SOLUBILITY OF FLUTICASONE PROPIONATE IN AQUEOUS SOLUTIONS MEASURED BY A METHOD AVOIDING ITS ADSORPTION TO EXPERIMENTAL TOOLS

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Abstract: A method for determining fluticasone propionate (FLT) in 50% methanol using HPLC, the adsorption of FLT, a method for avoiding such adsorption to experimental tools, and the solubility of FLT in aqueous solutions were investigated. In the developed determination method, the retention time of FLT was approximately 6.8 min, and linear regression analysis gave a slope, intercept, and correlation coefficient of Y = 99.173X + 21.513, r = 0.99999. The values of intra-day and inter-day precision and accuracy in this method were acceptable. The lower limit of quantification was established to be 10 ng/mL. Adsorption of FLT to experimental tools made of glass and polypropylene was observed. The adsorption to conventional experimental tools was examined using 100 ng/mL FLT solution. The adsorption characteristics of FLT and a method to avoid such adsorption were determined from these results. The solubilities of FLT were measured by the method avoiding the effect of adsorption, which in water, 0.1 N HCl, and buffer solutions at pH 2, 4, 6, and 8 were 0.1 µg/mL. It was found that, in order to determine the solubility of FLT was improved by the addition of △, β, γ, and δ-cyclodextrins.

Key words: Fluticasone propionate, Adsorption, Glass, Polypropylene, Solubility

INTRODUCTION

Inflammatory bowel diseases (IBDs) are a group of inflammatory conditions characterized by chronic, uncontrolled inflammation of the gastrointestinal tract. Crohn’s disease and ulcerative colitis are the two primary types of IBD. They are clearly distinct pathophysiological entities. Ulcerative colitis, the most common form of IBD worldwide, is a disease of the colonic mucosa only; it is less prone to complications and can be cured with colectomy. In contrast, Crohn’s disease is a transmural disease of the gastrointestinal mucosa which can affect the entire gastrointestinal tract from the mouth to the anus.

Systemic corticosteroids have been used to treat active IBD for over 50 years by virtue of their unquestionable efficacy in inducing clinical remission rapidly in the vast majority of patients. Nevertheless, traditional corticosteroids are associated to a plethora of potentially serious side effects due to their systemic metabolism; for this reason, interest has lately been growing in newer steroid compounds characterized by a high topical anti-inflammatory activity and a low systemic bioavailability. Budesonide has good efficacy and is an established treatment for Crohn’s disease; it has been shown to be beneficial for the induction of remission in these patients. On the other hand, the efficacy of fluticasone propionate in IBD is not well established.

Fluticasone propionate (FLT) is an inhaled corticosteroid with high anti-inflammatory potency, used for the topical treatment of asthma. Its high lipophilicity and associated low aqueous solubility result in high concentrations in pulmonary tissue as well as delayed absorption from the lung into the systemic circulation after inhaled administration. FLT has the same anti-inflammatory potency as budesonide as inhaled corticosteroids because the dose of FLT is the same. However, the efficacy of fluticasone propionate in IBD is not well established. The reason for the poor efficacy of FLT on IBD was considered to be its physicochemical properties, especially poor solubility. This property is good for producing high concentrations in pulmonary tissue, but may not be good for IBD. Therefore, study to improve the solubility of FLT was initiated. However, an obstacle to experimental work was identified, namely, the adsorption of FLT to experimental tools made of glass and polypropylene. No reports on such adsorption have been published. However, the solubility of FLT in water was reported to be 0.4 and 0.14 µg/mL, but the methods for measuring the solubility were not described enough in these previous reports. It was found that, in order to determine the solubility exactly, it had to be determined after its adsorption to experimental tools had been clarified. As a result, investigation of the adsorption was required. In this report, we first describe a method of determination of FLT in 50% methanol using HPLC, next the adsorption of FLT and a method to avoid such adsorption to experimental tools, and third the solubility of FLT in aqueous solutions measured by the method that avoids its adsorption. In addition, a result of preliminary study to enhance the solubility is described.
MATERIALS AND METHODS

Materials
Fluticasone propionate (FLT) and cyclodextrins were donated by Alps Pharmaceutical Ind. Co., Ltd. (Gifu, Japan) and NIHON SHOKUHIN KAKO Co., Ltd. (Tokyo, Japan), respectively. Other chemicals were of reagent or HPLC grade.

