolites of morphine and codeine in 20 necropsy cases from analysis of blood, bile, urine, gastric contents, and vitreous humor	Positive detection was identified in 17 of 20 cases in the vitreous humor matrix, being the second with the lowest concentration among those studied in this article

Application of TDA AAS to Direct Mercury Determination in Postmortem Material in Forensic Toxicology Examinations	Teresa Lech <i>et al</i> <sup>35</sup> . 2019 It is not open access (full text only on the university institutional network)	Detection of mercury by thermal decomposition, amalgamation and atomic absorption spectrometry (TDA AAS) in biological samples, with 13 cases being positive in the vitreous humor	Of the matrices studied (bile, blood, hair, urine and vitreous humor), the second lowest concentration of mercury was in the vitreous humor
Method for Postmortem Quantification of $\Delta$ 9-Tetrahydrocannabinol and Metabolites Using LC-MS-MS	Ahmed I Al-Asmari <sup>36</sup> . 2019 It is not open access (full text only on the university institutional network)	Detection of $\Delta$ 9-tetrahydrocannabinol (THC), 11-hydroxy- $\Delta$ 9-THC (THC-OH) and 11-nor- $\Delta$ 9-THC-9-carboxylic acid (THC-COOH) in biological samples, including vitreous humor, in 31 cases of necropsies	The vitreous humor showed positive detection of THC and THC-OH, with the majority of samples not presenting quantifiable values, thus being the least positive matrix of those analyzed.
A Case Series of Etizolam in Opioid-Related Deaths	Jirair Gevorkyan <i>et al</i> <sup>37</sup> . 2021 It is not open access (full text only on the university institutional network)	Detection of etizolam and opioids in urine, blood and vitreous humor in 10 necropsy cases	The vitreous humor had the lowest concentration of etizolam compared to the other samples
The distribution and redistribution of carfentanil in postmortem samples	CN Chatterton <i>et al</i> <sup>38</sup> . 2020 It is not open access (full text only on the university institutional network)	Review of 290 case reports of necropsies with detection of carfentanil in blood, urine and vitreous humor	Positive cases of carfentanil in urine and vitreous humor are mostly not positive in blood. This limits the use of these matrices in the analysis of this substance
A Gas Chromatography-Mass Spectrometry Method for Toxicological Analysis of MDA, MDEA and MDMA in Vitreous Humor Samples from Victims of Car Accidents	Aline Akemi Ishikawa <i>et al</i> <sup>39</sup> . 2018 It is not open access (full text only on the university institutional network)	Detection of ,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyethylamphetamine in vitreous humor from 5 necropsy cases from fatal car accident	Only MDMA was identified in all cases in the vitreous humor
Fatal intoxication with 1,4-butanediol: Case report and comprehensive review of the literature	Victoria Fischer <i>et al</i> <sup>40</sup> . 2023 It is not open access (full text only on the university institutional network)	Case report of fatal 1,4-butanediol poisoning. Investigation of chemical accumulation in different parts of the organism and in different samples. The vitreous humor was the one with the 2nd highest concentration of the drug in the body, second only to urine	The article does not inform the time of death until the necropsy and sample collection, only inferring that it was "fresh" and without signs of putrefaction
Postmortem tissue distribution of morphine and its metabolites in a series of heroin-related deaths	Peter D Maskell, <i>et al</i> <sup>41</sup> . 2019 It is not open access (full text only on the university institutional network)	Detection of morphine (M), morphine-3-glucuronide (M3G), and morphine-6-glucuronide (M6G) in 44 necropsies in biological samples, including vitreous humor	The correlation between the presence of morphine and its metabolites varied between samples from the same case, especially in femoral blood
Fatal intoxication with ivabradine: First case report	Knapp-Gisclon <i>et al</i> <sup>42</sup> . 2020 It is not open access (full text only on the university institutional network)	Report of a fatal case due to Ivabradine poisoning. Quantification of Ivabradine was performed in different organs and samples, including vitreous humor	The vitreous humor presented the second lowest concentration of Ivabradine among the matrices in this case (blood, lung, kidney, liver, heart and brain)
Post-mortem toxicology in violent fatalities in Cape Town, South Africa: A preliminary investigation	Marie Belle Kathrina Mendoza Auckloo, <i>et al</i> <sup>43</sup> . 2019 It is not open access (full text only on the university institutional network)	Detection of alcohol and other drugs in biological samples, including vitreous humor in a cohort of 104 autopsies resulting from violent criminal deaths	The sample collection strategy in the studied cohort did not standardize the type of matrix studied, varying between cases
Validation of a Fully Automated Immunoaffinity Workflow for the Detection and Quantification of Insulin Analogs by LC-MS-MS in Postmortem Vitreous Humor	Kevin M Legg <i>et al</i> <sup>44</sup> . 2019 It is not open access (full text only on the university institutional network)	Development of a method to identify different brands of exogenous insulins in the vitreous humor	Exogenous insulin is highly unstable in any standard condition, even in the vitreous humor, influencing detection and quantification by LC-MS/MS
Gabapentin-related Deaths: Patterns of Abuse and Postmortem Levels	Amy M. Tharp <i>et al</i> <sup>45</sup> . 2019 It is not open access (full text only on the university institutional network)	Survey of 104 cases of necropsies related to the detection of gabapentin in biological samples, including vitreous humor	Gabapentin tests are infrequent and only requested by court order or by the pathologist

