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Simple and rapid analytical method for carbapenems using capillary zone electrophoresis

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Abstract

A simple and rapid analytical method for carbapenems using high-performance capillary electrophoresis is described. All therapeutic carbapenem injections in Japan (imipenem, panipenem and meropenem) and four other β-lactams (piperacillin, cefotiam, cefotaxime, latamoxef) were separated and determined with good repeatability in about 10 min using simple free zone capillary electrophoresis. The electrophoresis buffer was 100 m*M* phosphate buffer of pH 8.0, and a fused-silica capillary of 25 μm I.D. and 47 cm length was adopted. The present method was successfully applied to monitor the degradation of carbapenems under various conditions (at various temperatures or in coexistence with other drugs prescribed in the case of methicillin-resistant *Staphylococcus aureus*).

Keywords: Carbapenems; β-Lactams

1. Introduction

Carbapenems are the newest β-lactam antibiotics developed in the last decade, which are used against serious infectious diseases or methicillin-resistant *Staphylococcus aureus* (MRSA). In the case of infectious disease, an adequate quantity of antibiotics must be administered to prevent the emergence of some drug-resistant bacteria. However, β-lactam antibiotics are unstable drugs [1–7], and are thought to degrade due to heat, light or mixing with other drugs [8–11]. Actually, therapeutic β-lactams are often administered in various combinations with other drugs taking into account the symptoms and age of the patients. Carbapenems are one of the last therapeutic drugs against MRSAs; therefore, it is important to measure the effective quantity of antibiotics just before the administration. However, in medical institutions, the lack of an appropriate simple and rapid method to quantify the carbapenems prevents the immediate inspection of the effective amount of the drug to be administered.

In this paper, a simple and rapid determination of carbapenems is described. High-performance capillary electrophoresis (HPCE) was used for rapid analysis with high resolution. As the sample, three carbapenem injections (imipenem, IPM; panipenem, PAPM; meropenem, MEPM) and several therapeutic β-lactams (piperacillin, PIPC; cefotiam, CTM; cefotaxime, CTX; latamoxef, LMOX) were used. Because of the simplicity of the method, capillary zone electrophoresis (CZE) was adopted, and various

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separation conditions concerning the electrophoresis buffer and the capillary itself were examined. With the present method, all carbapenem injections commercially available in Japan could be determined under the same analytical conditions for the first time. The CZE method developed was successfully applied to monitor the degradation of carbapenems under various conditions including the prescription for MRSAs.

2. Experimental

2.1. Reagents

The injections and drug substances of β-lactam antibiotics were supplied by pharmaceutical companies. Pentcillin (piperacillin; composition) and Carbenin (panipenem) were obtained from Sankyo (Tokyo, Japan), Pansporin (cefotiam) was from Takeda Chemical Industries, (Osaka, Japan), Claforan (cefotaxime) was from Hoechst Marion Roussel (Frankfurt, Germany), Shiomarin (latamoxef) was from Shionogi (Osaka, Japan), Tienam (imipenem) was from Banyu Pharmaceutical (Tokyo, Japan) and Meropen (meropenem) was from Sumitomo Pharmaceuticals (Osaka, Japan). The chemical structures of the β-lactams are listed in Fig. 1. Fosfomycin and vancomycin injections were commercially available. HPLC-grade methanol was purchased from Nakarai Tesque (Kyoto, Japan). Boric acid was obtained from Wako Pure Chemical Industries (Osaka, Japan). Sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dihydrate and sodium hydroxide solution were obtained from Merck (Darmstadt, Germany). All other chemicals were reagent grade. Water was purified by the Milli-Q II water purification system (Millipore, Bedford, MA, USA).

2.2. HPCE conditions

CZE was performed with a P/ACE system 5510 (Beckman Instruments, Fullerton, CA, USA) in a fused-silica capillary tube (Supelco, Bellefonte, USA) which was kept at 25°C. The electrical current was monitored throughout the operation. Detection was carried out by the measurement of UV absorption at 210, 280 or 300 nm at a position of 70 mm from the negative end of the tube with a P/ACE diode array detector. The absorption spectrum from 190 to 400 nm toward each peak was also measured. For data processing, an Epson dot-matrix printer was used. The sample solution was siphoned by pressure (0.5 p.s.i., 5 s). All solutions subjected to HPCE were filtered through a 0.45 μm membrane filter (Tosoh, Tokyo, Japan).

