Fentanyl is a highly potent synthetic opioid with a low molecular weight and high lipid solubility. A transdermal therapeutic system for fentanyl is available as a baseline opioid for cancer pain or chronic non-cancer pain. Although the transdermal fentanyl reservoir patch Durotep® has been available since the early 1990s, the United States Food and Drug Administration (FDA) has received numerous reports of serious adverse events due to its misuse or abuse of the fentanyl. Recent advances in transdermal technology permitted redesigning of the Durotep® in order to improve its structure and composition. The result of this effort is the fentanyl transdermal matrix patch (Durotep® MT, Durogesic®, DTrans®, and Durogesic® SMAT), which possesses a drug dissolving semi-solid polymer. Bioequivalence of the fentanyl transdermal matrix patch with its reservoir patch, in terms of its exposure and minimum and maximum plasma concentrations, was confirmed in a clinical study. The plasma disposition of fentanyl shows a large inter-individual variation because its metabolism varies markedly between patients. In addition, fentanyl transdermal absorption was also found to be variable among some individuals. Fever and exposure to an external heat source enhance fentanyl absorption while excessive sweating and certain systemic skin diseases limit fentanyl absorption. The determination of residual fentanyl in transdermal patches was helpful for evaluating its absorption rate in individuals.

In a previous report we described a validated method using a conventional octadecylsilane (ODS) column for the determination of residual fentanyl in a Durotep® transdermal reservoir patch. The patch preparation procedure cannot be employed in the Durotep® MT patch without a drug reservoir. A few methods are available for the determination of residual fentanyl in transdermal matrix patches. However, their application is limited in clinical settings. The earlier methods are time-consuming and involve complicated extraction procedures. With respect to fentanyl analysis, a fast HPLC separation using an ultrafine particle ODS is suitable for routine monitoring in clinical practice. Based on the van Deemter equation, the use of smaller particles can significantly reduce the height equivalent of a theoretical plate generated in a separation by improved mass transfer. Typically, shorter retention times can be achieved using ultrafine particles compared to using 5-μm particles. A simple and rapid analytical method for the determination of residual fentanyl in Durotep® MT patches is needed for clinical use. In addition, the assay validation parameters were scarcely reported in detail. The present HPLC-UV method is valid and the first one which is applicable to the applied Durotep® MT patches.

The transdermal absorption characteristics of the Durotep® MT patch still need to be clarified in clinical settings. The aim of this study was to develop a simple and rapid HPLC-UV method using an ultrafine particle octadecysilane (ODS) for the determination of residual fentanyl in applied Durotep® MT transdermal matrix patches. The transdermal absorption of fentanyl in Durotep® MT patches is needed for clinical use. In addition, the assay validation parameters were scarcely reported in detail. The present HPLC-UV method is valid and the first one which is applicable to the applied Durotep® MT patches.

Key words fentanyl; transdermal therapeutic system; matrix; HPLC-UV; ultrafine particle octadecysilane
with acetonitrile. One milliliter of the calibration standard solutions was evaporated to dryness in polypropylene tubes. Extraction solution containing 10 μg/mL papaverine as an internal standard was obtained by the dilution of stock solution with acetonitrile.

**Extraction of Transdermal Matrix Patches** Transdermal matrix patches (Durotep® MT, Janssen Pharmaceutical K.K., Tokyo, Japan) were stored at 4°C until patch extraction. After removal of the protective film, the patch was cut into pieces approximately 3.5 mm in width using multi-blade shredding scissors. The pieces were transferred into glass tubes (12×75 mm) containing 3 mL of acetonitrile extraction solution. For the calibration standard samples, drug-free Durotep® MT patches donated by Janssen Pharmaceutical K.K. were used as matrix. The pieces were extracted using sonicator (AU-225C, Aiwa Medical Industry Co., Ltd., Tokyo, Japan) at 28 kHz for 15 min and then centrifuged at 1670 g for 5 min. A 100 μL aliquot of the supernatant and 900 μL of mobile phase were added to the polypropylene tubes. For the calibration standard samples, the tubes were spiked with fentanyl. The mixtures were injected onto the HPLC system.

**Optimization of Extraction Condition** Extraction recoveries from 2.1, 4.2, and 8.4 mg of non-applied Durotep® MT patches (n=6) were evaluated in each extraction solution containing acetonitrile or methanol. In addition, the dispersed condition of the sonicated patch was also observed.