Critical Analysis of Laboratory Testing Methodologies When Interpreting Conflicting Results at Autopsy	Kimberly Margaret Johnson <i>et al</i> <sup>46</sup> . 2021 It is not open access (full text only on the university institutional network)	Case report of conflicting data on the detection of alcohol in different biological samples, including vitreous humor, based on enzymatic assay	The enzymatic assay in vitreous humor is not recommended and is poorly standardized due to degradation of the medium
Quantification of methadone and its metabolites: EDDP and EMDP determined in autopsy cases using LC-MS/MS	Karolina Nowak, <i>et al</i> <sup>47</sup> . 2021 It is not open access (full text only on the university institutional network)	Experimental study to determine the amount of methadone in 18 postmortem cases using blood, urine and vitreous humor samples	Only 3 cases were positive for methadone in the vitreous humor, with a lower concentration than in other matrices
Cannabinoid Hyperemesis Syndrome: Reports of Fatal Cases	Mahra Nourbakhsh <i>et al</i> <sup>48</sup> . 2019 It is not open access (full text only on the university institutional network)	Report of 3 cases of necropsies for detection of THC in the blood of 2, characterizing cannabinoid hyperemesis syndrome (CHS). Vitreous humor was also sampled	The THC administration route was not recorded and identified in the 3 cases
A fatal case of poisoning of a 19-year-old after taking 3-MMC	Joanna Margasińska-Olejak <i>et al</i> <sup>49</sup> . 2019 It is not open access (full text only on the university institutional network)	Report of a fatal case due to the use of 3-CMC. The presence of the substance was analyzed in samples of blood, gastric contents or urine and vitreous humor, with the substance showing higher concentrations in VH.	The autopsy was only completed 2 days after death, there is no mention of when the samples were collected and sent for analysis. The highest concentration of the substance was noted in the blood sample, with the vitreous humor in second place. It is not standardized in the writing whether the third matrix analyzed was urine or gastric contents.
Development and Validation of an Analytical Method for Volatiles with Endogenous Production in Putrefaction and Submersion Situations	Mélanie Pinto <i>et al</i> <sup>50</sup> . 2021 It is not open access (full text only on the university institutional network)	Experimental study. Development and validation of a method for analyzing the presence of volatile substances (during the putrefaction period) in different samples of blood, vitreous humor and urine.	VH showed the lowest concentration among the samples. There is no mention of when the samples were collected.
Fatal intoxication involving 4-methylpentadone (4-MPD) in a context of chemsex	Nathalie Cartiser <i>et al</i> <sup>51</sup> . 2021 It is not open access (full text only on the university institutional network)	Report of a fatal case due to the use of 4-CMC and its presence in samples of peripheral blood, cardiac blood, urine, bile and vitreous humor from both eyes. Sample collection time was described in the study.	VH presented the lowest concentration of the substance among the samples tested. Furthermore, there was a difference in concentration between the right and left eyes.
Oral Cavity Fluid as an Investigative Approach for Qualitative and Quantitative Evaluations of Drugs in Postmortem Subjects	Amy J Reisinger <i>et al</i> <sup>52</sup> . 2019 It is not open access (full text only on the university institutional network)	Experimental study. It proposes the use of fluids from the oral cavity in the context of forensic toxicology to identify legal and illicit substances. The results were compared between samples of blood, urine, bile, VH and/or liver tissue. The PM sample collection time was described	Despite highlighting the importance of VH analysis, the study did not do so for unspecified reasons.
Ethanol levels in legally autopsied subjects (2016-2017): Update of data and epidemiological implications in relation to violent deaths in Canary Islands (Spain)	M Almeida-González <i>et al</i> <sup>53</sup> . 2019 It is not open access (full text only on the university institutional network)	Retrospective study that identified the causes of deaths between 2016 and 2017 involving alcohol and its presence in different samples, blood or vitreous humor.	The highest concentrations of alcohol were found in the blood and not in the VH.
An ultra-sensitive UHPLC-QqQ-MS/MS method for determination of 54 benzodiazepines (pharmaceutical drugs, NPS and metabolites) and z-drugs in biological samples	Olga Wachetko <i>et al</i> <sup>54</sup> . 2023 It is not open access (full text only on the university institutional network)	Experimental study aiming to determine a method to identify benzodiazepine derivatives in blood, urine, bile and vitreous humor samples	VH proved to be a sample with the lowest concentration of substances. There is no mention of when the samples were collected. Of 21 cases, VH was only analyzed in 6.