2.3. HPLC conditions

A reversed-phase high-performance liquid chromatography (HPLC) system with a UV detector was used. The system was equipped with a 655-A12 pump (Hitachi, Tokyo), a 7125 injector (Rheodyne, Cotati, CA, USA), a UV-8010 detector (Tosoh) and an 807-IT integrator (Jasco, Tokyo, Japan). The HPLC conditions were as follows: analytical column: TSK-gel ODS-80Ts (4.6 mm I.D.×15 cm, Tosoh); column temperature: IPM and MEPM, 25°C; PAPM, 40°C; mobile phase: IPM, 10 m*M* MOPS buffer (pH 7.0)–acetonitrile=100/1 (v/v); PAPM, 10 m*M* MOPS buffer (pH 8.0)–acetonitrile=100/3 (v/v); MEPM, 20 m*M* sodium-phosphate buffer (pH 5.0)–methanol=85/15 (v/v); flow rate, 1 ml/min; detection: IPM, 280 nm; PAPM and MEPM, 300 nm.

2.4. Sample preparation

Each β-lactam antibiotic was dissolved in a saline solution corresponding to the therapeutic concentration of the injection drug. For HPCE analysis, these samples were analyzed without any treatment. For HPLC analysis, the samples were subjected to analysis after diluting 100 times with the mobile phase of the HPLC.

3. Results and discussion

3.1. Separation of carbapenems and β-lactams

The separation of carbapenems and four other β-lactams under various electrophoresis buffer conditions was investigated. Because the phosphate buffer gave better separation than the borate buffer in a preliminary experiment, phosphate buffer was used