**LC Conditions and UV Analysis** Residual fentanyl in the Durotep® MT patches was determined using a validated HPLC system (Shimadzu, Kyoto, Japan). The HPLC system consisted of an LC-20AD pump, SIL-20AFAST autoinjector, CTO-20A column oven, and SPD-M20A UV detector. The wavelength of the UV detector with 10 μL cell volume was set at 254 nm and its response time was 0.1 s. Data were collected and analyzed by Class-VP software (version 6.14, Shimadzu). Separation was performed using a 2.3-μm particle ODS-column (TSKgel Super-ODS, 50×4.6 mm i.d., TOSOH, Tokyo, Japan). The mobile phase consisted of 25% acetonitrile containing 5 mM ammonium acetate at pH 3.5. The flow rate was 1.5 mL/min and the column temperature was set at 40°C. The injection volume was 10 μL.

**Method Validation and Extraction Recovery** For the method validation, 2.1, 4.2, and 8.4 mg of non-applied Durotep® MT patches were used as quality control samples. Calibration curves were obtained by plotting the measured peak area ratio of fentanyl to papaverine. Linearity was observed over the range of 0.015—9.0 μg as a Durotep® MT patch (0.5—300 μg in polypropylene tubes). Accuracy and precision were calculated for three quality control samples at 2.1, 4.2, and 8.4 mg as non-applied Durotep® MT patches. The accuracy was determined by evaluating the analytical recovery of known amounts of non-applied Durotep® MT patches. The intra- and inter-assay precisions were expressed as relative standard deviation (R.S.D.). Extraction recoveries are also considered to indicate statistical significance. A p<0.05 was considered to indicate statistical significance.

**Results**

**Patch Extraction Conditions** Figure 1 shows the extraction recoveries from 2.1, 4.2, and 8.4 mg of non-applied Durotep® MT patches in each extraction solutions. There was no significant difference in extraction recovery between acetonitrile and methanol solvents. In addition, no significant difference was observed in extraction recovery between 2.1, 4.2, and 8.4 mg of non-applied Durotep® MT patches. Durotep® MT patches were destroyed to a greater degree and the extraction solution was clearer when sonicated in acetonitrile than in methanol.

**Chromatographic Separation** Figure 2 shows the UV chromatograms of a drug-free Durotep® MT patch, a drug-free Durotep® MT patch spiked with fentanyl, and an applied Durotep® MT patch in a cancer patient. Fentanyl and papaverine were eluted at 1.27 and 0.89 min, respectively, with a total run time of 2 min. No peaks interfering with fentanyl and papaverine were observed.

**Calibration Curve and Sensitivity** The calibration curve was linear over the range of 0.015—9.0 μg as Durotep® MT patches (0.5—300 μg in polypropylene tubes). The correlation coefficient was greater than 0.999 [y=(1.946±
0.012)x + (0.112±0.038), n=3, x=fentanyl amount in Durotep® MT patch (mg), y=area ratio of fentanyl to papaverine. The lower limit of quantification, defined as the concentration within 20% R.S.D., was 0.015mg as a Durotep® MT patch (0.5μg in polypropylene tubes, n=6). The accuracy of the lower limit of quantification was 90.4% (n=6).

Assay Accuracy and Precision Table 1 shows the assay accuracy and precision of fentanyl in each Durotep® MT patch. The intra- and inter-assay accuracies of fentanyl at 12.5, 25, and 50μg/h (2.1, 4.2, 8.4mg as a Durotep® MT patch, respectively) were 103.9—110.5% and 97.1—104.3%, respectively. The intra- and inter-assay precisions of fentanyl were 2.5—5.3% and 6.1—8.2%, respectively.

Stability Stock solutions of fentanyl and papaverine were stable at 4°C for at least 3 months (% of initial value, 102.3%, n=3). Fentanyl in opened Durotep® MT patches was stable at 4°C for at least 1 month (% of initial value, 97.1%, n=3). Fentanyl and papaverine in the mobile phase were stable at 4°C for at least 24h (% of initial value, 97.9%, n=3).

Plasma Fentanyl Concentration in Cancer Pain Patients Figure 3 shows the relationships between the theoretical delivery rate or measured absorption rate of fentanyl and its plasma concentration at 48h after the replacement of Durotep® MT patches in 35 cancer patients. Plasma fentanyl concentration was significantly correlated with its theoretical delivery rate in Durotep® MT patches (r=0.65, p<0.01). A slightly larger correlation coefficient was obtained between the plasma fentanyl concentration and its measured absorption rate in Durotep® MT patches (r=0.71, p<0.01). Figure 3 shows the variability of plasma fentanyl concentration in cancer patients. The plasma fentanyl concentration adjusted by the measured absorption rate also showed a large inter-individual variation in cancer patients (R.S.D., 81%).

