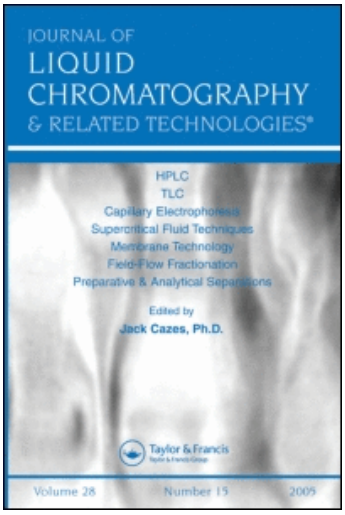

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DETERMINATION OF CYCLAMATE RADICAL BY SINGLE-COLUMN ION-CHROMATOGRAPHY WITH A BULK ACOUSTIC WAVE DETECTOR

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ABSTRACT

A chromatographic method based on a combination of single-column ion-chromatography separation with a bulk-acoustic-wave double-cell quartz crystal (DCQC) detector quantification for the determination of cyclamate in foodstuffs and body fluids was developed. The chromatographic system involves the use of a Shimadzu IC-A1 column with 5mM o-phthalic acid/methanol (90:10 V/V) as a mobile phase. The method exhibits an acceptable detection limit and anti-interference ability. The DCQC detector has a low temperature coefficient and high conductance sensitivity. Foodstuff and body fluid sample such as ice cream, beverage, dried fruit of *Areca catechu* L., and urine were analysed successfully.

INTRODUCTION

Sodium cyclamate ($C_6H_{12}NNaO_3S$) is a synthetic sweetener. Because its metabolism in the body has no relationship with insulin, it always used as a sweet additive in food for diabetics[1] (concentration <0.4% and about 40% is drained off in urine and 60% is drained off in stool for human). However, sodium cyclamate is also a non-nutritional sweetener and cannot provide heat energy to the human body. It is disadvantageous for a healthy human, espe-

cially for an infant who has used it as a replacement for sugar, and it is frequently absorbed.[2] In recent years, the viewpoint that sugar should be absorbed is generally agreed upon by nutritionists. And it is forbidden that synthetic sweeteners such as sodium cyclamate, saccharin etc. be used in infant food. The usage of sodium cyclamate in food has been strictly limited. For example, the concentration of this component in food must be kept less than 0.25g/kg in ice-cream and beverage, and 1.0g/kg in cake and candied fruit as stipulated by National Sanitation Standard of China (GB-2760-86).[3] Many methods have been proposed for the determination of sodium cyclamate, such as titration, photometry, thin layer chromatography (TLC), and high performance liquid chromatography (HPLC), etc.[4-8] In these methods, titration, photometry, and TLC have a poor anti-interference ability. As cyclamate has no appreciable UV absorbance, HPLC-UV method has a low sensitivity. Being anion, cyclamate is amenable to ion chromatography and detectable using a conductivity detector.[9]

The bulk acoustic wave (BAW) sensor has been developed rapidly because of many specific advantages such as high sensitivity, low cost, and conceptual simplicity etc. Based on its response ability with specific conductivity, several types of BAW detectors, such as series piezoelectric quartz crystal (SPQC), electrode-separated piezoelectric crystal (ESPC), and double cell quartz crystal (DCQC), which are used as a detector of liquid chromatography, have been developed and applied in many areas.[10-16] Recently, we reported the use of a BAW-DCQC detector to determine organic acids in Chinese medicine, etc.[12] Compared with the conventional conductivity detector, the BAW detectors have a particular advantage; that is the BAW detector cannot be interfered with double layer capacitance and Faradaic impedance without a multi-electrode technique etc. Thus, it is not surprising that the BAW detector has been used so widely.

In this paper, we described the determination of cyclamate in foodstuff and urine by ion chromatography with the BAW-DCQC detector. The method is simple, sensitive, and specific.

EXPERIMENTAL

Reagents and Materials

All chemicals and reagents were of analytical reagent grade, and the sodium cyclamate was of a chromatographic standard, purchased from Sigma Co. (St. Louis, MO, USA.). Deionized water was prepared with a Milli-Q Labo purification system (>80MΩ) (Millipore, Japan). The mobile phase and sample solutions were filtered by using 0.45μm filters (Millipore).