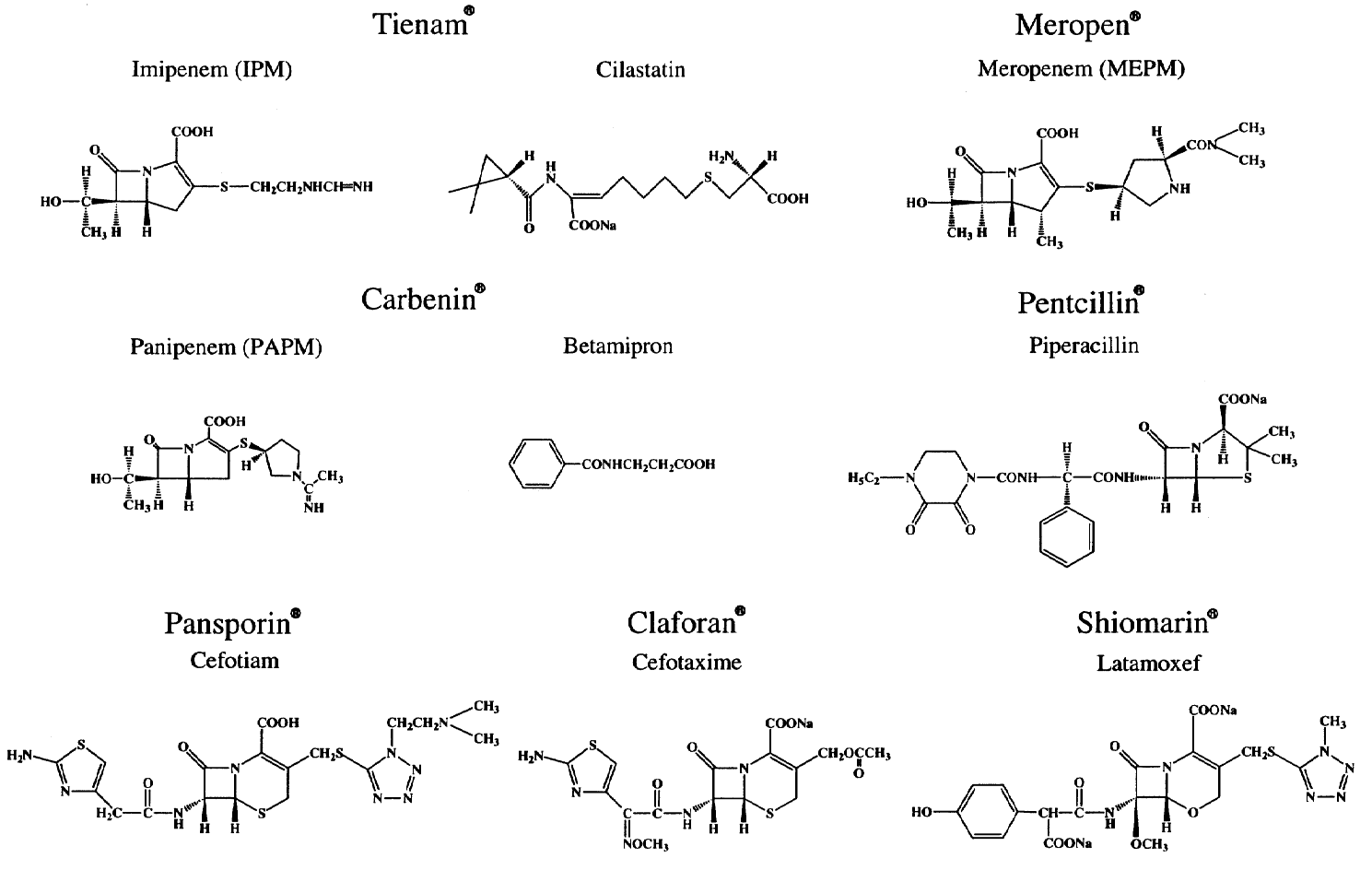

Fig. 1. Structures of carbapenems and β-lactams tested. Cilastatin and betamipron were added to moderate the renal toxicity.

for the separation. The effect of the pH of the electrophoresis buffer on the migration times of the antibiotics was examined and the optimum separation was achieved at pH 8.0. The effect of phosphate buffer concentration was also examined, and 100 m*M* was found to be the best for mutual separation of the drugs. Various internal diameters (I.D.) and lengths of the capillary were also investigated for rapid analysis accounting for the migration times, the number of theoretical plates (*N*) and the current observed. The voltage applied was 30, 15 and 10 kV for the capillary of 25, 50 and 75 μm I.D., respectively. A capillary with 25 μm I.D. showed the lowest current and accurate analysis in spite of the highest voltage (30 kV). The capillary with 25 μm I.D. also showed the shortest separation time. Capillary length was also investigated with the capillary of 25 μm I.D.. A capillary of 37 cm showed the shortest separation time but caused high electrical current which made the analyses inaccurate. The capillary of 47 cm showed a relatively short separation time with high *N* (26 000 plates/m). An appropriate current of about 50 μA was observed for this capillary. Thus, the capillary of 25 μm I.D. and 47 cm length was selected. For the detection of the antibiotics, UV absorbance at 210 nm was used. The electropherogram for carbapenems and four other β-lactams obtained under the optimum conditions of CZE (100 m*M* phosphate buffer of pH 8.0 using a capillary of 25 μm I.D.×47 cm) are shown in Fig. 2. Each peak was identified by comparing the migration time and the absorption spectrum with those of the drug substances.