Discussion Determination of residual fentanyl in applied transdermal patches is helpful for evaluating its transdermal absorption rate in individuals. In addition, estimation of the fentanyl absorption rate could be useful for predicting its efficacy and toxicity. In the present study, a simple and rapid HPLC-UV method using an ultrafine particle ODS for the determination of residual fentanyl in applied Durotep® MT patches has been developed. In the patch extraction, a shredded matrix patch was sonicated in 3mL of acetonitrile for 15min. Fentanyl separation using an ultrafine particle ODS and a short column was completed within 2min. The present validated method is simpler and faster than other reported methods that determine residual fentanyl in applied Durotep® MT patches.11,12)
In a previous report we demonstrated a validated method using a conventional ODS for the determination of residual fentanyl in Durotep® transdermal reservoir patches. Patch extraction involved filling acetonitrile into the drug reservoir and then collection into test tubes. The procedure cannot be employed for Durotep® MT patches without a drug reservoir. A few methods can determine residual fentanyl in applied Durotep® MT patches. Marier et al. extracted residual fentanyl using 2-propanol. Van Nimmen and Veulemans extracted fentanyl in shredded matrix patches using isotonic saline. These methods are not practical for clinical use due to difficulties in completing the extraction within 16 h. In contrast, in the present extraction, a shredded matrix patch was sonicated in acetonitrile for 15 min. Fentanyl dissolves faster in organic solvent than aqueous solution. A methanol based extraction protocol was reported for applied Durotep® MT patches. The protocol involved extraction for 15 min with 20 mL of methanol. In the present method, 3 mL of acetonitrile completely extracted residual fentanyl in non-applied matrix patches. Applied Durotep® MT patches were destroyed to a greater degree and the extraction solution was clearer when sonicated in acetonitrile than in methanol. Smaller volume of extraction solution was required in extraction solution containing acetonitrile. The extraction solution was diluted with the mobile phase and then injected directly onto the HPLC system. This method which lacks a clean-up process can be easily applied to the extraction of a great number of samples. HPLC separation of fentanyl and papaverine was completed within 2 min. If injection time is not included, it enables the measurement of 30 samples in 1 h due to the rapid HPLC separation. Plate number of chromatogram seems to be low in chromatograms appearing in Fig. 2. Although lower volume of flow cell would improve the plate number, no peaks interfering with analytes were observed in the present condition. In addition, more than 500 chromatographic runs could be performed with one ODS column without any deterioration of the separation performance. An earlier method employed a conventional HPLC column for the determination in residual fentanyl. The chromatographic conditions consisted of a 5 μm particle C8 column and a mobile phase of 35% acetonitrile containing 0.23% perchloric acid. In the HPLC separation, run

<table>
<thead>
<tr>
<th>Theoretical delivery rate (μg/h)</th>
<th>Amount in Durotep® MT patch (mg)</th>
<th>Intra-assay (n=6)</th>
<th>Intra-assay (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±S.D. (mg)</td>
<td>Accuracy (%)</td>
<td>R.S.D. (%)</td>
</tr>
<tr>
<td>12.5</td>
<td>2.1</td>
<td>2.26±0.13</td>
<td>110.5</td>
</tr>
<tr>
<td>25</td>
<td>4.2</td>
<td>4.46±0.32</td>
<td>107.1</td>
</tr>
<tr>
<td>50</td>
<td>8.4</td>
<td>8.43±0.47</td>
<td>103.9</td>
</tr>
</tbody>
</table>

S.D., standard deviation; and R.S.D., relative standard deviation.

<table>
<thead>
<tr>
<th>Table 2. Patient Demographic Characteristics</th>
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<tbody>
<tr>
<td>Sex, male/female</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
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<tr>
<td>Serum creatinine (mg/dL)</td>
</tr>
<tr>
<td>Aspartate aminotransferase (IU/L)</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
</tr>
<tr>
<td>Initial opioids (morphine/oxycodone)</td>
</tr>
</tbody>
</table>

Data are expressed as median and range in parentheses.

Fentanyl expands faster in organic solvent than aqueous solution. A methanol based extraction protocol was reported for applied Durotep® MT patches. The protocol involved extraction for 15 min with 20 mL of methanol. In the present method, 3 mL of acetonitrile completely extracted residual fentanyl in non-applied matrix patches. Applied Durotep® MT patches were destroyed to a greater degree and the extraction solution was clearer when sonicated in acetonitrile than in methanol. Smaller volume of extraction solution was required in extraction solution containing acetonitrile. The extraction solution was diluted with the mobile phase and then injected directly onto the HPLC system. This method which lacks a clean-up process can be easily applied to the extraction of a great number of samples.