Chromatographic System

The ion chromatographic system consisted of a model U6K fixed-volume (50μL) manual injector, a model 590 solvent deliver pump and a column heater. A model 810 chromatographic workstation was used to record and treat chromatographic data from the DCQC detector (all from Waters).

The column for separation was a Shim-pack IC-A1 stainless-steel column (10cm X 4.6mm ID) packed with an anion exchange resin of a polymethacrylate support with a particle size of 10 μm incorporating a quaternary ammonium base as a functional group. It was protected by a Shim-pack IC-GA1 guard column (Shimadzu).

The DCQC detector was made in this laboratory.[12] Figure 1 shows the schematic diagram of the DCQC detector. Cell 1 was the adjustment cell and one side of the crystal contacts with liquid in this cell. Cell 2 was the sample cell and mobile from the chromatographic column flows through the cell. The cell constant of cell 1 can be adjusted by changing the position of the PTFE column with the crystal and the cell constant of cell 2 is 0.85cm. The piezoelectric quartz crystal used was 9MHz and AT-Cut. A frequency-to-voltage converter (made in this laboratory)[14] was used to transform the frequency signal of the DCQC detector to a Baseline 810 chromatographic workstation, which was used to record chromatograms in real-time and to integrate peak areas.

Other pertinent IC conditions were as follows. A 5.0 mM solution of o-phthalic acid/methanol (90:10 V/V) was used as the mobile phase; flow rate, 2.0 mL/min.; injection volume, 25 μL; column temperature, 40°C; and detector temperature, 40°C.

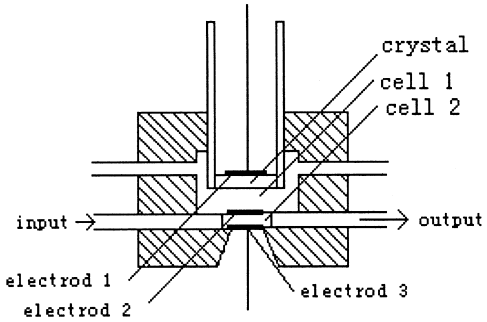

Figure 1. A diagram of the DCQC detector.

Sample Processing

Ice Cream and Beverage

50 mL of ice cream, thawed or beverage, was heated at 80°C for 30 min. After cooling, 1g of activated carbon (100-120 mesh) and 1g of aluminum oxide (neutral, 100-200 mesh) were added to the sample liquid. The liquid, after shaken for 15 min, was filtered through a filter paper. The filtrate was mixed with methanol (9:1 V/V). Then the mixture was filtered through the 0.45 μm filter membrane. The filtrate was injected on to the IC column.

Dried Fruit of Areca Catechu L.

5g of dried fruit of *Areca catechu* L. was extracted by petroleum ether (b.p. 30-60 °C) for three times to take out the fatty materials such as essential oils, etc., added in the manufacture process. After extracted, the dried fruit of *Areca catechu* L. was ground through a 1 mm screen. 15 mL of 1 mmol/L NaOH solution was added to the extract under ultrasonic wave condition. The extraction process was repeated 3 times. The extraction liquids were combined making the volume to 50 mL. The extraction liquid was mixed with methanol (9:1 V/V). Then the mixture was filtered through the 0.45 μm filter membrane.

Urine

Urine was adjusted to pH=9.0 by NaOH solution and heated at 70°C for 30 min under ultrasonic wave conditions. Then the urine was mixed with methanol (9:1 V/V). The mixture was filtered through the 0.45 μm filter membrane.

Procedure

The elution was pumped into the chromatographic system. After the baseline frequency (F0) was stabilized, the sample solution was injected into the IC, and the frequency (F1) was recorded versus time through the Baseline 810. The concentration of the tested ion was calculated from the peak area by comparison with the standard.