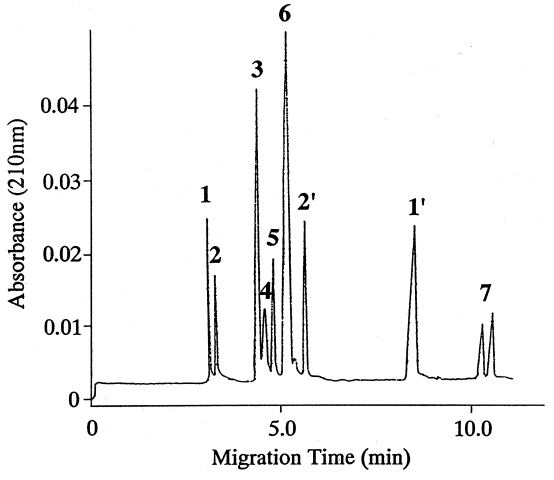

Fig. 2. Separation of carbapenems and four other β-lactams. Peak identities were as follows: 1, panipenem; 1′, betamipron; 2, imipenem; 2′, cilastatin; 3, cefotiam; 4, meropenem; 5, piperacillin; 6, cefotaxime; 7, latamoxef. Two peaks (diastereomers) were observed for latamoxef. The HPCE conditions were as follows: capillary, fused-silica (25 μm I.D.×47 cm); buffer, 100 m*M* sodium phosphate buffer (pH 8.0); electrophoresis, 30 kV constant voltage; detection, absorbance 210 nm.

3.2. Calibration curves and detection limits

The calibration curves of carbapenems and four other β-lactams were linear from the therapeutic concentration of the injection drugs (IPM, PAPM and MEPM, 0.5 g/100 ml saline; CTM, 0.5 g/20 ml saline; PIPC, 1 g/100 ml saline; CTX and LMOX, 1 g/4 ml saline) down to 2000-times diluted solution

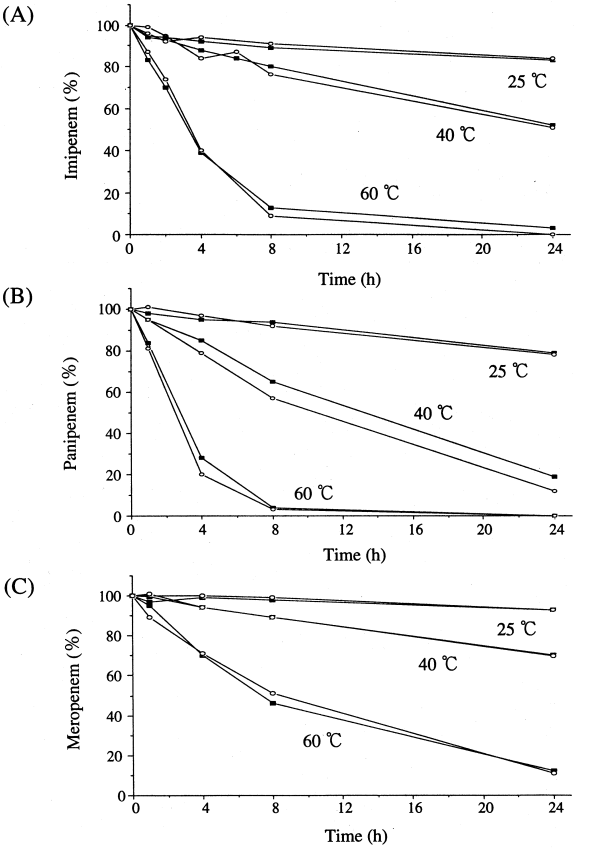

Fig. 3. Degradation of imipenem (A), panipenem (B) and meropenem (C) at various temperatures. Closed squares (■) represent the values obtained by the present method. Open circles (○) show the values obtained by the validated HPLC methods.

with correlation coefficients higher than 0.990. The detection limit of each antibiotic (injection amount, $S/N=3$) was as follows: IPM, 176 fmol; PAPM, 165 fmol; MEPM, 128 fmol; PIPC, 208 fmol; CTM, 94 fmol; CTX, 117 fmol; LMOX, 50 fmol.

3.3. Validation of the method

The repeatability of the present method was examined by repeating the quantification of the mixture of 7 antibiotics (IPM, PAPM and MEPM, 0.7 mg/ml saline; PIPC, 1.4 mg/ml saline; CTM and CTX, 3.5 mg/ml saline; LMOX, 0.35 mg/ml saline) eight times within a day. The relative standard deviation (RSD) of the quantities for the antibiotics were 0.00–3.04%, and the RSD values of the migration time were 0.16–0.54%. The day-to-day precision was determined by repeating the quantification for 8 days. The RSD of the quantity obtained

