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Determination of divalent carboxylic acids in traditional Chinese herbal and patent medicines using HPLC with double cell quartz crystal detector

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Abstract

A rapid, simple and sensitive high performance liquid chromatography (HPLC) method combined with a novel double cell quartz crystal (DCQC) detector is described for the determination of divalent carboxylic acids in traditional Chinese herbal and patent medicines. The chromatographic system involves the use of a μBondapak-NH_2 column with 20 mM KH_2PO_4 (pH 3.4, adjusted by H_3PO_4)/acetonitrile (95:5%) as a mobile phase. The DCQC detector has a low temperature coefficient and high conductance sensitivity independent of the background from 7.2 to 2500 μS. Analysis of sample is completed without the use of an ion suppression device.

Keywords: Double cell quartz crystal; High performance liquid cromatography; Organic acids

1. Introduction

Traditional Chinese herbal and patent medicines are quite effective. They are made from crude herbs and animal products. Many herbs and patents contain different organic acids. And organic acid content in the products can affect the effects of some Chinese medicines. Therefore, sometimes, determination of organic acids in the products is very important. But many of these acids, such as succinic acid and tartaric acid etc. lack chromophores and are difficult to detect and quantitate by UV detector. A wide variety of chromatographic methods have been used to accomplish the separation and detection of UV-inactive acids, including derivatization followed by gas chromatography [1], conventional liquid chromatography [2–4], and ion chromatography with conventional column, suppresser device, and conductivity detection [5].

The quartz crystal (QC) device has played an important role in probing interfacial processes [6]. The high sensitivity, low cost and conceptual sim-

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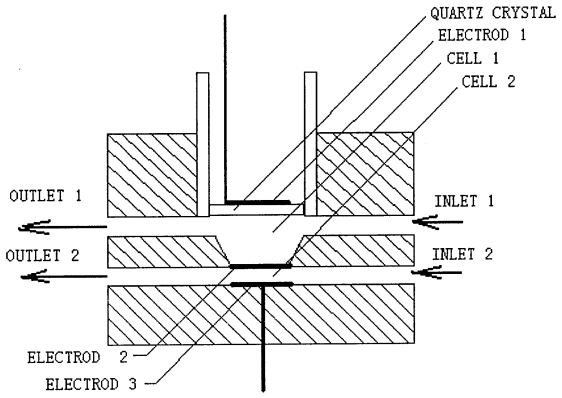

Fig. 1. Schematic diagram of the DCQC detector.

plicity of this method portend its development in a variety of commercial and research application. Several type forms of PQC have been used in chromatography successfully [7–11]. In these researches, series piezoelectric quartz crystal (SPQC) and electrode-separated piezoelectric crystal (ESPC) are used as detectors. Compared with conventional conductivity detector, the SPQC detector is considered to be advantageous in sensitivity and simplicity of construction. But the SPQC detector has also a disadvantage as its sensitivity is affected by background conductivity of the mobile. Recently we have proposed a double cell quartz crystal (DCQC) detector, which can overcome such disadvantage [7]. In this study, we describe the determination of some UV-inactive divalent carboxylic acids and fumaric acid in traditional Chinese herbal and patent medicines by high performance liquid chromatography (HPLC) with the DCQC detector. This method is simple, sensitive and specific, without the use of an ion suppression device.

2. Experimental

2.1. Materials

The standards were of chromatography grade (Sigma, USA). All other chemicals were of analytical reagent grade and were used as received without further purification. Distilled deionizing water was used for preparation of mobile phase and standard solutions. The mobile phase and sample solutions were filtered through a 0.45 μm filter membrane (Millipore, Bedford, MA).