RESULTS AND DISCUSSION

Chromatographic Calibration of Cyclamate

Figure 2 shows the chromatogram for a standard of cyclamate, urine, and dried fruit of *Areca catechu* L. The retention time of cyclamate is 5.28 min. The calibration graphs for cyclamate was linear over the ranges 1-30 μg/mL. The correlation coefficients of the calibration graphs were >0.9992. The detec-

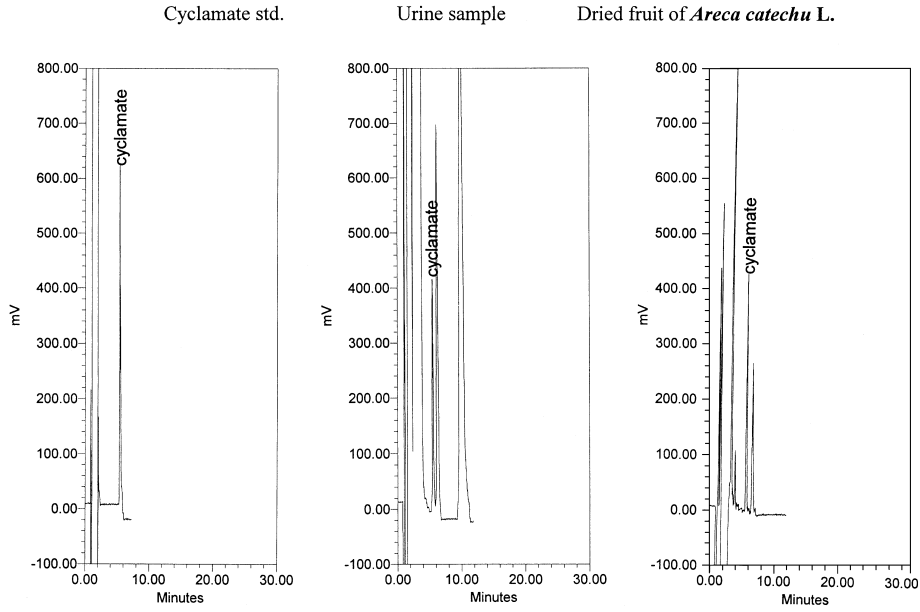

Figure 2. Chromatograms of sample analysis.

tion limits (signal-to-noise ratio = 3, noise 1Hz) were 0.2 μg/mL. The recovery of the method was determined through spiking known concentrations of cyclamate into dried fruit of *Areca catechu* L aFnd urine. The samples were chromatographed by the described procedure, yielding a mean recovery of 97.3% for five spiked fruit of *Areca catechu* L. and 98.5% for five spiked urine samples. For accuracy, analysis of five samples was repeated ten times. The results are given in Table 1. The coefficient of variation (C.V.) for cyclamate was less than 3%.

Working Condition of the BAW-DCQC

For the BAW-DCQC detector, the ΔF versus ΔG relationship (ΔF/ΔG) is linear when all other parameters are kept unchanged. And, we found that the response sensitivity of the DCQC is independent of the background conductivity of solution in cell 2 (G2) when conductivity of solution in cell 1 (G1) is ca.500μS. Cell constants can affect the detector performance. In this work, the cell constants were optimised k1=1.0cm, k2=0.85cm.

Table 1

Sample Analysis Accuracy

Sample	Results of Analysis (g/kg)					$\bar{X}$	C.V. (%)
Ice-cream 1#	0.177 0.176	0.179 0.177	0.178 0.172	0.175 0.176	0.177	0.176	1.44
Ice-cream 2#	0.093 0.092	0.094 0.091	0.093 0.093	0.093 0.095	0.094 0.094	0.093	1.22
Areca catechu L. 1#	1.271 1.289	1.240 1.284	1.262 1.292	1.218 1.286	1.232 1.228	1.260	2.25
Areca catechu L 2#	0.322 0.326	0.340 0.337	0346 0.342	0.348 0.345	0.338 0.347	0.339	2.61
Urine 1# (mg/L)	1.311 1.307	1.322 1.348	1.335 1.319	1.349 1.352	1.327 1.318	1.329	1.23
Urine 2# (mg/L)	2.162 2.143	2.175 2.103	2.091 2.187	2.107 2.121	2.156 2.182	2.143	1.64

For a conventional conductivity detector, its detection sensitivity depends on the background conductivity. So, the advantage of the DCQC detector over the conventional conductimetric detector is its independence on the background conductivity and its response stability. Figure 3 shows comparison results of detection sensitivity of these two detectors at different background conductivities. It is obvious that the response of the DCQC is more stable than the conventional conductimetric detector.

