

The determination of potassium concentration in vitreous humor by low pressure ion chromatography and its application in the estimation of postmortem interval

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Abstract

An analytical method was developed for the determination of potassium in vitreous humor by low pressure ion chromatography (LPIC). Experimental conditions for LPIC analysis were optimized. High sensitivity and selectivity were obtained using this method. The LOD and LOQ were 1 and 2 mmol l⁻¹, respectively. The linearity was demonstrated from 2 to 20 mmol l⁻¹. The intra- and inter-day precision (CV) based on three concentrations was less than 5.0%. It was a simple and fast method to measure potassium and was suitable for evaluating the postmortem interval (PMI) in relatively well-preserved bodies. Sixty-two samples from medical-legal autopsies with known PMI were analyzed. A linear correlation equation for potassium concentration in the vitreous humor and PMI was established: $[K^+] = 0.1702\text{PMI} + 5.5678$, $r = 0.8692$.

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1. Introduction

The estimation of the postmortem interval (PMI) is crucial to the practice of certain forensic cases. Many techniques have been used to estimate PMI. In the 1960s, a novel method was proposed to estimate PMI, which related to the potassium concentration in the vitreous humor. Researchers found that potassium released from the intracellular compartment increase its concentration in the vitreous humor in a time-dependant manner since an individual died [1,2]. Many equations have been established to describe the linear correlation between the potassium concentration in the vitreous humor and PMI. However, the values of the slope and intercept made significant difference from each other [3]. Many factors contribute to the significant variation, including the modalities of death, ambient temperature and putrefaction. Another important factor is the analytical method. Two major types of techniques, non-separation and separation techniques, have been used to measure potassium concentration [2,4]. Com-

pared with the non-separation analytical techniques, especially the flame photometry and ion selective electrode, the result of the separation analytical techniques were not influenced by the fluctuation of components concentration in the vitreous humor (such as ionic intensity, pH, amines, peptides, and so no). Capillary electrophoresis (CE) and ion chromatography, as two separation methods, have been used to measure the concentration of the inorganic ions. However, many applications have been limited because of the analytical costs and instrument complexity.

The purpose of our work was to develop and validate a simple, accurate and fast method for the determination of potassium in vitreous humor and estimation of postmortem interval. Sixty-two vitreous humor samples collected from selected medical-legal autopsies that had relatively well preservation conditions were subjected to analysis by low pressure ion chromatography (LPIC), a type of ion chromatography without the suppressor column that works at a low-pressure of 1.96×10^5 to 2.94×10^5 Pa [5–8]. Many inorganic cations, i.e., alkali metals, alkaline earth metals, transition-metals, and inorganic anions as well as organic acids have been well separated and analyzed by LPIC [9]. A significant linear correlation between the PMI

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and the potassium concentration in the vitreous humor has been established, which can be described by the following equation: $[K^+] = 0.1702PMI + 5.5678$, $r = 0.8692$.

2. Experimental

2.1. Apparatus

The analytical system consisted of a HL-2 electronic pump (Model ZJ-3, Shanghai, China), a high sensitivity self-made conductivity detector and a hand-control sampler. The data acquisition and processing was completed by the Data Working Station Software. A specific ion chromatography column made by our laboratory for detecting Na^+ , K^+ , NH_4^+ was used to measure the potassium concentration [9,10].

2.2. Chemicals and reagents

All reagents including nitric acid (HNO_3), potassium chloride (KCl) and sodium chloride (NaCl) of analytical grade were purchased from Jiulong Company (Chengdu, China). The deionized water was purified by “Easy pure RF compact” ultrapure water system (Barnstead/Thermolyne, American). The eluent (0.96 mmol l^{-1}) was prepared by diluting $66.7\text{ }\mu\text{l}$ nitric acid in 1 l deionized water and must be prepared daily. For the establishment of the linearity range, calibration curves in deionized water were prepared in the concentration range of $2\text{--}20\text{ mmol l}^{-1}$ (2, 4, 6, 8, 10, 12, 16 and 20 mmol l^{-1} , respectively).

2.3. Samples preparation

Sixty-two vitreous humor samples were collected from medical-legal autopsies. All the subjects studied were adults and the exact time since death was known (1–27 h). Those subjects who had ocular injury or diseases, or craniocerebral trauma were excluded from this study. A total volume of $100\text{ }\mu\text{l}$ of vitreous humor was collected. Four microliters was assayed by direct injection into the chromatography system and the rest were stored at $4\text{ }^\circ\text{C}$.

2.4. Chromatographic conditions

The separation of potassium was performed on a self-made LPIC column ($7.0\text{ cm} \times 0.6\text{ cm}$, I.D., $20\text{--}25\text{ }\mu\text{m}$), supplied by Xinshen Zhang, Ph.D. The eluent was 0.96 mmol l^{-1} nitric acid aqueous solution, and was delivered at a low flow rate of 0.8 ml min^{-1} . The column temperature was ambient temperature. The elution time for potassium was 6.7 min.

