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Rapid determination of 5-fluorouracil in plasma using capillary electrophoresis

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Abstract

A rapid, simple and sensitive capillary electrophoresis (CE) method used for the determination of 5-fluorouracil in rabbit plasma is described in the present paper. In this method, samples were simply pretreated by a solvent extraction procedure prior to injection. With a running buffer composed of 30 m*M* Tris–H_3PO_4 (pH 7.0) and 5% isopropanol, 5-fluorouracil was easily separated from the external standard α-phenethylol as well as other substances existed in the plasma. A linearity of 5-fluorouracil was determined in the range from 0.17 to 42.50 μg/ml with a correlation coefficient of 0.999. A limit of quantitation (LOQ) corresponding to signal-to-noise ratio of 10 was obtained (LOQ=0.08 μg/ml). The method was successfully used for determining the 5-fluorouracil in real plasma samples from rabbits.

Keywords: Drug analysis; 5-Fluorouracil

1. Introduction

5-Fluorouracil (5-FU) is a chemotherapeutic drug that has been widely used for the treatment of advanced gastrointestinal cancer, breast cancer and several other types of cancer for many years. So far the therapeutic mechanism of 5-FU remains unclear. It has been proved that there is a good relationship between concentration of 5-FU in plasma and toxicities and therapeutic for the treatment of different types of tumours. A high individual variability in 5-FU pharmacokinetics was observed [1,2]. Therefore, it is of great practical importance to determine concentration of 5-FU in plasma. Up to now, many analytical methods including gas chromatography–mass spectrometry (GC–MS) [3,4], magnetic resonance spectroscopy (MRS) [5], high-performance liquid chromatography (HPLC) [3,6–17], and HPLC–MS [18] have been reported for the determination of 5-FU in biological samples even in environment samples [19–21]. GC–MS and HPLC–MS methods need a time-consuming operation for sample derivatization. The MRS method is not popular because of its higher operational cost and large sample consuming. Also most of the HPLC methods use a low pH mobile phase that would shorten the life of the column. Moreover some HPLC methods are complicated including column switching [6], four column systems [7] or temperature gradient and gradient elution [8]. Since capillary electrophoresis (CE) has several advantage over

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HPLC, such as high separation efficiency, minimum sample treatment, short analysis time, low operational costs and low sample requirement, CE has been developed to determine 5-FU in body fluids. You [22] developed a CE method using amperometric detection to determine 5-FU in spiked blank body fluids. Reeuwijk [23] analyzed 5-FU as an external standard in plasma samples by CE. CE hyphenated with microdialysis was used to determine 5-FU in microdialyzates obtained from primary breast cancer patients [24]. Recently, a CE method was developed to determine 5-FU in biological materials, but the limit of detection was only 1.72 μg/ml [25]. The aim of this present paper is to develop a simple and rapid method for the quantitative determination of 5-FU in plasma with improved sensitivity as compared to the previously reported methods. To the best of our knowledge it is the first paper using CE method to determine 5-FU in real plasma samples taken from rabbits at different time.

2. Experimental

2.1. Chemicals and reagents

5-FU (purity: ≥99.8%) was obtained from China Institute for the Control of Pharmaceutical and Biological Products. α-Phenethylol was purchased from Beijing Chemical Plant (Beijing, China). Tris-(hydroxymethyl)amino-methane (Tris) was purchased from Shanghai Chemical Plant (Shanghai, China). All other chemicals were analytical reagents. The blank plasma and the real blood samples were provided by the First Affiliated Hospital of Lanzhou Medical College.

2.2. Instrumentation

All experiments were carried out on a BioFocus 3000 capillary electrophoresis system with a UV detector (Bio-Rad, USA). The applied voltage was held constant at 15 kV. Fused silica capillary was purchased from Yongnian Optical Fiber Factory (Hebei Province, China) with a total length of 35 cm and an effective length of 30 cm (50 μm I.D., 365 μm O.D.). Before use, the capillary was rinsed for 4 min with 0.1 mol/l NaOH, deionized water and running buffer, respectively. Between runs the capillary was only rinsed with running buffer for 2 min. The UV detector was operated at 260 nm at which 5-FU has a maximum absorption. The temperature of the capillary was maintained at 20 °C. Samples were pressure injected by 5.52×10^4 Pa*s.