Post-mortem diagnosis of kidney impairment: An experimental study	Peter D Maskell <i>et al</i> <sup>55</sup> . 2019 It is not open access (full text only on the university institutional network)	Experimental study. Quantification of creatine in VH to determine renal function close to the time of death. VH was the only biological sample used. The sample collection time was well described.	The specificity of creatine for determining renal failure was lower than that observed when measuring TGF.
The dark side of social media: Two deaths related to chloroform intoxication	Kaja Tusiewicz <i>et al</i> <sup>56</sup> . 2022 It is not open access (full text only on the university institutional network)	Identification and quantification of chloroform in 2 fatal cases of suicide. Samples used: blood, urine, VH and bile, kidney, liver and muscle samples.	The vitreous humor was the tested sample that showed the lowest concentration of the substance. There is no mention of the sample collection time or the origin of the muscle collected.
A fatal case involving the highest ever reported 4-CMC concentration	Kaja Tusiewicz <i>et al</i> <sup>57</sup> . 2023 It is not open access (full text only on the university institutional network)	Report of a fatal case due to the use of 4-CMC and its presence in blood and vitreous humor samples. HM showed a higher concentration of the substance than blood.	The PM time of sample collection was not reported.
Development and validation of a LC-MS/MS method for analysis of vortioxetine in postmortem specimens. First data from an authentic case	Pietro Zuccarello <i>et al</i> <sup>58</sup> . 2023 It is not open access (full text only on the university institutional network)	Experimental study - Development and validation of a vortioxetine method in samples of blood, VH, bile, brain, liver, kidney and gastric contents. VH showed the lowest concentration among the samples. It was reported when the body was found and when samples were taken.	Samples were collected 4 days after death.
High Correlation between Ethanol Concentrations in Postmortem Femoral Blood and in Alternative Biological Specimens, but Large Uncertainty When the Linear Regression Model Was Used for Prediction in Individual Cases	G Thelander <i>et al</i> <sup>59</sup> . 2020 It is not open access (full text only on the university institutional network)	Experimental study for the identification of ethanol in samples of venous blood, urine, VH, bile and pleural fluid.	The concentration of the substance analyzed in the VH was low compared to the other samples. There was no mention of the PM time for sample collection.
Use of the Randox Evidence Investigator immunoassay system for near-body drug screening during post-mortem examination in 261 forensic cases	Poppy McLaughlin <i>et al</i> <sup>60</sup> . 2019 It is not open access (full text only on the university institutional network)	Analysis of biological samples including VH from 261 necropsies to aid the development of a tool for substance detection	VH was the matrix that most varied in the positive detection of drugs and other substances of interest in the matrices studied
Comparison of the beta-hydroxybutyrate, glucose, and lactate concentrations derived from postmortem proton magnetic resonance spectroscopy and biochemical analysis for the diagnosis of fatal metabolic disorders	Jakob Heimer <i>et al</i> <sup>61</sup> . 2020 It is not open access (full text only on the university institutional network)	Experimental study in cases of fatal metabolic disorders, comparing the results of two different techniques for analyzing biological samples including VH	Need for future adaptation of the detection protocol between the tests used due to the high error rate, mainly due to the time of postmortem collection
The challenge of post-mortem GHB analysis: storage conditions and specimen types are both important	J Kietzerow <i>et al</i> <sup>62</sup> . 2020 It is not open access (full text only on the university institutional network)	Postmortem detection of gamma-hydroxybutyrate (GHB) in varied biological samples (at different temperatures) including VH	VH did not show variation in GHB between different storage temperatures
Ultraviolet-Visible and High-Resolution Mass Spectrometry for the Identification of Cyclopropyl-Fentanyl in the First Fatal Case in Spain	José Manuel Mateyet <i>al</i> <sup>63</sup> . 2020 It is not open access (full text only on the university institutional network)	Detection of cyclopropyl-fentanyl in biological samples from a fatal case in Spain, with VH being one of the samples analyzed	The necropsy was performed just 48 hours after death, inferring a reduction in positive quantification