Stock solution
An FLT solution at 1 mg/mL in methanol was prepared. This solution was diluted with 50% methanol to make an FLT solution at 10 μg/mL, which was stored at room temperature as a stock solution.

Determination of FLT in 50% methanol solution by HPLC
The concentration of FLT in 50% methanol solution was determined by an HPLC assay consisting of a Model LC-9A pump, equipped with a Model SCL-6B system controller, a Model SPD-6A UV spectrophotometric detector, a Model CTO-10A column oven, a Model R4AX Chromatopic, and a Model SIL-6B auto injector, all from Shimadzu (Kyoto, Japan). The mobile phase was acetonitrile-water-perchloric acid (60%)-sodium perchlorate monohydrate (660:340:1:5, V/V/V/W). The chromatographic column was a YMC Pack AM12S05-1506WT ODS (150 mm x 6 mm I.D., particle diameter 5 μm) from YMC Co. Ltd. (Kyoto, Japan). The flow rate, wavelength for determination, and temperature of the column were 1 ml/min, 240 nm, and 40°C, respectively. The concentrations of the prepared FLT solutions were 10–10,000 ng/mL. The solutions were prepared from the stock solution. The volume injected onto the HPLC column was 50 μL.

Adsorption studies
Adsorption of FLT to a 1 mL glass measuring pipette
The stock solution was diluted with water to make a 100 ng/mL FLT solution. Using a 1 mL glass measuring pipette, 1 mL of the 100 ng/mL FLT solution was added to a glass culture tube (100 mm x 13 mm I.D.) with 1 mL of methanol. This operation was performed 5 times using the same pipette. The first and fifth added solutions using the same pipette were well stirred and assayed by HPLC. Concentration ratio was calculated using the following equation:

\[
\text{Ratio} = \frac{\text{Peak area of the fifth solution}}{\text{Peak area of the first solution}}
\]

Adsorption of FLT to glass tubes
A total of 2 mL of the 100 ng/mL FLT solution was added to a glass culture tube (100 mm x 13 mm I.D.) using a 1 mL glass measuring pipette. The tube was left to stand at room temperature for 10 min. As a sample, 1 mL of the solution was withdrawn from the tube using a 1 mL glass measuring pipette, and added to the new tube with 1 mL of methanol. As a reference, the 100 ng/mL FLT solution was added to the tube using a 1 mL glass measuring pipette with 1 mL of methanol. The solutions in the tubes (sample and reference) were well stirred and assayed by HPLC. Recoveries (%) were calculated using the following equation:

\[
\text{Recovery} (%) = \frac{\text{Peak area of sample}}{\text{Peak area of reference}} \times 100
\]

Adsorption of FLT to glass tubes with operating conditions of stirring and changing tubes
A total of 2 mL of the 100 ng/mL FLT solution was added to a glass culture tube (100 mm x 13 mm I.D.) using a 1 mL glass measuring pipette. The solution in the tube was stirred for 15 s using a vortex mixer. The solution in the tube was transferred to a new tube (the second) using a 1 mL glass measuring pipette, and stirred in this tube in the same way. This operation was repeated a further 3 times. The operations for the solution were performed 5 times. As a sample, 1 mL of the solution was withdrawn from the fifth tube using a 1 mL glass measuring pipette, and added to the new tube with 1 mL of methanol. As a reference, the 100 ng/mL FLT solution was added to the new tube with 1 mL of methanol using a 1 mL glass measuring pipette. The solutions in the tubes (sample and reference) were well stirred and assayed by HPLC. Recoveries (%) were calculated using equation (1).

The same experiment using 100 ng/mL FLT in 50% methanol was performed and recoveries were calculated.