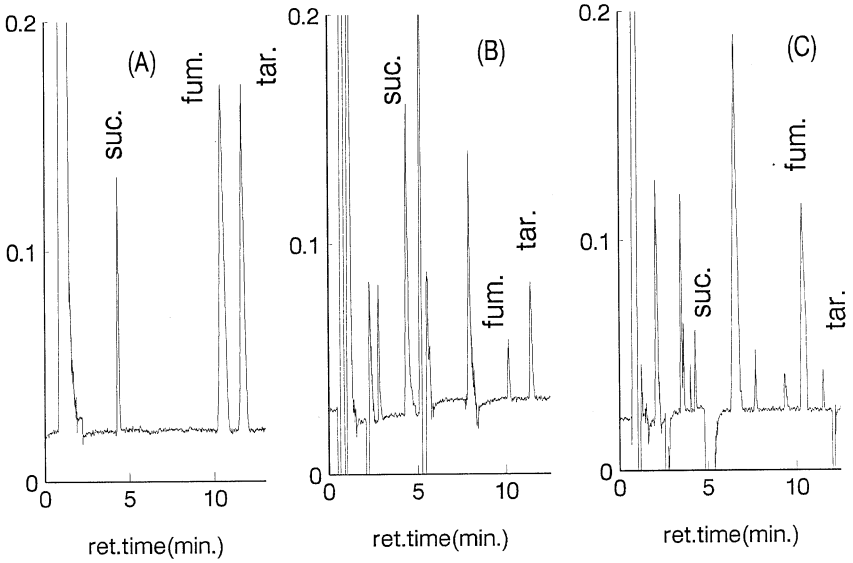

Fig. 2. (A) Standard chromatogram; (B) 1 # patent drug chromatogram; (C) leaf of *Vitis vinifera* L. chromatogram.

Table 1
Sample analysis accuracy

Sample	Acids	Results of analysis (μg/g)					$\overline{X}$	C.V.(%)
Herbal 1 #	Succinic	–[a]	–	–	–	–	–	–
	Fumaric	–	–	–	–	–	–	–
	Tartaric	117	123	115	120	109	116.8	4.1
Herbal 2 #	Succinic	327	341	334	351	339	338	2.3
	Fumaric	–	–	–	–	–	–	–
	Tartaric	104	111	103	115	112	109	4.3
Herbal 3 #	Succinic	–	–	–	–	–	–	–
	Fumaric	179	177	184	189	172	180	3.2
	Tartaric	37	39	42	38	40	39.2	4.4
Patent A	Succinic	78	82	77	77	84	79.6	3.6
	Fumaric	117	108	121	120	113	116	4.1
	Tartaric	43	47	45	43	48	45.2	4.5
Patent B	Succinic	247	261	249	252	259	254	2.2
	Fumaric	89	84	87	93	95	89.6	4.4
	Tartaric	74	82	74	77	73	76	4.3

[a] Content is smaller than 16 μg/g for fumaric acid and smaller than 60 μg/g for succinic acid.

2.2. Herbs and patents information

Herbs such as *Schefflera arboricola* Hayata, *Tamarindus indica* L., *Angelica sinensis* (Oliv.) Diels, *Vitis vinifera* L. were purchased from herbs market, and identified from tissue morphology by Hunan OSST Herbs-Extract Institute, People's Republic of China. 1 # patent and 2 # patent were pill medicines. 1 # contained *Angelica sinensis* (Oliv.) Diels, *Lycium chinense* Mill. etc. and 2 # contained *Sarcandra glabra* (Thunb.) Nakai, *Ficus carica* L. fruit etc.

2.3. Instrumentation

The HPLC system was used for composing a high pressure pump (Model 590, Waters Assoc.), a manual injector (U6K, Waters) provided with a 100-μl loop, a μBondapak-NH_2 column (3.9 × 300 mm), a guard-pak column (0.45 μm) (Waters) placed before the analytical column, and a Baseline 810 chromatographic workstation (Waters).

2.4. Double cell quartz crystal (DCQC) detector

The DCQC detector was made in this laboratory [7]. Fig. 1 shows the schematic diagram of the DCQC detector. The quartz crystal was mounted on the top of a Teflon column with one side facing liquid. The electrode on this side was removed, therefore the two opposite electrodes inducing an alternating electrical field across the crystal were separated by two flow-through conductivity cells, cell 1 and cell 2. Cell 1 was the adjustment cell and one side of the crystal was in contact with liquid in this cell. Cell 2 was the sample cell and mobile from the chromatographic column flowed through the cell. The cell constant of cell 1 can be adjusted by changing the position of PTFE column with the crystal and the cell constant of cell 2 is 0.85 cm. The piezoelectric quartz crystal was used for 9 MHz and AT-Cut.

