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# Determination of lactic acid and pyruvic acid in serum and cerebrospinal fluid by ion-exclusion chromatography with a bulk acoustic wave detector

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**Abstract**

A chromatographic method based on a combination of ion-exclusion chromatography separation and bulk acoustic wave series piezoelectric quartz crystal detector quantification for the determination of pyruvic acid and lactic acid in serum and cerebrospinal fluid (CSF) was developed. The separation was carried out using a Shim-pak SCR-102H ion-exclusion column with phosphoric acid solution as eluent. The method shows an acceptable detection limit and anti-interference ability. Serum and CSF from healthy individuals and patients were analysed successfully.

## 1. Introduction

The determination of lactic acid and pyruvic acid in biological fluids has recently gained considerable interest. Lactic acid concentration is an index of tissue oxygen deprivation and is helpful in grading the severity of shock [1]. Blood lactic acid concentration increases during physical work [2]. In patients with chronic heart failure and impaired cardiac output response to exercise, lactic acid kinetics may provide a useful and objective index of the severity of heart failure [3]. Pyruvic acid is a metabolite of sugar, fat and protein. In clinical diagnosis, pyruvic acid concentration can be used to diagnose metabolic block diseases such as diabetes, vitamin $B_1$ absence, some digestion blocks, etc. In cerebrospinal fluid (CSF), the lactic acid and pyruvic acid concentrations have important clinical value. In general, the lactic acid concentration in the CSF of a healthy individual is <180 ppm. The concentration level of lactic acid can be used to judge bacterial meningitis, viral meningitis, hydrocephalus, etc. Pyruvic acid can be used to diagnose epidemic encephalitis B at an early stage, poliomyelitis, cerebroma, etc.

Several types of detection methods, such as enzymatic methods [4,5], electrochemical detection or coupled enzymatic assay with high-performance liquid chromatographic (HPLC) separation [6,7], have been reported. HPLC has been applied to the determination of the two organic acids using reversed-phase [8] or anion-exchange

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columns. Ion-exclusion chromatography (IEC) is a technique used to separate weak acids, amino acids and other substances on an ion-exchange column. Several ion-exclusion chromatographic determinations of lactic acid and pyruvic acid have been reported. Conductivity cells and UV absorption spectrometers have been used as detectors [9,10].

The piezoelectric quartz crystal (PQC) has been developed rapidly and utilized widely as a chemical microsensor since its first application to chemistry by King [11] in 1964. It is a bulk acoustic wave (BAW) sensor. When a time-varying r.f. potential is applied to the electrodes, the crystal lattice undergoes particle displacement, the net result being a bulk elastic wave that propagates from one face of the crystal to the other. The wave velocity, and hence the oscillation frequency, are sensitive to changes in the mass or density, viscosity, specific conductivity and permittivity of the liquid medium in contact with the crystal surface [12–14]. Based on its response ability with specific conductivity, the BAW sensor has been used as a detector in liquid chromatography [15–17]. Recently, we reported the use of a BAW series piezoelectric quartz crystal (SPQC) detector to determine vitamin C [17].

In this paper, we describe the determination of lactic acid and pyruvic acid in human serum and CSF by IEC with a BAW series piezoelectric quartz crystal (SPQC) detector. This method is simple, sensitive and specific, without interferences.

## 2. Experimental

### 2.1. Reagents

All reagents and chemicals were of analytical-reagent grade. Standard lactic acid and pyruvic acid reagents were purchased from Sigma (U.S.A.). Deionized water (>80 MΩ) obtained with a Milli-Q system (Millipore) was used throughout. Filters (0.45 μm) for mobile phase filtration were obtained from Millipore.

### 2.2. Instrumentation and chromatographic conditions

The ion-exclusion chromatographic system consisted of a Model 590 pump, a Model U6K fixed-volume (100 μl) manual injector and a column heater (all from Waters).

The column for separation was a Shim-pak SCR-102H stainless-steel column (30 cm × 8 mm I.D.) packed with an H-type cation exchanger consisting of a semi-rigid styrene–divinylbenzene copolymer (Shimadzu). It was protected by a stainless-steel Guard-pak column (0.45 μm) (Waters).