Adsorption of FLT to pipette tips made of polypropylene
Pipette tips with the product names of Aibis for 1-200 µL and 100-1000 µL from AS One Co. Ltd. (Osaka, Japan) were used. A 100 ng/mL FLT solution was used for the experiment. For the 1-200 µL tip, the same tip was used 5 times upon setting a continuously adjustable air displacement pipette to 200 µL. A total of 200 µL was added to a glass tube with 200 µL of methanol. In addition, 1 mL of 100 ng/mL FLT solution was added to a glass tube with 1 mL of methanol using a 1 mL glass measuring pipette as a reference. The solutions of 200 µL and the reference were assayed by HPLC. The recovery was calculated using equation (1). In the case of the 100-1000 µL tip, the recovery was calculated the same as for the 1-200 µL tip. In this case, a continuously adjustable air displacement pipette was set at 1000 µL, and 1000 µL was added to a glass tube with 1000 µL of methanol.

Sample cups made of polypropylene for HPLC
There are two types of sample cup, IA and IIA, which were used for Shimadzu’s auto injector and made of polypropylene. We used sample cup IA. This was filled with 200 µl of sample solution. The 100 ng/mL FLT solution was added to the sample cup, which was left to stand at room temperature for 10 min. A total of 100 µL of the 100 ng/mL FLT solution in the sample cup (sample solution) was withdrawn using a continuously adjustable air displacement pipette with a 1-200 µL tip, and added to a sample cup with 100 µL of methanol. The sample solution and methanol were well mixed in the sample cup using the pipette with the tip. As a reference, 100 µL of 100 ng/mL FLT solution was added to a sample cup with 100 µL of methanol using the pipette with a 1-200 µL tip. The solution in the sample cup was well mixed, the same as the sample solution. The solutions in the sample cups were assayed by HPLC. The recovery was calculated using equation (1).
Then, 100 ng/mL FLT solutions in 10, 20, 30, and 40% methanol were prepared and the same experiment for the sample cup was performed.

**Syringe (5 mL) made of polypropylene**
A 5 mL syringe (ss-05szp) from Terumo (Tokyo, Japan) was used. A total of 5 mL of the 100 ng/mL FLT solutions was drawn into the syringe, and after 1 min, this was injected into a glass tube. This operation was performed 2 more times using the same syringe. From each glass tube, 1 mL of the solution was taken out using a 1 mL measuring pipette, and this solution was added to a glass tube with 1 mL of methanol, which was the sample solution. After stirring well, the concentration of FLT in the mixed solution was determined by HPLC. As a reference, 1 mL of the 100 ng/mL FLT solution was added to a glass tube with 1 mL of methanol using a 1 mL measuring pipette. After stirring well, the solutions in the tubes were assayed by HPLC. The recovery was calculated using equation (1).

**Syringe filters**
Syringe filters, E031, E032, E131, E132, E134, E254, 4406T, 4556T, 4525T, AP-4219, and AP-4585, from Pall Corporation (Tokyo, Japan) were used. An appropriate volume of the 100 ng/mL FLT solution was passed through these syringe filters. This solution was then discarded. The volume for E031 and E032 was 75 µL, for E131, E132, E134, 4452T, and 4556T was 2 mL, and for E254, 4406T, AP-4219, and AP-4585 was 7 mL. Next, the solution passed through the syringe filters was collected into a glass tube. The collected volumes for E031 and E032, for E131, E132, E134, 4452T, and 4556T, and for E254, 4406T, AP-4219, and AP-4585 were 50 µL, 1 mL, and 3.5 mL, respectively. These solutions were added to the glass tubes with the same volume of methanol. The mixed solutions after stirring well were assayed by HPLC as sample solutions. As a reference, 1 mL of the 100 ng/mL FLT solution was added to a glass tube with 1 mL of methanol using a 1 mL measuring pipette. After stirring well, the solutions in the tubes were assayed by HPLC. The recovery was calculated using equation (1).

The effect of the volume passed through the syringe filter of E131 on the recovery was investigated as follows. The 100 ng/mL FLT solution was passed through the syringe filters, and the filtrate was collected in 1 mL portions using glass tubes. The assay and the calculation of recoveries were performed following the above-described methods.