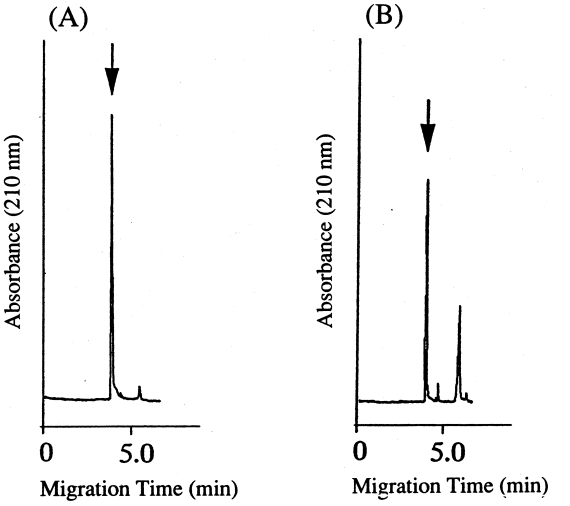

Fig. 4. Electropherograms of meropenem without heating (A) and heated at 40°C for 24 h (B). The peak of meropenem is indicated with an arrow.

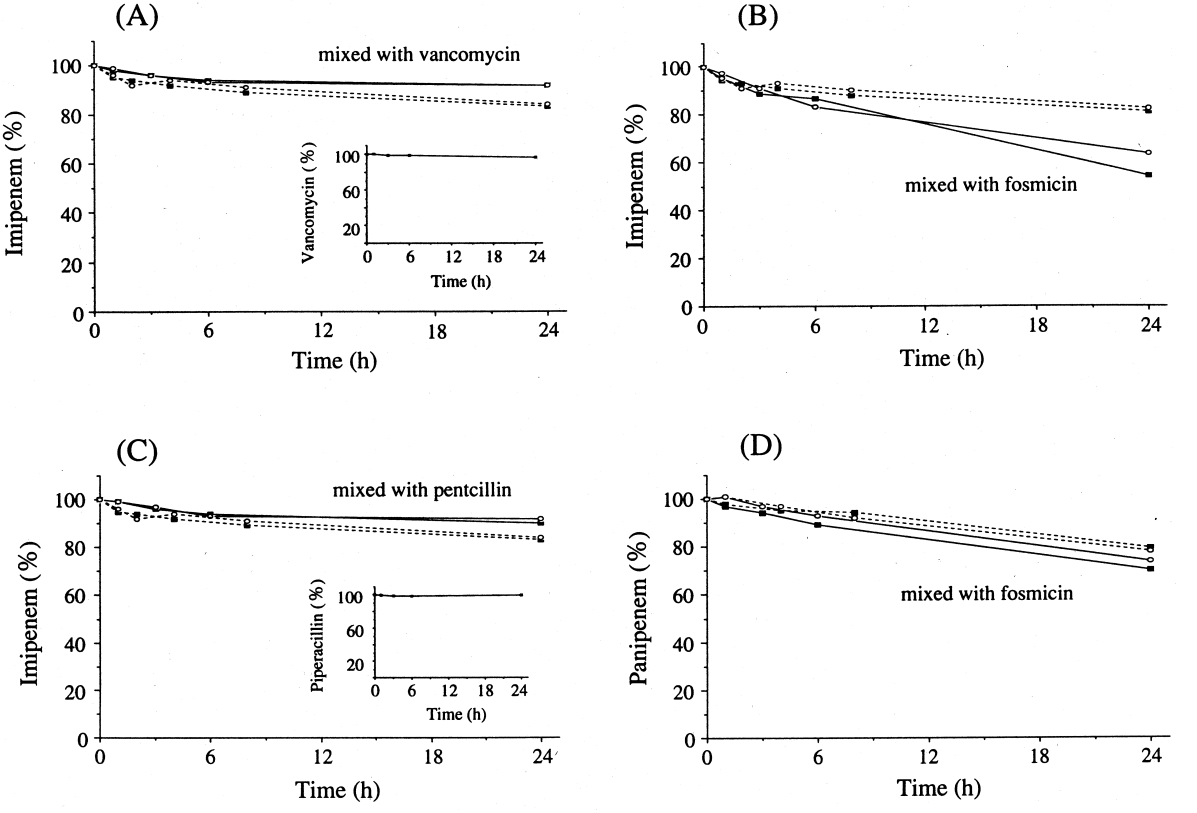

Fig. 5. Degradation of imipenem and panipenem in combination with various antibiotics. (A) IPM with vancomycin, (B) IPM with fosmicin, (C) IPM with pentcillin and (D) PAPM with fosmicin. Solid lines represent the values after mixing with the antibiotics. Dotted lines represent the degradation in the saline. The symbols are the same as described in Fig. 3. The small graphs show the degradation of the combination antibiotics (vancomycin and piperacillin).

were 1.52–2.42%, and the RSD of the migration times were 2.12–3.69%.