The bulk acoustic wave SPQC detector was made in this laboratory. The cell constant was 3.0 cm and the dead volume of the detector was 12 μl [17]. A frequency-to-voltage convertor (made in this laboratory [18]) was used to translated the frequency signal of the SPQC detector to the Baseline 810 chromatographic workstation (Waters).

Other pertinent IEC conditions were as follows: the mobile phase contained water and the pH was adjusted to about 3.0 with phosphoric acid; background conductivity of the mobile phase, 1.1 mS/cm; the mobile phases was filtered through a 0.45-μm filter; flow-rate, 1.0 ml/min; injection volume, 100 μl; column temperature, 30°C; and detector temperature, 30°C.

### 2.3. Preparation of standard solutions

Stock solutions (1.0 mg/ml) of lactic acid and pyruvic acid were prepared in water. Five mixed standards of lactic acid and pyruvic acid were prepared by pipetting 10, 20, 40, 80 and 100 μl of the pyruvic acid stock solution and 200, 400, 800, 1600 and 2000 μl of the lactic acid stock solution into separate 10-ml volumetric flasks and diluting to volume with water. The concentrations of standard series were pyruvic acid 1, 2, 4, 8 and 10 ppm and lactic acid 20, 40, 80, 160 and 200 ppm.

### 2.4. Sample processing

An aloquot of 0.5 ml of serum was added to 0.5 ml of methanol and mixed for 15 min, then

centrifuged at 4000 *g* for 10 min and the supernatant was collected. The supernatant was filtered with a 0.45-$\mu$m filter and 100 $\mu$l were injected into the IEC system.

An aliquot of 0.2 ml of CSF was added to 0.2 ml of methanol and mixed for 15 min, followed by steps as for serum.

## 3. Results and discussion

Figs. 1 and 2 show the chromatograms for a serum sample and a CSF sample obtained using the above conditions. The retention times of pyruvic acid and lactic acid are 4.8 and 6.5 min, respectively. No other peaks in serum and CSF interfered significantly with the detection of pyruvic acid and lactic acid. The calibration graphs for pyruvic acid and lactic acid were linear over the ranges 1–10 and 5–40 $\mu$g/ml, respectively. The correlation coefficients of the calibration graphs were >0.998. The detection limits (signal-to-noise ratio = 3, noise 1 Hz) were 0.3 $\mu$g/ml for pyruvic acid and 0.5 $\mu$g/ml for lactic acid. The method recovery was determined by spiking known concentrations of pyruvic acid and lactic into serum and CSF. The samples were chromatographed by the described procedure, yielding a mean recovery of 100.7% for pyruvic acid and 98.2% for lactic acid for nine spiked

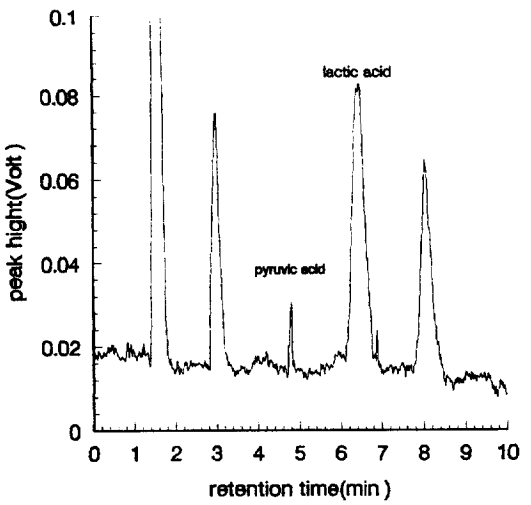


Fig. 1. Chromatogram for CSF analysis.

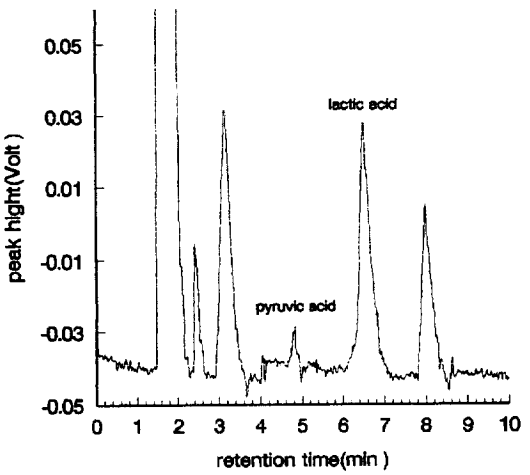


Fig. 2. Chromatogram for serum analysis.

samples. For accuracy, analyses of five samples were repeated ten times. The results are given in Table 1. The coefficient of variation (C.V.) for pyruvic acid was less than 5% and that for lactic acid was less than 4%.