3. Results and discussion

3.1. Selectivity

Typical chromatograms of a real vitreous humor sample and the standard potassium solution, obtained under the optimized experimental conditions, are shown in Figs. 1 and 2, respectively. R_s values, which were greater than 1.5, suggest good resolution.

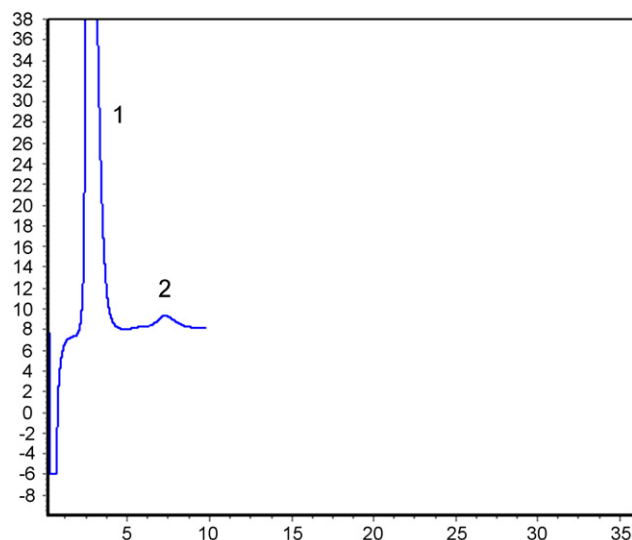


Fig. 1. Chromatogram of a sample of vitreous humor.

3.2. Calibration curves and detection limits

For the quantitative analysis of the potassium in this study, the peak height calibration curves were used. Eight calibration standards with the concentration of potassium varying from 2 to 20 mmol l^{-1} (2, 4, 6, 8, 10, 12, 16 and 20 mmol l^{-1} , respectively) were analyzed and the calibration curves were obtained by plotting peak height versus concentration. The linear relationship between peak height and potassium concentration was described by Table 1. LOD was determined as S/N of 3:1. LOQ was defined as the lowest concentration of the calibration curve. In this study, LOD was 1 mmol l^{-1} , LOQ was 2 mmol l^{-1} .

The number of points was eight and the data were based on three replicates.

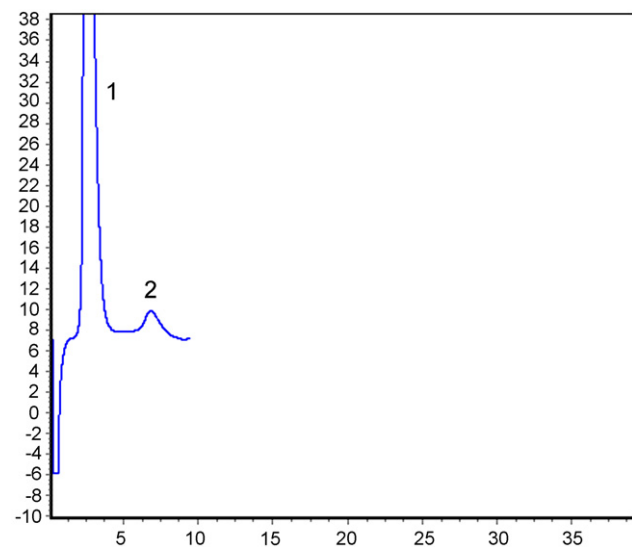


Fig. 2. Chromatogram of a sample of the standard potassium solution.

Table 1
Results of linearity tests

Sample	K ⁺
Linear range (mmol l ⁻¹)	2–20
Linear regression	
Slope	0.2121 ± 0.0054
Intercept	0.1007 ± 0.4236
RE ^a	0.9987
Equation	$y^b = 0.2120x^c + 0.1007$

^a RE: regression coefficient.

^b y: peak height.

^c x: concentration of K⁺.

Table 2
Intra-assay data

Added amount (mmol l ⁻¹)	Mean	S.D.	CV (%)	Accuracy (%)
4	4.0	0.1	2.5	1.0
10	10.1	0.2	1.5	0.8
20	20.1	0.1	0.6	0.7

The data were based on five replicates.

3.3. Precision and accuracy

Accuracy and precision of the method were investigated after measuring standards of the same concentration five times each. The intra- and inter-day precision were evaluated by the coefficient of variance (CV%). The data are shown in Tables 2 and 3.

3.4. Optimization of the chromatography conditions

For the length of the column, we chose a relatively short column because shorter columns have the following two advantages: reduced system pressure and improved sensitivity and precision. It was found that the resolution decreased sharply when the exchange capacity was less than 0.04 mmol g⁻¹, while higher exchange capacity will increase analysis time and decrease analytic sensitivity [4–10]. The optimal column exchange capacity is 0.04 mmol g⁻¹ in the present study. 0.96 mmol l⁻¹ HNO₃ was used as the eluent, which is optimal to reduce the background conductivity and to improve the sensitivity. Results of tests for precision and linear range showed that the system with the above parameters have good reproducibility and linear correlation. The linear correlation between the PMI and potassium concentration measured by LPIC is highly significant. As the concentration of sodium in the vitreous humor is about 0.1–0.15 mol l⁻¹, which is nearly 40 times higher than that of potassium in the vitreous humor, the chromatographic peak of potassium will be affected obviously by that of sodium

Table 3
Inter-assay data (n = 5)

Added amount (mmol l ⁻¹)	Mean	S.D.	CV (%)	Accuracy (%)
4	4.0	0.2	4.8	0.5
10	9.9	0.2	2.4	0.6
20	20.0	0.1	0.7	0.2

The data were based on five replicates.