Table 2
Sensitivity comparison of conventional conductivity and DCQC detector on different background

Background (μs)	Response of detector (μV s)	
	DCQC	Conventional
287	8.42E+05	7.14E+05
450	8.27E+05	5.27E+05
1100	7.71E+05	6.47E+04
1870	7.02E+05	8.03E+03

Table 3
Determination results comparison by different methods[a]

Sample	Acids	Determination results (μg/g)	
		DCQC	Conventional
1 #	Succinic	274	283
	Fumaric	177	174
	Tartaric	454	442
2 #	Succinic	543	560
	Fumaric	478	477
	Tartaric	217	208
3 #	Succinic	70	75
	Fumaric	743	762
	Tartaric	317	305
4 #	Succinic	117	115
	Fumaric	443	451
	Tartaric	80	77

[a] The mixed standard was added in a sample. 1 #, 2 # and 3 # were *Angelica sinensis* (Oliv.) Diels. Sample 4 # was root of *Schefflera arboricola* Hayata.

A frequency-to-voltage converter (made in this laboratory ([10]) 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.

2.5. Chromatographic condition

Chromatographic runs consisted of isocratic elution. The flow-rate of mobile phase, 20 mM KH_2PO_4 (pH 3.4, adjusted by H_3PO_4)/acetonitrile (95:5%), was 1.0 ml/min. All solutions were filtered through a type HA 0.45 μm membrane filter (Millipore) and degassed in vacuum. The flow cell of the DCQC detector was maintained at 40°C. No detector drift due to thermal effects was observed.

The eluant was pumped into the chromatographic system. The sample solution was introduced after the baseline was stabilised ($f0$ and $v0$), and the voltage signals ($v1$) was recorded versus time by Baseline 810. The concentration of the tested ion was calculated from the obtained frequency shift versus time chromatogram by comparing it with the standard.

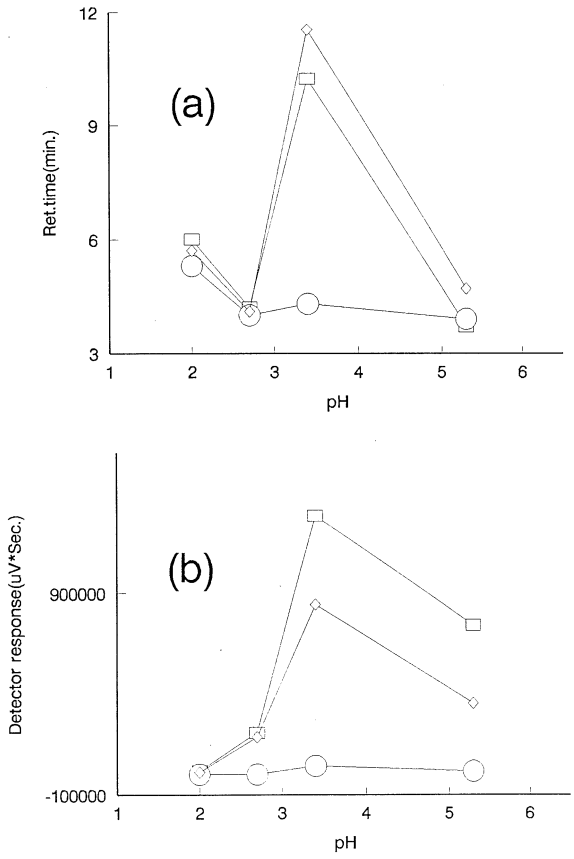

Fig. 3. pH of mobile effect on separation (a) and detection sensitivity (b). –○– succinic; –□– fumaric; –◇– tartaric.