For the SPQC detector, the response sensitivity is good when the mobile conductivity is on a range of 150-1200μS.[10] When the mobile conductivity is over 3000μS, the detector does not work satisfactorily. In the DCQC detector, because there are solution cells between the crystal and electrode, the total conductivity between them can be adjusted through changing solution conductivity in these two cells to meet the requirement of the crystal oscillation. In this way, the working region of the DCQC detector is much wider than that of the SPQC detector reported earlier.[12] It extends to 7.2-2500μS. Crystal does not contact with the mobile directly, so the crystal oscillation stability is not affected by the liquid flowing in the sample cell. Therefore, the DCQC oscillation is very stable.

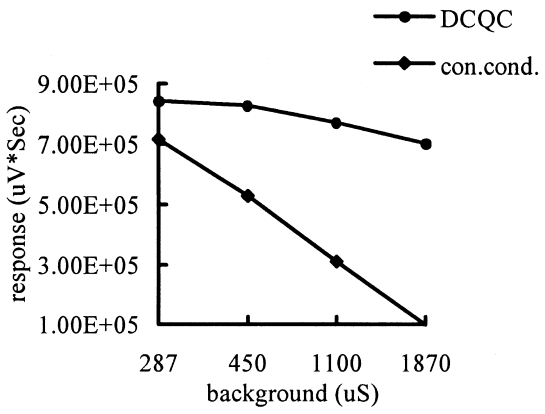

Figure 3. Comparison of detection sensitivity of two detectors.

Temperature can affect the response stability of the DCQC detector. Table 2 shows the drift of baseline and the noise level under different temperatures of the detector and a constant column temperature of 40°C. The drift results were obtained within 1 hour. When the detector temperature was the same as that of the column, the noise level was the least. Hence, the detector temperature must be kept equal to that of the column. In our experiments, to keep the thermal equilibrium, the detector temperature was maintained at 40°C.

Chromatographic Conditions

Methanol and o-phthalic acid concentration in the mobile can affected the retention characteristics of the components on an anion exchange column. We

Table 2

Dependence of Noise and Drift on Detector Temperature at a Colum Temperature of 40°C

Temperature (°C):	25	30	35	40	45	50	55	60	65
Noise (Hz)	2	1	1	1	1	2	2	4	6
Drift (Hz)	5	4	4	3	3	4	5	6	8

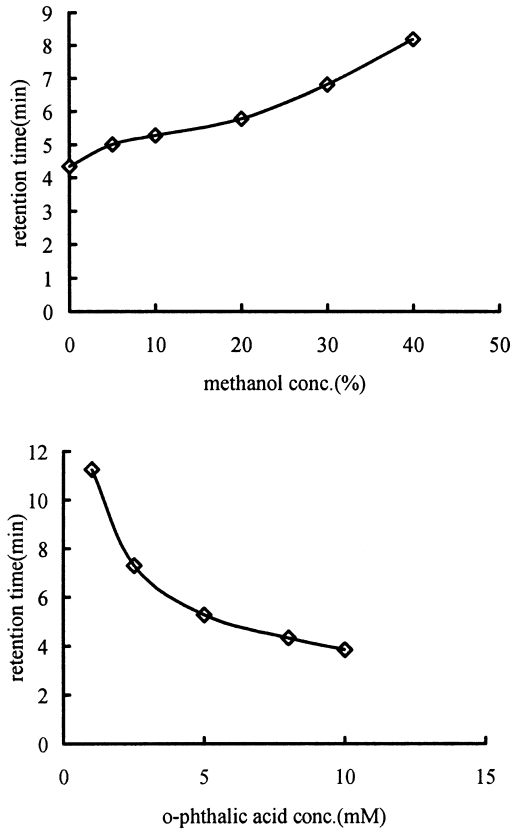

Figure 4. Effects of methanol and o-phthalic acid concentration in mobile on retention: (a) effect of methanol concentration; (b) effect of o-phthalic acid concentration.