Chronic alcohol abuse may lead to high skin iron content, but not to hepatic siderosis	Alexander Paulke <i>et al.</i> <sup>64</sup> . 2019 It is not open access (full text only on the university institutional network)	Experimental study to evaluate the presence of iron ions and their relationship with chronic alcoholism. Various biological samples were used including VH, from 53 necropsies	VH did not present detectable quantification of iron
Fatal poisoning by intravenous injection of castor bean ( <i>Ricinus communis</i> L.) extract-a case study	Marie Staňková <i>et al.</i> <sup>65</sup> . 2020 It is not open access (full text only on the university institutional network)	Report of a fatal case caused by injection of homemade castor bean extract. Different biological samples were analyzed for confirmation, including VH, with this matrix presenting a higher concentration than the others.	The pharmacodynamics and pharmacokinetics of ricin in human tissue have not yet been fully elucidated, so variable deposition in matrices may be a consequence of other factors.

## Discussion

The results presented the following distribution per year: 1 article published in 2019, 1 article published in 2020, 4 articles published in 2021, 4 articles published in 2022 and 2 articles published in 2023.

Some results are classified as descriptive and retrospective population studies (n=2)<sup>9,15</sup>. Four articles were found that carried out a bibliographic survey also covering the application of VH in forensic toxicology<sup>5,6,7,12</sup>. The case reports found using VH as a detection matrix (n=4) are divided into accidental deaths resulting from fatal intoxication with some chemical substance, not necessarily classified as an illicit drug<sup>10,11,13,16</sup>.

Articles on analytical methodologies involving the use of VH were also found (n=2)<sup>8,11</sup>. The substances identified vary between legal and illicit drugs and other toxic molecules (ethyl sulfate - EtS), and positive putrefied alcohols - PA's<sup>9</sup>; oleander leaf extract<sup>10</sup>; ethanol<sup>9,11,13,15</sup>; beta-hydroxybutyrate<sup>15</sup>; Rocuronium<sup>13</sup>;  $\alpha$ -PiHP<sup>14</sup>; isopropyl alcohol<sup>16</sup>). The main advantage of the vitreous humor is its ability to preserve metabolites or other toxic substances of interest, due to the closed characteristic of the structure of the eyeball, also avoiding contamination by pathogens<sup>4,40</sup>.

With the presence of water, collagenous matrix and hyaluronic acid, VH can retain molecules of toxicological interest, slowing down their metabolism and consequent excretion from the body<sup>66</sup>.

Only one of the open access articles found in the 12 results has VH as the only biological sample analyzed in forensic toxicology<sup>8</sup>, with the others also presenting the analysis of other matrices, such as blood and urine. As the objective was the development and standardization of a new analytical method exclusively for this matrix, there is an interest in the forensic field in the use of vitreous humor as a preferred sample.

The remaining 50 articles reviewed that are not open access, requiring use of the university institutional network provided by the college, vary their categories similar to the 12 open access. Most of these articles are case reports of identification of one or more molecules of interest or retrospective study. The second most frequent category is the development of analytical methods or standardization and optimization of existing methods in experimental studies.

The main analytical method used in the other articles evaluated through access made by the university institutional network was LC-MS/MS, applied in all studies, with gas chromatography (GC) also being used, coupled or not with mass spectrometry (MS). Other methods, such as vitreous humor blood gas analysis, were also used. Other articles reviewed in these results, classified as comments on another article<sup>67</sup> and position article<sup>68</sup>, do not fall within the scope of the analysis of VH as a biological matrix, although the first is open access.

The results obtained from this review indicate a small number of articles available in open access in the last 5 years, with the majority of them being classified as bibliographic reviews and case reports. However, the focus of forensic toxicology on the vitreous humor is not only on molecules classified as illicit or legal drugs, but other intoxicants and also biomarkers of interest, such as electrolytes and carnitines.

## Conclusion

The vitreous humor is a biological matrix capable of detecting substances of toxicological interest in forensic situations. It has advantages over other biological samples due to its structural and anatomophysiological characteristics, closed inside the eyeball and composed of a collagenous matrix and water, mainly. However, among the disadvantages, the main one is the mandatory collection only postmortem, unlike peripheral blood, urine, hair and other matrices.

With the relevance of this matrix in toxicological investigation, with the number of indexed publications growing from 2018 to date, the forensic application of this sample is increasingly requested in criminal cases that cannot use conventional matrices, such as peripheral blood. The review of other articles published, with full text through the university network, points to the use of VH mainly in forensic toxicology of postmortem illicit drugs, with positive detection of the analyte of interest frequently occurring through LC-MS.

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