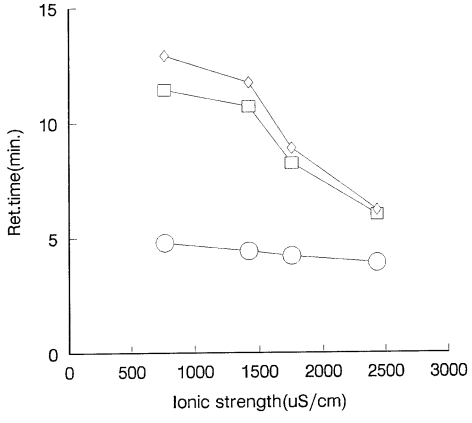

Fig. 4. Ionic strength of mobile effect on separation. –○– succinic; –□– fumaric; –◇– tartaric.

Table 4
Sample analysis results

Sample	Succinic (μg/g)	Fumaric (μg/g)	Tartaric (μg/g)
Root of *Schefflera arboricola* Hayata	–[a]	457	82
Fruit of *Tamarindus indica* L.	–[a]	46	734
Leaf of *Vitis vinifera* L.	253	112	341
Angelica sinensis. (Oliv.) Diels	–[a]	–[a]	211
Patent 1 #	891	63	439
Patent 2 #	117	1284	293

[a] Content is smaller than 16 μg/g for fumaric acid and smaller than 60 μg/g for succinic acid.

2.6. Sample preparation

All herbal and patent drug samples were ground through a 1-mm screen. The sample (0.1–1.0 g) was accurately weighed and 25 ml of petroleum ether (b.p. 30–60°C) was added to extract fatty components in the sample, the extraction process being repeated three times. The residue after extraction was dried under nitrogen flow at 40°C. Then 5 ml of 0.01 M NaOH was added to the dried residue. The mixture was shaken under nitrogen, left at room temperature overnight and filtered off, and the solution pH was adjusted with 0.1 M H_3PO_4 to 5. Then the solution was diluted to 10 ml with water and filtered through a 0.45-μm filter, 50 μl of the filtrate was injected on to the HPLC column.

3. Results and discussion

3.1. Chromatographic calibration of organic acids

Fig. 2 shows the chromatogram for a mixed standard of several organic acids and herbal, patent drugs analysis chromatograms obtained under the after-mentioned conditions. The retention times of succinic, fumaric, and tartaric acids are 4.30, 10.24 and 11.54 min, respectively. No other components in the sample interfered significantly with the detection of organic acids. The calibration graphs for succinic acid, fumaric acid and tartaric acid were linear over the ranges 20–100, 5–30 and 10–50 μg/ml, respectively. The correlation coefficients of the calibration graphs were >0.9992. The detection limits (signal-to-noise ratio, 3; noise, 1 Hz) were 3.0 μg/ml for succinic acid, 0.8 μg/ml for fumaric acid and 1.5 μg/ml for tartaric acid. The recovery of the method was determined by spiking known concentrations of the organic acids into the herbal drug, leaf of *Vitis vinifera* L., and 1 # patent drug. The samples were chromatographed by the described procedure, yielding a mean recovery of 97.3% for succinic acid, 104.8% for fumaric acid and 98.1% for tartaric acid for five spiked 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 succinic acid was less than 4%, for fumaric acid was less than 5% and tartaric acid was less than 5%.

3.2. Working condition of the DCQC

For the DCQC detector, the ΔF versus ΔG relationship ($\Delta F/\Delta G$) 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. 500 μS. Cell constants can affect the detector performance. In this work, the cell constants were optimised, $k1 = 1.0$ cm, $k2 = 0.85$ cm.

For 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. Table 2 shows

comparison results of detection sensitivity of these two detectors at different background conductivity. It is obvious that the response of the DCQC is more stable than that the conventional conductimetric detector.

For the SPQC detector, the response sensitivity is good when the mobile conductivity is on a range 150–1200 μs [8]. When the mobile conductivity is over 3000 μs, 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 by 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 [7]. It extends to 7.2–2500 μS. Because the crystal does not come in contact with the mobile directly, the crystal oscillation stability is not affected by the liquid flowing in the sample cell. Therefore, the DCQC oscillation is very stable.