investigated the relationship of the retention time of cyclamate with concentration of methanol and o-phthalic acid in the mobile. The results are shown in Figure 4. From the results, the retention time of cyclamate is decreased with decreasing of methanol and increasing of o-phthalic acid concentration in the mobile. When the mobile does not contain o-phthalic acid, the retention time is larger than 30 min. And when the concentration of o-phthalic acid is smaller than 0.5mM, the retention time of cyclamate is larger than 25min. This is disadvantageous for rapid analysis. From analysis of samples such as urine and dried fruit of *Areca catechu* L. etc, the optimum mobile is a mixture of 5.0 mM solution of o-phthalic acid and methanol (90:10 V/V). Under this condition, the cyclamate can be separated completely with interference components.

Comparison of Methods

An HPLC method[7] was used as a comparison method. The method is an isocratic method with pre-column derivatization and UV detection for the quantification of cyclamate. Cyclamate is analyzed as cyclohexylamine, after the simple process of oxidation of the sample by means of hydro peroxide. Trinitrobenzenesulfonic acid (TNBS) appears to be a reagent for the pre-column derivatization. The results for six samples were compared with those obtained by an HPLC method (Table 3). For ice cream and beverage samples, the values of both methods were in agreement. However, cyclamate contents of urine and dried fruit of *Areca catechu* L. obtained by the HPLC method were somewhat higher than those obtained by the proposed method. We think that when cyclamate is separated on an IC-A1 column, its retention principle is ion exchange. The interference components should be ionic compounds mostly, and interference of non-ionic components is small. In contrast, reverse phase columns use the molecular interaction to separate the organic components. And in urine and plant samples, organic components are complex. So, anti-interference ability for organic components of IC is higher than that of RP-HPLC.

Food and Human Urine Sample Analysis

We used the posed method to analyze food and human urine. Dried fruit of *Areca catechu* L. is a food of the common people. Adding sweeteners is a necessary technology within manufacture process. In general, sugar is a com-

Table 3

Results of Sample Analysis and Methods Comparison

Sample	IC-DCQC (g/kg or g/L)	HPLC (g/gk or g/L)
Ice-cream 1	0.177	0.182
Ice-cream 2	0.093	0.010
Beverage 1	0.021	0.021
Dried fruit of *Areca catechu* L. 1	1.271	1.320
Dried fruit of *Areca catechu* L.2	0.342	0.359
Dried fruit of *Areca catechu* L. 3	1.284	1.474
Urine 1	0.0013	0.0027
Urine 2	0.0021	0.0034

mon sweeter. But from the results, many products of the food used sodium cyclamate to replace sugar as a sweetener, and content level of the constitute in the food is always higher than the level which is affirmed by the National Sanitation Standard of P. R. China. Meanwhile, sodium cyclamate was detected in most ice cream samples (Table 3).

Over 70% of the human population are unable to metabolize cyclamate, while only 3 to 5% of the population metabolize more than 20% of their daily intake. Cyclamate is excreted unchanged in urine. So, the determination results of human urine samples can reflect the information of cyclamate intake. From obtained results of urine determined by the proposed method, we found when blank urine from people without cyclamate in their diet was processed, no peaks appeared in the cyclamate region of the chromatogram. We investigated cyclamate concentration in urine of the population who likely eat dried fruit of *Areca catechu* L. The highest value is 132 mg/L and the mean value is 32.1 mg/L. This is related with the high concentration of cyclamate in the food.

CONCLUSIONS

A method based on the conjunction of a double cell quartz crystal (DCQC) detector with signal column ion chromatographic technology for the determination of cyclamate radical has been developed. The optimum experimental parameters of the detector were chosen. The method has high sensitivity and accuracy, independently of the background conductivity of the mobile, from 8.5 to 2450 μS. This makes the detector more advantageous than the conventional conductimetric method. The method has been applied to analyze cyclamate radical in food and human urine successfully.

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