3.4. Degradation of carbapenems

Degradation of carbapenems under various conditions (at various temperatures or in combination with other drugs) were investigated using the present method described above. The quantities obtained using the present method were compared to those obtained by the HPLC method, which was described in 2.3. HPLC conditions. Fig. 3 shows the degradation of IPM, PAPM and MEPM at 25, 40 and 60°C. The three carbapenems were almost stable at 25°C; however, they were unstable at 40 and 60°C. The quantities and the time course of degradation of each carbapenem obtained by the present method were almost the same as those obtained by HPLC. The typical electropherograms (MEPM, 40°C) are shown in Fig. 4.

The degradation of carbapenems in combination with 5% (w/v) glucose solution were investigated. Each carbapenem was mixed with the glucose solution for 24 h at 25°C, and the quantity was evaluated as the percentage of that obtained just after the combination. The quantities of IPM, PAPM and MEPM were 58, 74 and 83% of those obtained at 0 h, respectively, and the values were almost the same as those obtained by the HPLC methods.

The actual prescription of carbapenems against MRSAs were also investigated. Fig. 5(A)–(C) represent the degradation of IPM mixed with vancomycin, fosmicin and pentcillin respectively. Fig. 5(D) shows the degradation of PAPM mixed with fosmicin. In each experiment, the temperature was kept at 25°C. Pentcillin is a kind of β-lactam antibiotic, which was also used for the investigation of separation conditions described above. Fosmicin and vancomycin were also antibiotics effective for MRSAs. As shown in this Figure, the stability of the carbapenems changed in combination with other drugs; however, the quantity and the time course of carbapenem were almost the same as those obtained by the HPLC method. Fosfomycin (composition of fosmicin) does not have strong UV absorbing groups; therefore, the quantity of fosfomycin was not measured by the present methods. Vancomycin and pentcillin were well separated from the carbapenems and could be determined using the present method. The electropherograms of IPM mixed with vancomycin and PIPC are shown in Fig. 6.

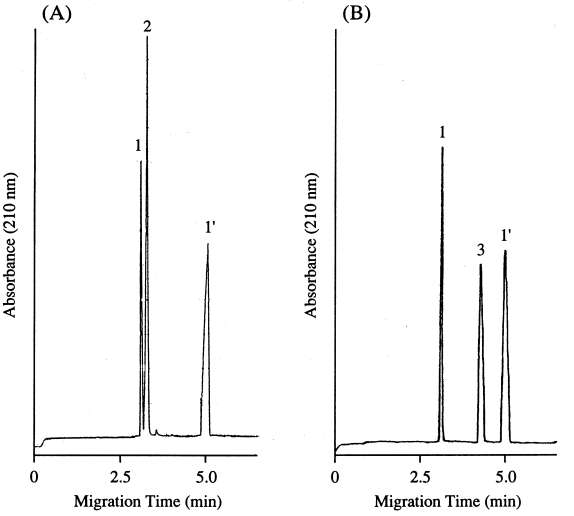

Fig. 6. Separations of imipenem/vancomycin (A) and imipenem/piperacillin (B). Peaks: 1, IPM; 1′, cilastatin; 2, vancomycin; 3, piperacillin.

4. Conclusions

In the present method, all therapeutic carbapenem injections in Japan and other β-lactams tested could be well separated and determined in about 10 min with good repeatability. This method was valuable because all therapeutic carbapenem injections could be analyzed under the same simple and rapid analytical conditions. In addition, vancomycin, which is the most widely used antibiotic against MRSAs, could also be analyzed by the present method. The results indicated in this paper suggested that the present method should be valuable for use in medical institutions.

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