**Solubility study**
Three milligrams of FLT was added to glass tubes. Then, 10 mL of solvents, which were water, 0.1 N HCl, and buffer solutions at pH 2, 4, 6, and 8, were added to the tubes. After the tubes were stoppered, they were kept at 37°C for 7 days. The buffer solutions used were prepared by mixing 0.2 M solutions of Na3PO4 and H2PO4. After 7 days, 5 mL of the solution in each tube was added to a syringe (ss-05SZP) to saturate the adsorption, and after discarding the solution in the syringe, 5 mL of the solution was then added to the syringe with the syringe filter of E131. A total of 3 mL of filtrate for each syringe was discarded, and next 1 mL of filtrate was added to a glass tube at 37°C. A sample solution of 200 µL was taken out from the tube and added to glass tubes with 200 µL of methanol. Concentrations of FLT in the mixed solutions were determined by HPLC. The standard solutions for determination of the solubility were prepared as follows. An FLT solution of methanol at 1 mg/mL was diluted with methanol to make solutions at 1000, 500, 100, and 50 ng/mL. A total of 200 µL of each methanol solution was added to a glass tube with 200 µL of water and stirred well. These mixed solutions were used for preparing the standard curve for the solubility study.

**Preliminary study for enhancement of FLT solubility**
Three milligrams of FLT was added to glass tubes. Then, 10 mL of solvents, which were water and 20 mM solutions of α-, β- and γ-cyclodextrins were added to the tubes. The tubes were stoppered. After FLT in the tubes was dispersed by an ultrasonic treatment for 1 min, they were kept at 37°C for 1 h. Preparing filtrates for the solution in the tubes and the determination of FLT in the filtrates was performed by the same method described in solubility study.

**RESULTS AND DISCUSSION**
**Determination of FLT in 50% methanol solution**
The retention time of FLT was approximately 6.8 min. Linear regression analysis gave a slope, intercept, and correlation coefficient of $Y = 99.173X + 21.513$, $r = 0.99999$. The intra-day precision and accuracy were determined by analyzing five replicates at each drug concentration, which are shown in Table 1. The precision was found to range from 0.1% to 3.3%. The accuracy value ranged from –5.1% to 0.2%. The inter-day precision and accuracy were determined by analyzing duplicates at each standard concentration over six different days. The result for the calibration curve is shown in Table 2. The precision from 10 ng/mL to 10,000 ng/mL was from 37.8% to 0.3%. The accuracy ranged from –3.6% to 9.3%. The values of the intra-day and inter-day precision and accuracy except lowest concentration ranged within 10% and from –10% to 10%, respectively, which were acceptable. The lower limit of quantification was established to be 10 ng/mL from the validation data, as shown in Table 2, because the precision at 10 ng/mL was greater than 10%.

An attempt was made to prepare a calibration curve for FLT in water, but a linear relationship was not obtained. The reason for the non-linear relationship was considered to be the adsorption of FLT in water to the experimental apparatus. Because non-specific adsorption of drugs to plastic or glass containers used in clinical use is well known. Therefore, an adsorption study for FLT was initiated.
Table 1: Intra-day precision and accuracy of the measurement of FLT in 50% methanol solution

<table>
<thead>
<tr>
<th>Actual concentration (ng/mL)</th>
<th>Concentration found (ng/mL) (mean ± SD, n=5)</th>
<th>Precision (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9.5 ± 0.3</td>
<td>3.3</td>
<td>-5.1</td>
</tr>
<tr>
<td>50</td>
<td>48.8 ± 0.8</td>
<td>1.6</td>
<td>-2.3</td>
</tr>
<tr>
<td>100</td>
<td>98.1 ± 0.2</td>
<td>0.2</td>
<td>-1.9</td>
</tr>
<tr>
<td>500</td>
<td>498.2 ± 1.1</td>
<td>0.2</td>
<td>-0.4</td>
</tr>
<tr>
<td>1000</td>
<td>1001.0 ± 1.8</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>5000</td>
<td>5008.7 ± 7.2</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>10000</td>
<td>9995.7 ± 34.9</td>
<td>0.4</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Precision and accuracy values were calculated using the following equations:
Precision (%) = \((\text{SD}/\text{mean}) \times 100\);
Accuracy (%) = \(((\text{concentration found} - \text{actual concentration})/\text{actual concentration}) \times 100\).