HPLC separation of fentanyl and papaverine was completed within 2 min. If injection time is not included, it enables the measurement of 30 samples in 1 h due to the rapid HPLC separation. Plate number of chromatogram seems to be low in chromatograms appearing in Fig. 2. Although lower volume of flow cell would improve the plate number, no peaks interfering with analytes were observed in the present condition. In addition, more than 500 chromatographic runs could be performed with one ODS column without any deterioration of the separation performance. An earlier method employed a conventional HPLC column for the determination in residual fentanyl. The chromatographic conditions consisted of a 5 μm particle C8 column and a mobile phase of 35% acetonitrile containing 0.23% perchloric acid. In the HPLC separation, run

![Fig. 3. Relationships between the Theoretical Delivery Rate (A) or the Measured Absorption Rate (B) and Fentanyl Plasma Concentration at 48 h after the Replacement of Durotep® MT Patches in 35 Cancer Patients](image)

Correlation coefficients were analyzed by Pearson’s test.
times exceeding 10 min were required because of the larger particle ODS and longer column.

Method validation revealed the assay precision and accuracy of each patch were within 8.2% and 97.1—110.5%, respectively. The results obtained with this method met the standards of the international FDA guideline. The validated method can be helpful for evaluating the absorption rate of fentanyl in patients receiving Durotep® MT patches. Although the suitability of the present method has not been confirmed for 75 and 100 μg/h Durotep® MT patches, their matrices possess the same semi-solid polymer as 12.5, 25, and 50 μg/h.

Residual fentanyl in 75 and 100 μg/h Durotep® MT patches is measurable by patch extraction with a 2-fold volume of extraction solution based on the linearity in combination with 25 and 50 μg/h Durotep® MT patches.

In the study, the present method employed 2.1, 4.2, and 8.4 mg of non-applied patches to evaluate the validation results. Ideally, the same area of patches with different amount of fentanyl should be applied in method validation. The various amounts of Durotep® MT patches are employed in clinical settings. In addition, we confirmed that the fentanyl spiked drug-free Durotep® MT patches have the same results as non-applied Durotep® MT patches over the calibration range. Method validation using ready-made non-applied Durotep® MT patches is more practical in clinical settings.

The present method, like an earlier method, used a conventional HPLC system for the determination of residual fentanyl. A sensitive method possessing pictogram level accuracy such as mass spectrometry was not required for the determination of residual fentanyl because it was present at a milligram level. An analytical method using GC-MS for the determination of residual fentanyl has been reported. The assay precision and accuracy were within 8.9% and 92.8—107.1%, respectively, and were comparable to our present method using an ultrafine particle ODS.

The suitability of the present method in cancer patients receiving Durotep® MT patches was confirmed in our clinical study. Although plasma fentanyl concentration was correlated with the measured absorption rate, its concentration showed a large inter-individual variation like in earlier reports. In a previous report, we also found that plasma fentanyl concentrations adjusted according to the measured absorption rate were variable among cancer patients receiving Durotep® transdermal reservoir patches. Age, body mass index, sex and cancer type have been shown to be associated with fentanyl pharmacokinetics. Transdermal fentanyl resulted in lower plasma concentrations of fentanyl in cachectic patients as compared to non-cachectic patients. Transdermal absorption of fentanyl was impaired in cachectic cancer patients. In addition, fentanyl is metabolized by cytochrome P450 (CYP) 3A, and its metabolism varies markedly between patients. It remains to be clarified whether or not polymorphisms of the CYP3A gene contribute to the pharmacokinetic variability of fentanyl in patients receiving Durotep® MT patches.

Conclusions

A simple and rapid HPLC-UV method using an ultrafine particle ODS for the determination of residual fentanyl in applied Durotep® MT patches has been developed. The method can be suitable for evaluating the absorption rate of fentanyl in patients receiving Durotep® MT patches.

Acknowledgement This work was supported by a Grant-in-Aid for Young Scientists (B) provided by The Japanese Ministry of Education, Culture, Sports, Science and Technology.

References and Notes
4) Roy S. D., Gutierrez M., Flynn G. L., Cleary G. W., J. Pharm. Sci.,

Fig. 4. The Variability of Plasma Fentanyl Concentration Adjusted by Its Theoretical Delivery Rate (A) or Its Measured Absorption Rate (B) in Cancer Patients Receiving Durotep® MT Patches

Box plots represent the median, 25th, and 75th percentiles. The whiskers indicate the range and extend within 1.5 times the length of the inner quartiles.