2.3. Preparation of running buffer and standard solutions

All solutions were prepared with redistilled water. Stock solutions of 250 m*M* Tris and 200 m*M* phosphoric acid were prepared. The running buffer solutions were composed of 10–40 m*M* Tris and a certain amount of organic additive (methanol, isopropanol, or acetonitrile) and the pH was adjusted to 6.0–8.0 with H_3PO_4. The running buffers were filtered through a 0.45-μm syringe filter prior to use. Stock solution of 5-FU was prepared by dissolving 8.5 mg 5-FU in 50.00 ml water, resulting in a solution containing 0.17 mg/ml. A stock solution of the external standard at a concentration of 1% (v/v) was prepared by diluting 100 μl α-phenethylol to 10.00 ml with water.

Calibration curve solutions were prepared by spiking nine different concentrations of 5-FU in 100 μl solution to 0.5 ml drug-free rabbit plasma. The 5-FU was extracted by adding 2 ml ethyl acetate followed by shaking for 2 min. After centrifuging (4000 rpm) for 10 min, 1.5 ml supernatant was carefully transferred into a tapered tube. The resultant extract was evaporated to dryness in a vacuum drying oven at 60 °C. Finally the residue was redissolved in 100 μl water and directly injected for determination by HPLC. However, 40 μl of this sample solution was mixed with 10 μl 1% α-phenethylol prior to inject into CE.

2.4. Real blood samples

Five rabbits with weight 1.65–2.10 kg were used and marked randomly with A, B, C, D and E in this study. 5-FU was injected through the marginal vein of ear (A), portal vein (B), superior mesenteric artery (C) and arteriae lienalis (D and E), respectively, and the injection dose was 15 mg/kg. Blood samples were taken from rabbits after injection of 5-FU for 5, 10, 15, 20, 30, 60, 90, 120, 150 and 180 min,

respectively. The real blood samples were pretreated as described in Section 2.3.

3. Results and discussion

3.1. Method development and optimization

A standard solution containing 5-FU and external standard was easily separated with high resolution with the running buffer composed of 40 m*M* Tris–H_3PO_4 (pH 6.0). However, when the spiked plasma samples were analyzed, the 5-FU was almost co-eluted with an unknown substance existed in the plasma. Therefore effort must be done to improve the separation. Initially, an approach of using isopropanol as buffer modifier was tried out. With increasing the concentration of isopropanol in the buffer from 0 to 25% resolution factor (between the peak of unknown substance and that of 5-FU) increased from 0.74 to 1.30. No any improvement of the resolution was achieved with increasing the concentration of isopropanol to 30%. However, the migration time of 5-FU was prolonged from 3.18 to 14.78 min due to the increase of viscosity of the buffer. Therefore the buffer solution containing 25% isopropanol was selected for the following experiment.

The effect of Tris concentration on the separation of the spiked plasma samples was studied in the concentration ranging from 10 to 40 m*M*. The resolution increased from 0 to 1.73 as the concentration of Tris increased from 10 to 30 m*M*. The resolution became to decrease with further increasing the concentration of Tris to 40 m*M*. Furthermore, the effect of buffer pH on the separation of the spiked plasma samples was also studied in a pH ranging from 6.0 to 8.0. In this case, the resolution factor of 1.73, 2.60 and 1.76 was obtained at pH 6.0, 7.0 and 8.0, respectively. Based on the above described experiments, it was found that the buffer pH and the concentration of Tris played more important role for improvement of the separation. Therefore the concentration of isopropanol was optimized again to short the separation time. Keep the concentration of Tris at 30 m*M* and buffer pH at 7.0, respectively, change the isopropanol concentration from 0 to 25%. It was found that the separation was good enough using the buffer only containing 5% isopropanol. In this case, the resolution factor of 2, and the separation time shorter than 6 min was obtained.

Finally, a buffer system composed of 30 m*M* Tris–H_3PO_4 (pH7.0) containing 5% isopropanol was selected as the running buffer for the determination of 5-FU in real plasma samples (Fig. 1, the sample was from rabbit A injected after 15 min).