Temperature can affect the response stability of the DCQC detector. Hence, the detector temperature must be kept constant. In our experiments, to keep the thermal equilibrium, the detector temperature was maintained at 25°C.

3.3. Method comparison

In our investigation on determination of UV-inactive organic acids in Chinese herbal and Chinese patent medicines, HPLC-conventional conductimetric detection technique [12] was used as a method for comparison. The results are shown in Table 3. No significant differences were observed between the two methods.

3.4. Chromatographic conditions

Because the DCQC detector response depends on the change in conductivity of the mobile, the charge detected must be an anion. The pH of the mobile not only affects the separation, but also the detection sensitivity. Fig. 3 shows effect of pH on separation and detection sensitivity. From results, there is an optimum pH value to meet separation and detection. In this experiment, the optimum pH is 3.4.

Organic content and ionic strength of the mobile are also important parameters for the separation. Fig. 4 shows the effects of these parameters on the separation. Under different pH values, the organic content effects in mobile for separation is not same. When pH of mobile is 3.4, the optimum organic content is 5% and the optimum ionic strength of the mobile is 20 mM potassium dihydric phosphate.

3.5. Sample preparation

Before extracting sample, taking off fatty components step must be employed. Otherwise, many negative peaks present in the sample chromatogram may affect quantitation of the organic acids. Maybe the neutral components produced these negative peaks. After taking off fatty components, the negative peaks obviously decreased and the analysis results were always higher (about 4–10%) than non-taking off fatty components step.

3.6. Sample determination

We determined fumaric acid, succinic acid and tartaric acid in herbal drugs such as *Schefflera arboricola* Hayata, *Tamarindus indica* L., *Angelica sinensis* (Oliv.) Diels, *V. vinifera* L. and some patent drugs. The patent drugs were made in our laboratory according to ancient formulas and traditional manufacturing technology. Table 4 shows the analysis results of samples. It can be seen from the results that the concentration of the organic acids in herbal is always lower than that in patent drugs. Specially, fumaric acid, because of its strong analgesic effect, is a major bioactive component in some patent drugs. So, its concentration in patent drugs is higher than in herbal sources.

4. Conclusions

The HPLC-DCQC method described here has sufficient sensitivity to determine UV-inactive or-

ganic acids in traditional Chinese herbal and patent medicines. The method is simple, rapid and accurate, and the possibility of application of the DCQC detector in HPLC has been demonstrated. In this method, the use of the ion suppression device is not necessary. The method can be used to monitor the quality of traditional Chinese patent medicine and investigate the acids content in traditional Chinese herbal medicine.

Acknowledgements

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References

[1] H. Katoaka, et al., J. Chromatogr. 473 (1) (1989) 276.
[2] D.J. Pietrzyk, et al., J. Chromatogr. Sci. 27 (8) (1989) 485.
[3] P.G. Rigas, et al., Anal. Chem. 59 (1987) 1388.
[4] L. Hackzell, et al., Acta Pharm. Suec. 18 (5) (1981) 271.
[5] J.S. Fritz, J. Chromatogr. 546 (1991) 111.
[6] M.D. Ward, D.A. Buttry, Science 249 (1990) 1000.
[7] K. Chen, P. Chen, L. Nie, S. Yao, J. Chromatogr. A 753 (1996) 171–176.
[8] P. Chen, L. Nie, S. Yao, J. Chromatogr. Sci. 33 (1995) 268–272.
[9] P. Chen, L. Nie, S. Yao, J. Chromatogr. B 673 (1995) 153–158.
[10] P. Chen, L. Nie, S. Yao, X. Fan, Instrum. Sci. Technol. 23 (1995) 137–145.
[11] B. Yu, P. Chen, L. Nie, S. Yao, Anal. Lett. 291 (1996) 43–57.
[12] S.L. Barbara, et al., Anal. Chem. 68 (1996) 1067–1070.