Table 2: Inter-day precision and accuracy of the measurement of FLT in 50% methanol solution

<table>
<thead>
<tr>
<th>Actual concentration (ng/mL)</th>
<th>Concentration found (ng/mL) (mean ± SD, n=8)</th>
<th>Precision (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10.9 ± 4.1</td>
<td>37.8</td>
<td>9.3</td>
</tr>
<tr>
<td>50</td>
<td>48.2 ± 2.8</td>
<td>5.8</td>
<td>-3.6</td>
</tr>
<tr>
<td>100</td>
<td>98.3 ± 2.4</td>
<td>2.4</td>
<td>-1.7</td>
</tr>
<tr>
<td>500</td>
<td>499.9 ± 5.5</td>
<td>1.1</td>
<td>0.0</td>
</tr>
<tr>
<td>1000</td>
<td>994.6 ± 9.8</td>
<td>1.0</td>
<td>-0.5</td>
</tr>
<tr>
<td>5000</td>
<td>5016.1 ± 20.3</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>10000</td>
<td>9995.8 ± 27.7</td>
<td>0.3</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Precision and accuracy values were calculated using the following equations:
Precision (%) = \((\text{SD}/\text{mean}) \times 100\);
Accuracy (%) = \(((\text{concentration found} - \text{actual concentration})/\text{actual concentration}) \times 100\).

Adsorption of FLT to glassware

When the same glass measuring pipette was used 5 times, the concentration of FLT in 1 mL of the solution was unchanged because the ratio was 0.996±0.044 (mean ± SD, n=3). It was found from this result that the 1 mL glass measuring pipette could be used for FLT solutions not containing methanol.

The recovery of FLT from the solution in the tube left to stand at room temperature for 10 min was 94.1±3.8% (mean±SD, n=3). The adsorption of FLT was thus observed, but it was limited.

The recovery of FLT from the solutions in the tube with operating conditions of stirring and changing tubes was 12.3 ± 2.5% (mean ± SD, n=3). It was found that the low recovery was caused by the increase of surface area of glass contacting the solution. On the other hand, in the same experiment using 50% methanol as a solvent, the recovery was 100.0 ± 2.8% (mean ± SD, n=3), which showed that the adsorption of FLT to glass was completely avoided.

These results indicated that FLT was adsorbed to the surface of glass, but that the decrease of the concentration using glass tubes and measuring pipettes without stirring and changing glass tubes might be neglected. The adsorption was considered to be prevented by using 50% methanol solution. For this reason, the calibration curve of HPLC assay was prepared using 50% methanol solution.

Adsorption of FLT to experimental apparatus made of polypropylene

The recoveries from two kinds of pipette tips are shown in Table 3. The recoveries in the first operation were both low. The values for the 2 tips in the second operation were around 100%, which showed that the adsorption to the tips was easily saturated. It was found from these results that pipette tips could be used after several operations using the same pipette tip, at which time saturation had been reached.

Table 3: Recovery of FLT from pipette tips made of polypropylene

<table>
<thead>
<tr>
<th>Operation times</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tips (1-200 µl)</td>
<td>95.0 ± 1.2</td>
</tr>
<tr>
<td>Tips (100-1000 µl)</td>
<td>95.5 ± 0.9</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD for 3 experimental runs.
The same tip was used 5 times upon setting a continuously adjustable air displacement pipette at 200 and 1000 µL for 1-200 and 100-1000 µL tips, respectively.