3.2. Method validation

The linearity of the present method was prepared using blank plasma samples spiked with different amounts of 5-FU in order to obtain the concentrations of 0.17, 0.33, 0.66, 1.33, 2.66, 5.31, 10.63, 21.25, 42.50 μg/ml in plasma, respectively. The samples were extracted as described in Section 2.3. The ratio of the peak area of 5-FU to the external standard was used as assay parameter. Peak–area ratios were plotted against theoretical concentrations. The relationships between theoretical concentrations and peak–area ratios were linear in the concentration range 0.17–42.50 μg/ml. The regression equation for 5-FU was $y = 0.0142x + 0.0007$, $r = 0.999$ (x represented concentration of 5-FU in plasma; y represented peak–area ratio). Limit of quantitation

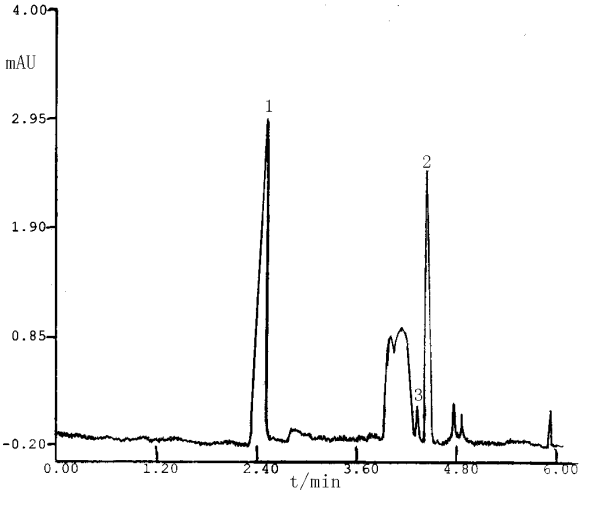

Fig. 1. Electropherogram of real blood sample from rabbit A injected after 15 min. Buffer: pH 7.0, 30 m*M* of Tris–H_3PO_4, containing 5% isopropanol; 1 = α-phenethylol; 2 = 5-FU; 3 = unknown substance.

Table 1
Reproducibility of the CE method (RSD%)

	5-FU concentration (μg/ml)		
	1.3	10.6	42.5
RSD of t_{FU}	2.1	3.0	4.0
RSD of A_{FU}	7.3	6.2	8.0
RSD of t_{FU}/t_{ES}	0.6	0.9	1.1
RSD of A_{FU}/A_{ES}	2.8	1.4	2.8

t_{FU} and t_{ES} represented migration times of 5-FU and external standard, respectively. A_{FU} and A_{ES} represented peak areas of 5-FU and external standard, respectively.

(LOQ) corresponding to signal-to-noise ratio of 10 was also determined (LOQ = 0.08 μg/ml).

To investigate the reproducibility of the CE method, three spiked plasma samples of concentration 1.33, 10.63 and 42.50 μg/ml were continuously analyzed nine times, respectively. Then reproducibilities of migration time and peak area in terms of the relative standard deviation (RSD) were evaluated, and the results were shown in Table 1. Whichever concentration level was used RSD <4.1 and <8.0% were obtained for migration time and peak area, respectively. Moreover RSD <1.2 and <2.9% were gained for the ratio of eluted time of 5-FU to that of external standard and the ratio of the peak area of 5-FU to that of external standard, respectively. Therefore, the reproducibility of the method was quite satisfactory.

Recovery was determined using four spiked plasma samples with concentration levels of 0.37, 1.70, 3.40, 17.00 μg/ml. The absolute recovery was calculated using the ratios of the peak area of 5-FU to that of external standard which were determined in the extracted plasma and directly injected aqueous solutions of the same concentration, respectively. The determined recoveries of 82.6, 82.9, 87.1 and 86.7%, respectively, were gained at those different concentrations.