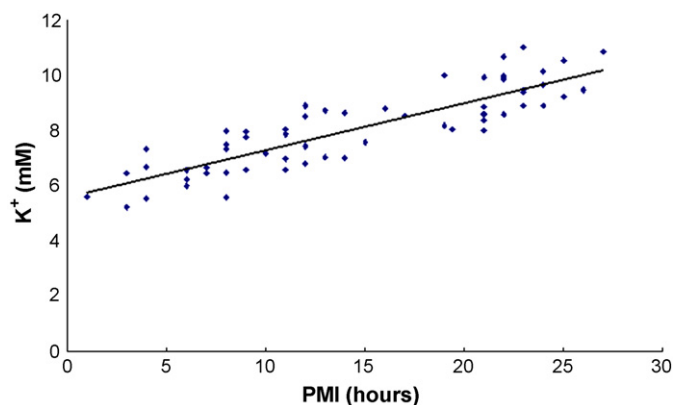


Fig. 3. Plot of PMI vs. K⁺ concentration.

when more than 10 μ l vitreous humor samples were injected and analyzed by the LPIC system. Different volumes of vitreous samples were measured by the LPIC system and in the present analytic system only 4 μ l sample of vitreous humor is sufficient to obtain a clear result.

3.5. Application

Many equations for the correlation between potassium concentrations in the vitreous humor and PMI have been reported by different groups. The primary difference among these equations is the intercept, which varies from 2.3587 to 8. Besides the modalities of death, the state of putrefaction, ambient temperature, analytical techniques, the sampling method and sample storage procedures were also reported to affect the slope and intercept of the correlation equations [11]. It has also been reported that the potassium concentration in the vitreous humor in one eye differ from the other one of the same corpse [12]. To minimize the effect of the above factors, we measured the potassium concentration in the vitreous humor in selected medical-legal bodies. These bodies had relatively good preservation conditions with least effects of putrefaction. These subjects have not ocular injury or diseases, or craniocerebral trauma. We did not detect a significant difference between two eyes of same subject. A linear correlation between the PMI and the K⁺ concentration in vitreous humor was found, and the correlation can be described by the following equation: $[K^+] = 0.1702\text{PMI} + 5.5678$, $r = 0.8692$. The concentrations of potassium measured in this study ranged from 5.6 to 10.87 mmol l⁻¹ with the PMI values varying from 1 to 27 h. Fig. 3 shows the plot for PMI versus K⁺ concentration of all the 62 samples.

4. Conclusion

This study was the first to measure the potassium concentration in the vitreous humor by LPIC. In this study, the factors affecting the results were discussed, and optimized conditions have been obtained.

Compared to other separative method, this method is less complex, cheaper and time-saving. The results show that the PMI

can be valuably estimated based on potassium concentration of the vitreous humor measured by LPIC.

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References

- [1] L. Hansson, U. Uotila, R. Lindfors, K. Laiho, J. Forensic Sci. 11 (1966) 390.
- [2] F. Tagliaro, F. Bortolotti, G. Manetto, F. Cittadini, V.L. Pascali, M. Marigo, J. Chromatogr. A 924 (2001) 493.
- [3] J.I. Munoz, J.M. Suarez-Penaranda, X.L. Otero, M.S. Rodriguez-Calvo, E. Costas, X. Miguens, L. Concheiro, J. Forensic Sci. 46 (2001) 209.
- [4] B. Madea, C. Kreuser, S. Banaschak, Forensic Sci. Int. 118 (2001) 29.
- [5] X.S. Zhang, Analysis and Application of Ion Chromatography, Sichuan Press of Science and Technology, Chengdu, 1986.
- [6] X.S. Zhang, X.P. Jiang, J. Chromatogr. A 671 (1994) 23.
- [7] X.S. Zhang, Chin. J. Chromatogr. 8 (1990) 128.
- [8] X.S. Zhang, High-Performance Liquid Chromatographic Analysis, Academic Periodical Press, Beijing, 1990, p. 128, Chapter 6.
- [9] X.P. Jiang, X.S. Zhang, M.H. Liu, J. Chromatogr. A 857 (1999) 175.
- [10] M.H. Liu, X.S. Zhang, X.P. Jiang, J. Petrochem. Univ. 12 (1999) 26.
- [11] J.I. Coe, Am. J. Forensic Med. Pathol. 14 (1993) 91.
- [12] D.J. Pounder, D.O. Carson, K. Johnston, Y. Orihara, J. Forensic Sci. 43 (1998) 604.