For BAW-SPQC, if a solution with conductivity $G_0$ is chosen as the reference, and the change in conductivity in solution ($\Delta G$) is much less than $G_0$, the frequency shift can be expressed as

$$\Delta F = -BFs^2\,\Delta G$$

where *Fs* is the resonant frequency of the crystal and *B* is a constant which is dependent on the crystal parameters, the solution capacitance and $G_0$. It can be seen that the frequency shift of the BAW-SPQC decreases linearly with increasing conductivity. In the presence of a foreign electrolyte, the sensitivity to conductivity tends to increase owing to the increase in *B* until a maximum range is achieved [17,19]. However, when the background conductivity is over about 3000 $\mu$S, the detector does not work satisfactory. Generally, the concentration of the mobile phase in IEC is 0.1%, but the specific conductivity of 0.1% phosphoric acid is about 3000 $\mu$S/cm. Hence under this mobile phase condition, the detector cannot work satisfactorily. In this work, we found that when the pH of the mobile phase is less than 3, the separation of lactic acid and

Table 1
Results for accuracy

| Sample | Acid | Results of analysis (ppm) | | | | | | | | | | C.V. (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CSF 1 | Lactic | 102, | 105, | 103, | 109, | 101, | 105, | 105, | 108, | 107, | 102 | 2.59 |
| | Pyruvic | 7.2, | 7.9, | 8.1, | 7.4, | 7.3, | 7.2, | 7.9, | 8.2, | 7.1, | 7.8 | 5.12 |
| CSF 2 | Lactic | 91, | 94, | 93 | 96, | 96, | 99, | 91, | 92, | 98, | 93, | 2.85 |
| | Pyruvic | 8.9, | 8.0, | 9.2, | 9.3, | 8.7, | 8.6, | 8.1, | 9.0, | 8.6, | 8.4 | 4.72 |
| Serum 1 | Lactic | 113, | 115, | 113, | 119, | 120, | 115, | 115, | 117, | 121, | 114 | 2.37 |
| | Pyruvic | 8.1, | 8.7, | 8.9, | 8.5, | 8.2, | 8.2, | 8.3, | 8.8, | 8.4, | 8.3 | 3.08 |
| Serum 2 | Lactic | 120, | 127, | 131, | 121, | 125, | 122, | 129, | 124, | 127, | 123 | 2.71 |
| | Pyruvic | 11.0, | 11.3, | 11.0, | 11.1, | 12.1, | 11.7, | 11.3, | 11.5, | 11.2, | 11.9 | 3.16 |
| Serum | Lactic | 187, | 194, | 181, | 188, | 191, | 183, | 185, | 186, | 189, | 181 | 2.15 |
| | Pyruvic | 5.6, | 6.1, | 5.9, | 5.7, | 5.2, | 5.7, | 5.7, | 6.0, | 5.2, | 5.4 | 5.13 |

pyruvic acid proceeds successfully. The background conductivity of the mobile phase is 1.1 mS/cm, which fits the optimum sensitivity range of the background [17]. Fig. 3 shows the relationship between the sensitivity and background conductivity.

When the cell constant of the detector is decreased, the response tends to increase, but its noise tends to increase also. Hence the detected limit is not improved (Table 2). On the other hand, the drift of the baseline and the noise level of the SPQC detector are affected by the temperature. The frequency–temperature coefficient increases with increasing concentration of the background electrolyte. In this work, we chose 30°C for the column and the detector was kept at the same temperature. The accuracy of temperature control of the detector was within ±0.5°C. Under these conditions, the temperature of the column does not affect the separation of lactic acid and pyruvic acid significantly, and the detector yields a very stable response.