Table 4 shows the recoveries of FLT from the sample cups. In the case of water, the value was 45.5 ± 8.0% (mean ± SD, n=3), which showed that the sample cups were not suitable for sample solutions of water. The values increased with increasing concentration of methanol. In the sample solutions containing 30% or more methanol, adsorption was found to be negligible.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>45.5 ± 8.0</td>
</tr>
<tr>
<td>10% methanol</td>
<td>67.4 ± 8.1</td>
</tr>
<tr>
<td>20% methanol</td>
<td>93.1 ± 8.2</td>
</tr>
<tr>
<td>30% methanol</td>
<td>99.9 ± 2.4</td>
</tr>
<tr>
<td>40% methanol</td>
<td>98.4 ± 1.1</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD for 3 experimental runs.

When syringes were used for the 100 ng/mL FLT solution, the recoveries for the first, second, and third operations were 91.2 ± 3.1, 97.1 ± 4.1, and 97.3 ± 4.0%, respectively. This result showed that the syringe required about 5 mL of the 100 ng/mL FLT solution in order for negligible adsorption to occur.

Adsortion of FLT to syringe filters
The recoveries of FLT in filtrates after using syringe filters, E031, E032, E131, E132, E134, E254, 4452T, 4556T, 4406T, AP4219T, and AP4585T, were 27.9, 2.7, 78.3, 26.9, 56.8, 0, 0, 6.3, 15.6, and 0% (n=1), respectively. It was found that E131 was the best filter for FLT among those used.

The relationship between the recovery of filtrate and the volume of filtration for the syringe filter E131 was investigated. The recovery of the first portion was 19.1 ± 7.4% (mean ± SD, n=3). The values for the second, third, fourth, and fifth portions were 72.2 ± 4.8, 78.3 ± 3.3, 83.8 ± 2.8, and 82.5 ± 5.1%. It was found from these results that, before using this filter, 3 mL of filtrate should be discarded.

Solubility
The solubilities of FLT in water, 0.1 N HCl, and buffer solutions at pH 2, 4, 6, and 8 at 37°C were all 0.1 µg/mL, as shown in Table 5. No effect of pH of the solutions on the solubility was observed. Hoegger and Rohdewald, and Baumann et al. reported that the solubility of FLT was 0.4 and 0.14 µg/mL in water, respectively. However, these reports did not show the procedure applied for the solubility study. Our presented solubility values were determined using a procedure avoiding the effect of adsorption. Therefore, the values are considered to be more exact than those previously reported.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Solubility (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.1</td>
</tr>
<tr>
<td>0.1 N HCl</td>
<td>0.1</td>
</tr>
<tr>
<td>Buffer solution pH2</td>
<td>0.1</td>
</tr>
<tr>
<td>Buffer solution pH4</td>
<td>0.1</td>
</tr>
<tr>
<td>Buffer solution pH6</td>
<td>0.1</td>
</tr>
<tr>
<td>Buffer solution pH8</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Preliminary study for enhancement of FLT solubility
The solubility of FLT in water, and α-, β-, and γ-cyclodextrins were 0.16, 0.91, 3.3, and 81 µg/mL, respectively. The solubility was improved by the addition of cyclodextrins, and γ-cyclodextrin was found to be most effective. The result of water was higher than 0.1 µg/mL. The reason was considered to be super saturation by the ultrasonic treatment. Further study for improving FLT solubility will be performed.

CONCLUSION
Adsorption of FLT to experimental tools made of glass and polypropylene was revealed. The adsorption to conventional experimental tools was examined using 100 ng/mL FLT solution. The adsorption characteristics of FLT and a method to avoid such adsorption were determined from these results. The solubility of FLT were measured by the method avoiding the effect of adsorption, which in water, 0.1 N HCl, and buffer solutions at pH 2, 4, 6, and 8 were 0.1 µg/mL. It was found from the result of a preliminary experiment that the solubility of FLT was improved by the addition of α-, β-, and γ-cyclodextrins.

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REFERENCES
2. Hanauer S. Inflammatory bowel disease:
epidemiology, pathogenesis, and therapeutic opportunities. *Inflamm Bowel Dis* 2006;12 (Suppl. 1):s3-s9.