Table 3
Determination results of rabbit E (μg/ml)

t (min)	Concentration (μg/ml)	
	CE	HPLC
5	4.6	121.6
10	2.2	106.4
15	9.3	31.7
20	5.6	18.5
30	2.5	10.3
60	2.3	2.1
90	1.2	1.4
120	4.2	4.4

3.3. Determination of real blood samples

The quantitative results of the real blood samples by CE and HPLC methods were summarized in Table 2. The trend of the concentrations obtained from CE method was approximately similar to those from HPLC method in all four rabbits injected with different mode. And the data of CE were in consistent with those of HPLC in most of the time points. However the concentrations of 5-FU in real plasma samples obtained from rabbit E showed much different between CE and HPLC (the results were shown in Table 3). The results obtained by CE were

Table 2
Determination results of rabbits A, B, C and D (1 and 2 represented CE and HPLC method, respectively, μg/ml)

t (min)	A(1)	A(2)	B(1)	B(2)	C(1)	C(2)	D(1)	D(2)
5	56.0	52.7	48.7	48.1	132.1	138.5	106.9	103.3
10	30.7	28.3	19.4	19.1	30.2	28.1	108.7	104.7
15	12.8	13.3	8.6	8.5	26.2	25.4	30.5	27.2
20	7.0	6.9	5.2	4.9	4.1	5.2	17.7	18.7
30	4.3	4.5	–	–	2.3	2.2	4.8	4.9
60	1.2	1.4	–	–	1.4	1.8	1.5	2.0
90	0.4	0.5	0.6	0.6	0.4	0.7	0.7	1.3
120	2.5	2.5	0.4	0.3	0.3	0.6	–	–
150	0.4	0.3	0.3	0.4	0.5	0.9	0.3	0.4
180	1.8	2.1	1.2	1.3	3.2	3.4	1.4	1.8

The concentrations >45.5 μg/ml were obtained by determining the diluted samples.
(–): lacked these samples.

much lower than those of HPLC, at the following time 5, 10, 15, 20 and 30 min after injecting rabbit E. These might be illustrated that some substances could not be separated from the main component by HPLC. Comparing the electropherogram with the HPLC chromatogram (Fig. 2, the sample was from rabbit B injected after 15 min), it was obviously shown that more substances were found in electropherogram.

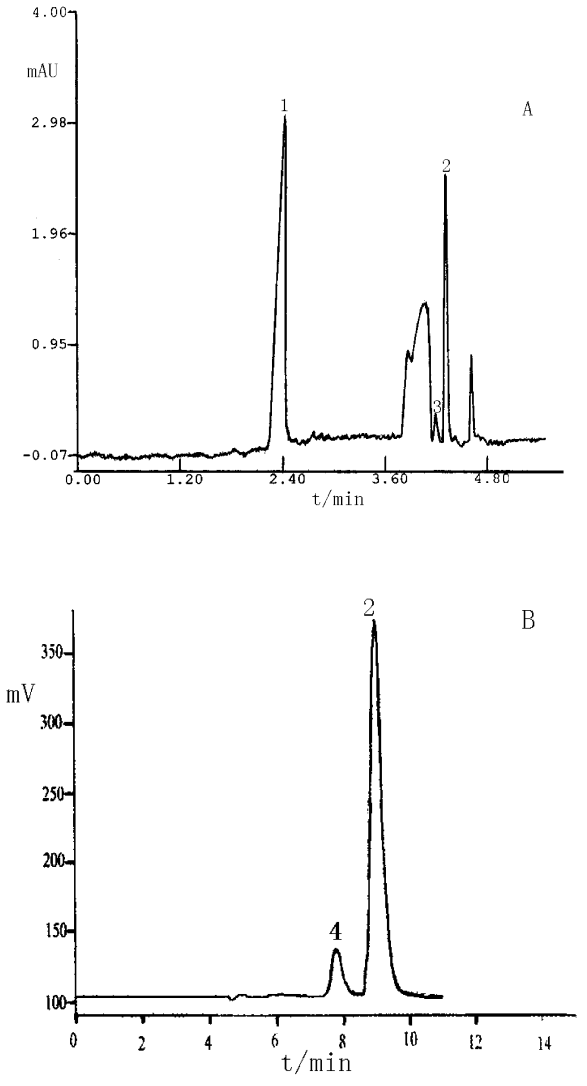

Fig. 2. Electropherogram(A) and HPLC chromatogram (B) of real blood sample from rabbit B injected after 15 min. (A) Buffer: pH 7.0, 30 m*M* of Tris–H_3PO_4, containing 5% isopropanol; 1=α-phenethylol; 2=5-FU; 3=unknown substance. (B) Column: Diamonsil C_{18} 5 μm, 150×4.6 mm; protect column: ODS 10 μm, 50×4.6 mm; mobile phase: methanol–water (3:97, v/v), adjusted to pH 3.5 with acetic acid; flow-rate: 0.5 ml/min; 2=5-FU; 4=unknown substance.