We determined lactic acid and pyruvic acid in serum and CSF from healthy individuals and patients (Table 3). HPLC–UV detection [20,21] was used as a method for comparison. No significant differences were observed between the two methods. It can be seen from the results that the concentration of lactic acid in CSF from bacterial meningitis patients is much higher than that for healthy individuals. However, in the case of viral meningitis, the concentration increase is not

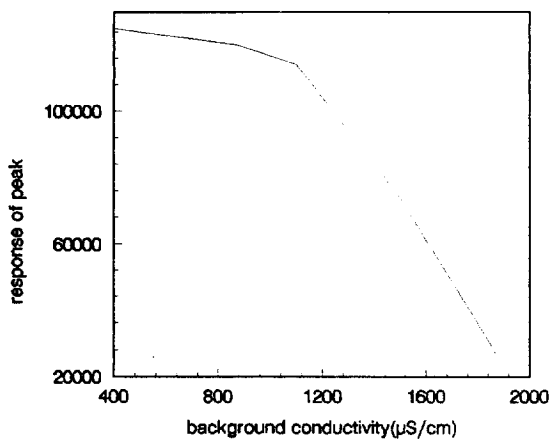


Fig. 3. Relationship between response sensitivity and background conductivity. Tested sample: 8 ppm pyruvic acid (100 μl). Peak response in μV·s.

Table 2
Relationship between the response and noise of the BAW-SPQC detector with its cell constant

| Cell constant (cm) | Response (Hz) | Noise (Hz) |
|---|---|---|
| 0.62 | 261 | 3 |
| 0.97 | 191 | 3 |
| 1.74 | 142 | 2 |
| 3.00 | 103 | 1 |
| 4.72 | 97 | 1 |

Sample tested: 8 ppm pyruvic acid (100 μl).

Table 3
Results for determination of lactic acid and pyruvic acid (ppm) in serum and CSF by IEC–SPQC and HPLC–UV

| Compound | Method | Healthy serum | Bacterial meningitis, CSF | Viral meningitis, CSF | Cerebral death, CSF | Vitamin $B_1$ absence, serum | Acute virus hepatitis, serum |
|---|---|---|---|---|---|---|---|
| Lactic acid | IEC–SPQC | 110 ± 34 | 349 ± 90 | 143 ± 39 | 562 | 153 ± 21 | 223 ± 40 |
| | HPLC–UV | 113 ± 43 | 329 ± 86 | 136 ± 40 | 547 | 150 ± 18 | 230 ± 44 |
| Pyruvic acid | IEC–SPQC | 6.0 ± 3.2 | 10.3 ± 2.1 | 9.2 ± 3.4 | 9.8 | 30.0 ± 6.0 | 13.2 ± 2.9 |
| | HPLC–UV | 5.9 ± 3.0 | 10.5 ± 2.4 | 9.1 ± 3.2 | 10.1 | 29.3 ± 5.4 | 12.8 ± 2.7 |

significant. For one CSF sample obtained following cerebral death, the lactic acid content is especially high, i.e., up to 562 ppm. For the case with absence of vitamin $B_1$, the serum pyruvic acid concentration rises to about 30 ppm. For acute virus hepatitis samples, both lactic acid and pyruvic acid levels rise markedly.

As the proposed method is based on the conductivity change in the course of chromatographic separations, it is of interest to compare this method with the conventional conductimetric detection technique. In an electrolytic conductance cell, such phenomena as double-layer capacitance formation or Faradaic impedance may take place and may cause the effective potential applied to the cell to change, thus affecting the determination of ions. Techniques involving the use of a sinusoidal wave potential, a bipolar pulse conductance technique, etc. [22], have been developed to solve these problems, but the instrument is complex [23]. In the BAW-SPQC system, because the oscillation frequency of the electric field applied between the electrodes of the resonator is very high (9 MHz), there is no effect of the double electric layer capacitance of the electrode on the measurement of frequency (which is dependent on the conductivity between the electrodes), and because the magnitude of the potential difference between the electrodes is very small, electrolysis could not take place. Hence, BAW-SPQC detection need not use an alternative measuring technique or multiple electrodes, as is the case with conductimetric detection. In addition, its construction is simple and the results are accurate (Table 1).

## 4. Conclusion

The IEC-BAW SPQC method described here has sufficient sensitivity to determine pyruvic acid and lactic acid in biological fluids. The method is simple, rapid and accurate, and the possibility of application of the BAW-SPQC detector in IEC has been demonstrated. However, the chromatographic operating parameters must be selected carefully to fit the requirements of sensitivity and stability of the detector. From the analytical results, the lactic acid and pyruvic acid concentrations in serum and CSF of patients reflect the symptoms to some extent.

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