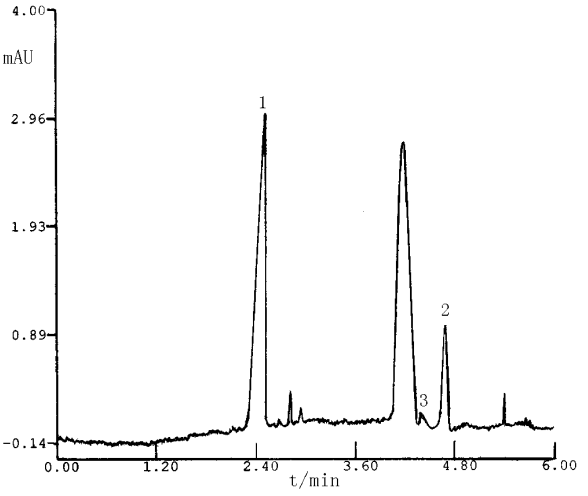

Fig. 3. Electropherogram of real blood sample from rabbit E injected after 60 min. Buffer: pH 7.0, 30 m*M* of Tris–H_3PO_4, containing 5% isopropanol; 1=α-phenethylol; 2=5-FU; 3=unknown substance.

When the height of the substance marked as peak 3 was lower, then the result of CE was very close to that of HPLC method (Fig. 3, sample from rabbit E

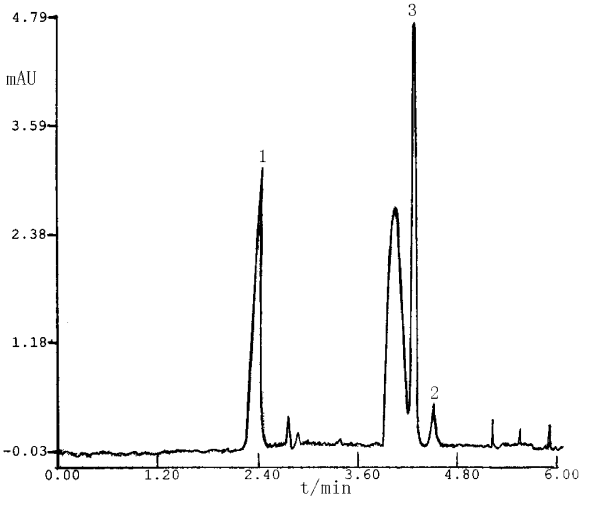

Fig. 4. Electropherogram of real blood sample from rabbit E injected after 10 min. Buffer: pH 7.0, 30 m*M* of Tris–H_3PO_4, containing 5% isopropanol; 1=α-phenethylol; 2=5-FU; 3=unknown substance.

injected after 60 min). This phenomenon was proved by the other electropherograms in which the peak 3 was lower and the concentrations determined by CE and HPLC were similar (Figs. 1 and 2A). While the peak 3 in electropherograms was higher, then the results of CE were much lower than those of HPLC (Fig. 4, sample from rabbit E injected after 10 min). It might indicate that the peak of 5-FU coeluted with some substances in HPLC. And these substances should have the same character of UV absorption as 5-FU because the peak of 5-FU was identified to be purity by photodiode array detector. Figs. 1–4 also illustrated that the number or amount of substances in the real blood samples varied for different rabbits or even the same rabbit at different time.

4. Conclusion

The rapid and effective CE method established in this paper might be popularized or at least be used as a supplementary method for HPLC by the hospitals in which many plasma samples containing 5-FU should be analyzed.

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References

[1] C. Katona, J. Kralovanszky, A. Rosta, E. Pandi, G. Fonyad, K. Toth, A. Jeney, Oncology 55 (1998) 468.
[2] E. Gamelin, M. Boisdron-Celle, R. Delva, C. Regimbeau, P.E. Cailleux, C. Alleaume, M.L. Maillet, M.J. Goudier, M. Sire, M.C. Person-Joly, M. Maigre, P. Maillart, R. Fety, P. Burtin, A. Lortholary, Y. Dumesnil, L. Picon, J. Geslin, P. Gesta, E. Danquechin-Dorval, F. Larra, J. Robert, J. Clin. Oncol. 16 (1998) 1470.
[3] E. Matsushima, K. Yoshida, R. Kitamura, K. Yoshida, J. Chromatogr. B 691 (1997) 95.
[4] D. Anderson, D.J. Kerr, C. Blesing, L.W. Seymour, J. Chromatogr. B 688 (1997) 87.
[5] P.M.J. McSheehy, M.T. Seymour, A.S.E. Ojugo, L.M. Rodrigues, M.O. Leach, I.R. Judson, J.R. Griffiths, Eur. J. Cancer 33 (1997) 2418.
[6] T. Maeda, S. Sumi, K. Hayashi, K. Kidouchi, T. Owaki, H. Togari, S. Fujimoto, Y. Wada, J. Chromatogr. B 731 (1999) 267.
[7] L.K. House, J. Ram'rez, M.J. Ratain, J. Chromatogr. B 720 (1998) 245.
[8] J. Escoriaza, A. Aldaz, E. Calvo, J. Giráldez, J. Chromatogr. B 736 (1999) 97.
[9] W. Wattanatorn, H.L. Mcleod, J. Cassidy, K.E. Kendle, J. Chromatogr. B 692 (1997) 233.
[10] J.M. Joulia, F. Pinguet, P.Y. Grosse, C. Astre, F. Bressolle, J. Chromatogr. B 692 (1997) 427.
[11] M. Jung, G. Berger, U. Pohlen, S. Päuser, R. Reszka, H.J. Buhr, J. Chromatogr. B 702 (1997) 193.
[12] C.G. Zambonin, A. Aresta, M. Grano, J. Pharm. Biomed. Anal. 17 (1998) 11.
[13] W.J. Loos, P. de Bruijn, L. van Zuylen, J. Verweij, K. Nooter, G. Stoter, A. Sparreboom, J. Chromatogr. B 735 (1999) 293.
[14] M.A. Fruitwala, N.M. Sanghavi, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 471.
[15] E. Gamelin, M. Boisdron-Celle, A. Turcant, F. Larra, P. Allain, J. Robert, J. Chromatogr. B 695 (1997) 409.
[16] E. Gamelin, M. Boisdron-Celle, F. Larra, J. Robert, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 3155.
[17] S.P. Ackland, M.B. Garg, R.H. Dunstan, Anal. Biochem. 246 (1997) 79.
[18] K. Wang, M. Nano, T. Mulligan, E.D. Bush, R.W. Edom, J. Am. Soc. Mass Spectrom. 9 (1998) 970.
[19] L. Floridia, A.M. Pietropaolo, M. Tavazzani, F.M. Rubino, A. Colombi, J. Chromatogr. B 724 (1999) 325.
[20] G. Micoli, R. Turci, M. Arpellini, C. Minoia, J. Chromatogr. B 750 (2001) 25.
[21] T. Kiffmeyer, H.J. Goetze, M. Jursch, M. Jursch, U. Lueders, Fresenius' J. Anal. Chem. 361 (1998) 185.
[22] T. You, X. Wang, X. Yang, E. Wang, Anal. Lett. 32 (1999) 1109.
[23] H.J.E.M. Reeuwijk, U.R. Tjaden, J. van der Greef, J. Chromatogr. B 726 (1999) 269.
[24] R.M. Mader, M. Brunner, B. Rizovski, C. Mensik, G.G. Steger, H.G. Eichler, M. Mueller, Electrophoresis 19 (1998) 2981.
[25] A. Prochazkova, S. Liu, H. Friess, S. Aebi, W. Thormann, J. Chromatogr. A 916 (2001) 215.